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(54) Title: METHODS AND BIOMARKERS FOR DETECTION AND TREATMENT OF MATURE T-CELL LEUKEMIA

(57) Abstract: The present invention relates to methods and biomarkers for detection and characterization of mature T-cell neoplasias / leukemias (e.g., T-cell prolymphocytic leukemia, Sezary syndrome) in biological samples (e.g., tissue samples, blood samples, plasma samples, cell samples, serum samples).

METHODS AND BIOMARKERS FOR DETECTION AND TREATMENT OF MATURE T-CELL LEUKEMIA

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under CA136905 and CA140806 awarded by the National Institutes of Health. The Government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to methods and biomarkers for detection and characterization of mature T-cell neoplasias / leukemias (e.g., T-cell prolymphocytic leukemia, Sezary syndrome) in biological samples (e.g., tissue samples, blood samples, plasma samples, cell samples, serum samples).

BACKGROUND OF THE INVENTION

The genetic alterations underlying the pathogenesis of mature (post-thymic) T-cell leukemias are largely unknown and treatment options are often ineffective and patient outcomes poor. Among the mature T-cell leukemias, T-cell prolymphocytic leukemia (T-PLL) is an aggressive neoplasm of mature T-lymphocytes characterized by a rapid clinical course, resistance to conventional chemotherapy and poor median survival (less than 7.5 months) (see, e.g., Matutes, E. et al. Blood 78, 3269-3274 (1991); herein incorporated by reference in its entirety). An additional T-cell leukemia, Sezary Syndrome (SS), is an aggressive mature T-cell leukemic disease with median 5-year survival of less than 20% (see, e.g., Willemze, R. et al. Blood 105, 3768-3785, doi:2004-09-3502 (2005); herein incorporated by reference in its entirety).

Additional insight into the pathogenesis of mature T-cell leukemias is needed. In addition, better, more effective non-invasive tests for early detection of mature T-cell leukemias are needed to lower the morbidity and mortality associated with such cancers.

SUMMARY OF THE INVENTION

The present invention relates to methods and biomarkers for detection and characterization of mature T-cell neoplasias / leukemias (e.g., T-cell prolymphocytic

leukemia, Sezary syndrome) in biological samples (e.g., tissue samples, blood samples, plasma samples, cell samples, serum samples).

In certain embodiments, the present invention provides methods for detecting one or more JAK/STAT pathway variants associated with a mature T-cell leukemia in a subject, comprising contacting a sample from a subject with a JAK/STAT pathway variant detection assay under conditions that the presence of a JAK/STAT pathway variant associated with a mature T-cell leukemia is determined; and diagnosing the subject with a mature T-cell leukemia when one or more of the JAK/STAT pathway variants are present in the sample.

Similarly, in certain embodiments, the present invention provides uses of a variant JAK/STAT pathway nucleic acid or polypeptide for detecting a mature T-cell leukemia in a subject.

In certain embodiments, the present invention further provides methods for determining a decreased time to adverse outcome in a subject diagnosed with a mature T-cell leukemia, comprising contacting a sample from a subject with a JAK/STAT pathway variant detection assay under conditions that the presence of a JAK/STAT pathway variant associated with a mature T-cell leukemia is determined; and detecting a decreased time to adverse outcome in the subject when the JAK/STAT pathway variants are present in the sample. In some embodiments, the adverse outcome is selected from the group consisting of relapse of the mature T-cell leukemia, metastasis, or death.

In some embodiments for such methods and uses, the subject is a human (e.g., a human subject being screened for a mature T-cell leukemia) (e.g., a human subject at risk for developing a mature T-cell leukemia) (e.g., a human subject assessing the effectiveness of a mature T-cell leukemia treatment regimen).

In some embodiments, the biological sample is selected from the group consisting of a tissue sample, a cell sample, and a blood sample.

In some embodiments, the one or more JAK/STAT pathway variants encodes a loss of function mutation and/or a gain of function mutation.

In some embodiments, the JAK/STAT pathway variant is one or more variants selected from a JAK1 variant, a JAK3 variant, a STAT5B variant, and an IL2RG variant.

In some embodiments, the JAK1 variant is a JAK1 polypeptide having an amino acid sequence differing from a wild type JAK1 amino acid sequence. In some embodiments, the JAK1 variant is a JAK1 nucleic acid sequence encoding a JAK1 polypeptide having an amino acid sequence differing from a wild type JAK1 amino acid sequence. In some embodiments, the one or more JAK1 variants is a JAK1 polypeptide having, in comparison to wild type, an

amino acid variation selected from the group consisting of JAK1 p.F636L, JAK1 p.G646C, JAK1 p. Y654F, JAK1 p.V658F, JAK1 p.S703I, and JAK1 p.T901R.

In some embodiments, the JAK3 variant is a JAK3 polypeptide having an amino acid sequence differing from a wild type JAK3 amino acid sequence. In some embodiments, the JAK3 variant is a JAK3 nucleic acid sequence encoding a JAK3 polypeptide having an amino acid sequence differing from a wild type JAK3 amino acid sequence. In some embodiments, the one or more JAK3 variants is a JAK3 polypeptide having, in comparison to wild type, an amino acid variation selected from the group consisting of JAK3 p.ΔKNC563, AK3 p.M511I, JAK3 p. A573V, JAK3 p.R657. JAK3 p.Y980, JAK3 p.G662W, JAK3 p.P664T, JAK3 p. Y981, and JAK3 p.S989I.

In some embodiments, the STAT5B variant is a STAT5B polypeptide having an amino acid sequence differing from a wild type STAT5B amino acid sequence. In some embodiments, the STAT5B variant is a STAT5B nucleic acid sequence encoding a STAT5B polypeptide having an amino acid sequence differing from a wild type STAT5B amino acid sequence. In some embodiments, the one or more STAT5B variants is a STAT5B polypeptide having, in comparison to wild type, an amino acid variation selected from the group consisting of STAT5B p.T628S, STAT5B p.N642H, STAT5B p.Y699, STAT5B p.R659C, STAT5B p.Q706L, and STAT5B p.Y665H.

In some embodiments, the IL2RG variant is a IL2RG polypeptide having an amino acid sequence differing from a wild type IL2RG amino acid sequence. In some embodiments, the IL2RG variant is a IL2RG nucleic acid sequence encoding a IL2RG polypeptide having an amino acid sequence differing from a wild type IL2RG amino acid sequence. In some embodiments, the one or more IL2RG variants is a IL2RG polypeptide having, in comparison to wild type, an amino acid variation selected from the group consisting of IL2RG p.Y325, IL2RG p.ΔGSM268, and IL2RG p. K315E.

In some embodiments, the mature T-cell leukemia is T-cell prolymphocytic leukemia and the one or more JAK/STAT pathway variants is JAK / STAT pathway polypeptide having, in comparison to wild type, an amino acid variation selected from the group consisting of JAK1 p.V658F, JAK1 p.S703I, JAK1 p.T901R, JAK3 p.ΔKNC563, JAK3 p.M511I, JAK3 p. A573V, JAK3 p.R657, STAT5B p.T628S, STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.N642H, STAT5B p.Y665H, IL2RG p.ΔGSM268, and IL2RG p. K315E.

In some embodiments, the mature T-cell leukemia is Sezary syndrome and the one or more JAK/STAT pathway variants is JAK / STAT pathway polypeptide having, in comparison to wild type, an amino acid variation selected from the group consisting of JAK1 p. Y654F, JAK3 p. A573V, JAK3 p.Y980, JAK3 p. Y981, JAK3 p.S989I, STAT5B p.Y699, STAT5B p.N642H, and Il2RG p.Y325.

In some embodiments, the determining comprises detecting variant JAK1, JAK3, STAT5B, and IL2RG nucleic acids or polypeptides.

In some embodiments, the detecting variant JAK1, JAK3, STAT5B, and IL2RG nucleic acids comprises one or more nucleic acid detection method selected from the group consisting of sequencing, amplification and hybridization.

In some embodiments, the determining comprises a computer implemented method. In some embodiments, the computer implemented method comprises analyzing JAK1, JAK3, STAT5B, and IL2RG variant information and displaying the information to a user.

In some embodiments, the methods and uses further comprise the step of treating the subject for a mature T-cell leukemia and monitoring the subject for the presence of JAK1, JAK3, STAT5B, and IL2RG variants associated with the mature T-cell leukemia. For example, in some embodiments, the methods and uses further comprise the step of treating the subject for a mature T-cell leukemia under condition such that at least one symptom of the mature T-cell leukemia is diminished or eliminated.

In some embodiments, the treating comprises inhibiting JAK1, JAK3, STAT5B, and/or IL2RG expression and/or activity. In some embodiments, inhibiting STAT5B expression and/or activity is accomplished through administration of an agent configured to inhibit STAT5B expression (e.g., pimozide). In some embodiments, inhibiting JAK1 and/or JAK3 expression and/or activity is accomplished through administration of an agent configured to inhibit JAK1 and/or JAK3 expression and/or activity (e.g., ruxolitinib, tofacitinib, baricitinib, CYT387, and/or lestaurtinib).

In some embodiments, the methods further comprise administering one or more agents for treating a mature T-cell leukemia. In some embodiments, the one or more agents is selected from the group consisting of a purine analog (e.g., pentostatin, fludarabine, cladrbine), chlorambucil, cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP), cyclophosphamide, vincristine, prednisone (COP), and vincristine, doxorubicin, prednisone, etoposide, cyclophosphamide, bleomycin, alemtuzumab, and vorinostat.

In some embodiments, the methods and uses further comprise the step of detecting a variant in one or more additional genes and/or polypeptides associated with a mature T-cell

leukemia. In some embodiments, the one or more genes or polypeptides are selected from the group consisting of CHEK2, EZH2, and FBXW10.

Additional embodiments will be apparent to persons skilled in the relevant art based on the teachings contained herein.

DESCRIPTION OF THE DRAWINGS

Fig. 1 (gray-scaled) shows a phosphoproteomic screen of mature T-cell leukemia cell line HUT78. a, Schematic of phosphoproteomic screening analysis. Further details of experimental technique can be found in the Supplemental Information. b, Tyrosine-phosphorylated proteins identified by mass spectrometry in HUT78 T-cell leukemia cell line; pY indicates phosphorylated residue, boxed entries highlight JAK3 and STAT5B peptides.

Fig. 2 (gray-scaled) shows dual phosphoproteomic and genome sequencing screen identifies JAK-STAT activation in the mature T-cell leukemia cell line HUT78. a-c, Tandem mass-spectra confirming tyrosine phosphorylation of IL2RG p.Y325, JAK3 p.Y980/Y981 and STAT5B p.Y699 residues in HUT78 cells. d, Summary of results of phosphotyrosine proteomic screening of HUT78 cells (left) and summary of novel mutations in kinases and their targets identified by WES (right) highlighting the presence of altered JAK-STAT pathway in both datasets. e-g, *JAK1* (p.Y654F) and *JAK3* (p.A573V) mutations in HUT78 cells discovered through WES and confirmed by Sanger sequencing. HUT78 cells are haploinsufficient at the *JAK3* locus leading to monoallelic amplification.

Fig. 3 (gray-scaled) shows representative diagnostic material for T-PLL samples. a, Cytology of representative T-PLL case. b, TCR locus florescence in situ hybridization (FISH) analysis demonstrating typical inv(14) in a representative T-PLL sample.

Fig. 4 (gray-scaled) shows structural alterations involving *TCL1B/MTCP* locus in index T-PLL samples. Ideogram of structural alterations in 4 index T-PLL cases subjected to genomic sequencing involving chr14 (left) and/or chrX (right); the smaller regions (non-gray scaled "red") and the 22,976,660, 22,974,099, 22,962,913, 96,175,046, 96,082,911, 96,153,448 values represents the intrachromosomal translocation, inv(14); the second region for the chr14 (non-gray scaled "blue") and the values 22,946,702 and 154,299,773 indicates the interchromosomal translocation, t(14;X); breakpoint positions for each translocation are shown.

Fig. 5 (gray-scaled) shows activating IL2R-JAK1/3-STAT5B axis mutations in T-cell leukemia. a-d, Representative *IL2RG, JAK1, JAK3* and *STAT5B* mutations identified in primary T-PLL cells by WGS/WES and confirmed somatic acquisition by Sanger sequencing

of tumor (upper panels) and paired normal tissue (lower panels). Schematic representation of mutations in *IL2RG* (e), *JAK1* (f), *JAK3* (g) and *STAT5B* (h and l) identified through WGS, WES or targeted Sanger sequencing of HUT78 cells (diamond) and primary T-cell leukemias T-PLL (circles) and SS (squares) and the cutaneous T-cell lymphoma MF (triangles). Mutations with confirmed somatic acquisition are shown as filled symbols, with mutations at residues otherwise previously identified in hematopoietic malignancy shown as grey symbols; variants where adequate matched constitutional DNA was not available are shown as open symbols. For T-PLL and MF, the mutations are concentrated in the pseudo-kinase domains (f and g, purple; JAK1 and JAK3) or the SH2 domain (light blue; STAT5B). Two additional variants were detected in the kinase domains of JAK1 and JAK3 (red); a single case of T-PLL harbored a somatic three amino acid deletion in the transmembrane domain of IL2RG (e, purple) as well as a somatic missense mutation in the cytoplasmic domain. Δ indicates small deletion of several contiguous amino acid residues. i, Frequency of JAK-STAT pathway mutations in mature T-cell leukemia/lymphoma. j, SH2 domain of the STAT5A protein (1y1u) highlighting analogous residues of the STAT5B mutations p.N642H (gray-scaled blue), p.Y665H (gray-scaled purple) and p.T628S (gray-scaled red) demonstrating close 3-dimensional proximity of mutated STAT5B residues based on STAT5A crystal structure. k, Pseudokinase domain of JAK2 (4fvp) highlighting V617 (gray-scaled light blue) and analogous residues for the JAK3 mutations p.A573V (gray-scaled red), p.M511I (gray-scaled dark blue), p.ΔKNC563 (gray-scaled purple).

Fig. 6 (gray-scaled) shows copy-number variations in T-PLL. Circos diagram depicting aCGH data for 43 individual T-PLL samples (inner data tracks) and a histogram representation of all samples to show areas of recurrent gain or loss of chromosomal material (outer data tracks; gray-scaled blue represents loss, gray-scaled red indicates gain); arrow indicates recurrent loss of ATM locus on 11q; arrowhead represents alterations of chromosome 8 present in a majority of T-PLL genomes. Histogram data is presented as the number of cases with –logR values less than -0.2 (loss, blue) or greater than 0.2 (gain, red).

Fig. 7 (gray-scaled) shows distribution of *ATM* mutations in T-PLL. a, Protein diagram depicting all mutations in ATM identified by genome and exome sequencing of T-PLL samples. b-d, Representative Sanger sequencing electropherograms confirming the existence of the mutation in tumor samples (upper panels) and the absence of mutations in paired normal samples (lower panels).

Fig. 8 (gray-scaled) shows targeted inhibition of activated STAT5B signaling in primary T-cell leukemias. a-b, Mutated IL2RG (p.ΔGMS628), JAK1 (p.S703I), JAK3 (p.Q507P) and STAT5B (p.T628S and p.N642H) leads to increased activation of STAT5B transcriptional activity (a, bar graph; n=3 for each mutant in separate experiments; asterisk indicates $p<0.05$; dagger indicates $p<0.001$) and increased phosphorylation of STAT5B (b, Western blot; arrowhead indicates exogenous STAT5B, arrow indicates endogenous STAT5B) in HeLa cells. c, Enhanced cell proliferation in the presence of mutant p.T628S STAT5B protein in Ba/F3 cells (n=6; asterisk indicates $p<0.01$). d, Enhanced colony forming capacity in Jurkat T-cells (n=3; asterisk indicates $p<0.01$). e, Upregulation of pSTAT5B in representative primary T-PLL samples demonstrated by immunocytochemistry. Targeted inhibition of pSTAT5 with the small molecule inhibitor Pimozide leads to causes reduced proliferation (f), decreased viability (g) and diminished pSTAT5 levels (h) in primary T-PLL samples (n=3 independent replicates for f and g, asterisk indicates $p<0.005$; representative data is shown for T-PLL 25 in h; arrowhead in h indicates cleaved PARP associated with apoptotic activation). i, Pathway diagram illustrating the interaction of IL2RG, JAK1, JAK3 and STAT5B during IL2 cytokine activation. Cytokine binding to the extracellular portion of membrane-associated interleukin receptors induces conformational changes in the intracellular portion. Associated JAK non-receptor tyrosine kinases then auto-phosphorylate leading to STAT recruitment and activation through tyrosine phosphorylation. Activated STAT proteins then dimerize and translocate to the nucleus to regulate transcription of numerous genes involved in differentiation, proliferation and survival. Pimozide treatment inhibits STAT5 phosphorylation delimiting downstream transcriptional activity of activating mutations in cytokine receptor-JAK-STAT proteins (highlighted green).

Fig. 9 (gray-scaled) shows read alignment of WGS data supporting *JAK1* mutations in T-PLL index cases. The total individual reads supporting variant calling of the p.V658F (a) and p.S703I (b) mutations in index T-PLL samples is shown. Nucleotides with deviation from reference sequence are highlighted. The mutations as well as a synonymous single nucleotide polymorphism are boxed.

Fig. 10 (gray-scaled) shows three-dimensional apposition of selected residues affected by *JAK1*, *JAK3* and *STAT5B* mutation in T-PLL. Amino acid sequence alignment of STAT5B (top) versus STAT5A (a, bottom), JAK2 (top) versus JAK1 (b, bottom) and JAK3 (c, bottom) in the region of recurrent mutations in T-PLL. Colored fill indicates identical

amino acid; white, minus indicates disparate residues; white with colored text, + indicates similar residues); selected mutated residues are indicated in red.

Fig. 11A and 11B provide the homo sapiens wild type nucleic acid sequence and amino acid sequence for JAK1, respectively.

Fig. 11C and 11D provide the homo sapiens wild type nucleic acid sequence and amino acid sequence for JAK3, respectively.

Fig. 11E and 11F provide the homo sapiens wild type nucleic acid sequence and amino acid sequence for STAT5B, respectively.

Fig. 11G and 11H provide the homo sapiens wild type nucleic acid sequence and amino acid sequence for IL2RG is provided at Fig. 11G and 11H, respectively.

Figure 12 (gray-scaled) shows selected recurrently mutated genes in T-PLL by WES and WGS.

Fig. 13 shows JAK-STAT mutational status associated with patient demographic and clinical diagnostic and prognostic information. Index cases subjected to WGS are listed first. Deletions or point mutations in 11q23 locus or *ATM* gene, respectively are indicated. Karyotypic or immunohistochemical evidence of rearrangements involving the *TCL1A/B* or *MTCP1* loci or TCL1 protein are indicated. Mutations identified in T-PLL cases are shown by gene. Cases for which no mutation was identified are indicated according to which method of mutation detection was employed. The patient's treatment status at time of specimen collection is also indicated as are the times from disease diagnosis to relapse or death and the patients' survival status.

DEFINITIONS

To facilitate an understanding of the present invention, a number of terms and phrases are defined below:

As used herein, the term "mature T-cell leukemia" or "mature T-cell neoplasia" refers to a type of lymphoid leukemia that which affects mature T-cells. Examples of mature T-cell leukemia include, but are not limited to, T-cell prolymphocytic leukemia (T-PLL) and Sezary syndrome (SS). T-cell prolymphocytic leukemia (T-PLL) is a mature T-cell leukemia with aggressive behavior and predilection for blood, bone marrow, lymph nodes, liver, spleen, and skin involvement. SS is a type of cutaneous lymphoma wherein the affected cells are T-cells that have pathological quantities of mucopolysaccharides. As described herein, the terms "mature T-cell leukemia" and "mature T-cell neoplasia" are interchagable.

The term "JAK/STAT signaling pathway" or "JAK/STAT pathway" refers to a signaling pathway for transmitting information from chemical signals outside a cell, through the cell membrane, and into gene promoters on DNA in the cell nucleus, which causes DNA transcription and activity in the cell. The JAK-STAT system consists of three main components: (1) a receptor (2) Janus kinase (JAK) and (3) Signal Transducer and Activator of Transcription (STAT). Examples of JAK include JAK1, JAK2, JAK3, and TYK2. Examples of STAT include STAT1, STAT3, STAT5A, STAT5B, and STAT6. In some embodiments, the cytokine that bind the receptor is IL2RG.

As used herein, the term "JAK / STAT pathway variant" refers to an aberrant or mutated or non-wild-type member of the JAK / STAT pathway. Examples of JAK / STAT pathway variants include any JAK1, JAK3, STAT5B, and/or IL2RG nucleic acid sequence and/or amino acid sequence differing in any manner from its respective wild type sequence. The homo sapiens wild type nucleic acid sequence and amino acid sequence for JAK1 is provided at Fig. 11A and 11B, respectively. The homo sapiens wild type nucleic acid sequence and amino acid sequence for JAK3 is provided at Fig. 11C and 11D, respectively. The homo sapiens wild type nucleic acid sequence and amino acid sequence for STAT5B is provided at Fig. 121 and 121, respectively.The homo sapiens wild type nucleic acid sequence and amino acid sequence for IL2RG is provided at Fig. 11G and 11H, respectively.

Specific examples of JAK1 variants include, but are not limited to, JAK1 polypeptides having an amino acid sequence differing from its respective wild type sequence (or nucleic acid sequence encoding such an amino acid) in the following manner: JAK1 p.F636L, JAK1 p.G646C, JAK1 p. Y654F, JAK1 p.V658F, JAK1 p.S703I, and JAK1 p.T901R. Specific examples of JAK3 variants include, but are not limited to, JAK3 polypeptides having an amino acid sequence differing from its respective wild type sequence (or nucleic acid sequence encoding such an amino acid) in the following manner: JAK3 p.M511I, JAK3 p. A573V, JAK3 p.R657., JAK3 p.G662W, JAK3 p.P664T, JAK3 p.Y980, JAK3 p.ΔKNC563, JAK3 p. Y981, and JAK3 p.S989I. Specific examples of STAT5B variants include, but are not limited to, STAT5B polypeptides having an amino acid sequence differing from its respective wild type sequence (or nucleic acid sequence encoding such an amino acid) in the following manner: STAT5B p.T628S, STAT5B p.N642H, STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.Y699, and STAT5B p.Y665H. Specific examples of IL2RG variants include, but are not limited to, IL2RG polypeptides having an amino acid sequence differing from its respective wild type sequence (or nucleic acid sequence encoding

such an amino acid) in the following manner: IL2RG p.ΔGSM268, Il2RG p.Y325 and IL2RG p. K315E.

As used herein, the term "JAK inhibitor" refers to an agent (e.g., a pharmaceutical agent) that functions by inhibiting the activity of one or more of the Janus kinase (JAK) family of enzymes (e.g., JAK1, JAK2, JAK3, TYK2), thereby interfering with the JAK/STAT signaling pathway. Examples of JAK inhibitors include, but are not limited to, ruxolitinib, tofacitinib, baricitinib, CYT387, and lestaurtinib.

As used herein, the term "STAT inhibitor" refers to an agent (e.g., a pharmaceutical agent) that functions by inhibiting the activity of one or more of the Signal Transducer and Activator of Transcription (STAT) family, thereby interfering with the JAK/STAT signaling pathway. An example of a STAT inhibitor (e.g., STAT5B inhibitor) is pimozide.

As used herein, the term "biomarker" refers to an organic biomolecule which is differentially present in a sample taken from a subject of one phenotypic status (e.g. having a disease) as compared with another phenotypic status (e.g., not having the disease). A biomarker is differentially present between different phenotypic statuses if the mean or median expression level of the biomarker in the different groups is calculated to be statistically significant. In some embodiments, biomarkers, alone or in combination, provide measures of relative risk that a subject belongs to one phenotypic status or another. Therefore, they are useful as markers for disease (diagnostics), therapeutic effectiveness of a drug (theranostics) and drug toxicity.

Examples of mature T-cell leukemia biomarkers established through the experiments conducted during the present invention include, for example, variants of JAK1 having amino acid sequences differing its respective wild type sequence (e.g., JAK1 p.F636L, JAK1 p.G646C, JAK1 p. Y654F, JAK1 p.V658F, JAK1 p.S703I, and JAK1 p.T901R), variants of JAK3 having amino acid sequences differing its respective wild type sequence (e.g., JAK3 p.M511I, JAK3 p.ΔKNC563, JAK3 p. A573V, JAK3 p.G662W, JAK3 p.P664T, JAK3 p.R657. JAK3 p.Y980, JAK3 p. Y981, and JAK3 p.S989I), variants of STAT5B having amino acid sequences differing its respective wild type sequence (e.g., STAT5B p.T628S, STAT5B p.N642H, STAT5B p.Y699, STAT5B p.R659C, STAT5B p.Q706L, and STAT5B p.Y665H), and variants of IL2RG having amino acid sequences differing its respective wild type sequence (e.g., IL2RG p.ΔGSM268, Il2RG p.Y325 and IL2RG p. K315E).

As used herein, the term "measuring" means methods which include detecting the presence or absence of biomarker(s) in the sample, quantifying the amount of marker(s) in

the sample, and/or qualifying the type of biomarker. Measuring can be accomplished by methods known in the art and those further described herein. Any suitable methods can be used to detect and measure one or more of the markers described herein.

As used herein, the term "detect" refers to identifying the presence, absence or amount of the object to be detected (e.g., a biomarker).

As used herein, the term "diagnostic" means identifying the presence or nature of a pathologic condition, i.e., a mature T-cell leukemia (e.g., T-PLL, SS). Diagnostic methods differ in their sensitivity and specificity. As used herein, the term "sensitivity" is defined as a statistical measure of performance of an assay (e.g., method, test), calculated by dividing the number of true positives by the sum of the true positives and the false negatives. As used herein, the term "specificity" is defined as a statistical measure of performance of an assay (e.g., method, test), calculated by dividing the number of true negatives by the sum of true negatives and false positives.

As used herein, the term "informative" or "informativeness" refers to a quality of a marker or panel of markers, and specifically to the likelihood of finding a marker (or panel of markers) in a positive sample.

As used herein, the term "metastasis" is meant to refer to the process in which cancer cells originating in one organ or part of the body relocate to another part of the body and continue to replicate. Metastasized cells subsequently form tumors which may further metastasize. Metastasis thus refers to the spread of cancer from the part of the body where it originally occurs to other parts of the body.

The term "neoplasm" as used herein refers to any new and abnormal growth of tissue. Thus, a neoplasm can be a premalignant neoplasm or a malignant neoplasm. The term "neoplasm-specific marker" refers to any biological material that can be used to indicate the presence of a neoplasm. Examples of biological materials include, without limitation, nucleic acids, polypeptides, carbohydrates, fatty acids, cellular components (e.g., cell membranes and mitochondria), and whole cells.

As used herein, the term "adverse outcome" refers to an undesirable outcome in a patient diagnosed with a mature T-cell leukemia. In some embodiments, the patient is undergoing or has undergone treatment for a mature T-cell leukemia. Examples of adverse outcome include but are not limited to, recurrence of a mature T-cell leukemia, metastasis, transformation, or death.

As used herein, the term “amplicon” refers to a nucleic acid generated using primer pairs. The amplicon is typically single-stranded DNA (e.g., the result of asymmetric amplification), however, it may be RNA or dsDNA.

The term "amplifying" or "amplification" in the context of nucleic acids refers to the production of multiple copies of a polynucleotide, or a portion of the polynucleotide, typically starting from a small amount of the polynucleotide (*e.g.*, a single polynucleotide molecule), where the amplification products or amplicons are generally detectable.

As used herein, the term "primer" refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, that is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product that is complementary to a nucleic acid strand is induced (*e.g.*, in the presence of nucleotides and an inducing agent such as a biocatalyst (*e.g.*, a DNA polymerase or the like) and at a suitable temperature and pH). The primer is typically single stranded for maximum efficiency in amplification, but may alternatively be double stranded. If double stranded, the primer is generally first treated to separate its strands before being used to prepare extension products. In some embodiments, the primer is an oligodeoxyribonucleotide. The primer is sufficiently long to prime the synthesis of extension products in the presence of the inducing agent. The exact lengths of the primers will depend on many factors, including temperature, source of primer and the use of the method. In certain embodiments, the primer is a capture primer.

A "sequence" of a biopolymer refers to the order and identity of monomer units (e.g., nucleotides, etc.) in the biopolymer. The sequence (e.g., base sequence) of a nucleic acid is typically read in the 5' to 3' direction.

As used herein, the term "subject" refers to any animal (*e.g.*, a mammal), including, but not limited to, humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

As used herein, the term "non-human animals" refers to all non-human animals including, but are not limited to, vertebrates such as rodents, non-human primates, ovines, bovines, ruminants, lagomorphs, porcines, caprines, equines, canines, felines, aves, etc.

As used therein, the term “locus” as used herein refers to a nucleic acid sequence on a chromosome or on a linkage map and includes the coding sequence as well as 5’ and 3’ sequences involved in regulation of the gene.

As used therein, the term "gas phase ion spectrometer" refers to an apparatus that detects gas phase ions. Gas phase ion spectrometers include an ion source that supplies gas phase ions. Gas phase ion spectrometers include, for example, mass spectrometers, ion mobility spectrometers, and total ion current measuring devices. "Gas phase ion spectrometry" refers to the use of a gas phase ion spectrometer to detect gas phase ions.

As used therein, the term "mass spectrometer" refers to a gas phase ion spectrometer that measures a parameter that can be translated into mass-to-charge ratios of gas phase ions. Mass spectrometers generally include an ion source and a mass analyzer. Examples of mass spectrometers are time-of-flight, magnetic sector, quadrupole filter, ion trap, ion cyclotron resonance, electrostatic sector analyzer and hybrids of these. "Mass spectrometry" refers to the use of a mass spectrometer to detect gas phase ions.

As used therein, the term "laser desorption mass spectrometer" refers to a mass spectrometer that uses laser energy as a means to desorb, volatilize, and ionize an analyte.

As used therein, the term "tandem mass spectrometer" refers to any mass spectrometer that is capable of performing two successive stages of m/z-based discrimination or measurement of ions, including ions in an ion mixture. The phrase includes mass spectrometers having two mass analyzers that are capable of performing two successive stages of m/z-based discrimination or measurement of ions tandem-in-space. The phrase further includes mass spectrometers having a single mass analyzer that is capable of performing two successive stages of m/z-based discrimination or measurement of ions tandem-in-time. The phrase thus explicitly includes Qq-TOF mass spectrometers, ion trap mass spectrometers, ion trap-TOF mass spectrometers, TOF-TOF mass spectrometers, Fourier transform ion cyclotron resonance mass spectrometers, electrostatic sector-magnetic sector mass spectrometers, and combinations thereof.

As used therein, the term "mass analyzer" refers to a sub-assembly of a mass spectrometer that comprises means for measuring a parameter that can be translated into mass-to-charge ratios of gas phase ions. In a time-of-flight mass spectrometer the mass analyzer comprises an ion optic assembly, a flight tube and an ion detector.

As used therein, the term "ion source" refers to a sub-assembly of a gas phase ion spectrometer that provides gas phase ions. In one embodiment, the ion source provides ions through a desorption/ionization process. Such embodiments generally comprise a probe interface that positionally engages a probe in an interrogatable relationship to a source of ionizing energy (e.g., a laser desorption/ionization source) and in concurrent communication at atmospheric or subatmospheric pressure with a detector of a gas phase ion spectrometer

Forms of ionizing energy for desorbing/ionizing an analyte from a solid phase include, for example: (1) laser energy; (2) fast atoms (used in fast atom bombardment); (3) high energy particles generated via beta decay of radionuclides (used in plasma desorption); and (4) primary ions generating secondary ions (used in secondary ion mass spectrometry). A preferred form of ionizing energy for solid phase analytes is a laser (used in laser desorption/ionization), in particular, nitrogen lasers, Nd-Yag lasers and other pulsed laser sources. "Fluence" refers to the energy delivered per unit area of interrogated image. A high fluence source, such as a laser, will deliver about 1 mJ/mm2 to 50 mJ/mm2. Typically, a sample is placed on the surface of a probe, the probe is engaged with the probe interface and the probe surface is struck with the ionizing energy. The energy desorbs analyte molecules from the surface into the gas phase and ionizes them. Other forms of ionizing energy for analytes include, for example: (1) electrons that ionize gas phase neutrals; (2) strong electric field to induce ionization from gas phase, solid phase, or liquid phase neutrals; and (3) a source that applies a combination of ionization particles or electric fields with neutral chemicals to induce chemical ionization of solid phase, gas phase, and liquid phase neutrals.

As used therein, the term "solid support" refers to a solid material which can be derivatized with, or otherwise attached to, a capture reagent. Exemplary solid supports include probes, microtiter plates and chromatographic resins.

As used therein, the term "probe" in the context of this invention refers to a device adapted to engage a probe interface of a gas phase ion spectrometer (e.g., a mass spectrometer) and to present an analyte to ionizing energy for ionization and introduction into a gas phase ion spectrometer, such as a mass spectrometer. A "probe" will generally comprise a solid substrate (either flexible or rigid) comprising a sample presenting surface on which an analyte is presented to the source of ionizing energy.

As used therein, the term "surface-enhanced laser desorption/ionization" or "SELDI" refers to a method of desorption/ionization gas phase ion spectrometry (e.g., mass spectrometry) in which the analyte is captured on the surface of a SELDI probe that engages the probe interface of the gas phase ion spectrometer. In "SELDI MS," the gas phase ion spectrometer is a mass spectrometer. SELDI technology is described in, e.g., U.S. Patent Nos. 5,719,060 and 6,225,047; each incorporated herein by reference in its entirety.

As used therein, the term "Surface-Enhanced Affinity Capture" or "SEAC" is a version of SELDI that involves the use of probes comprising an absorbent surface (a "SEAC probe"). "Adsorbent surface" refers to a surface to which is bound an adsorbent (also called a "capture reagent" or an "affinity reagent"). An adsorbent is any material capable of binding

an analyte (e.g., a target polypeptide or nucleic acid). "Chromatographic adsorbent" refers to a material typically used in chromatography. Chromatographic adsorbents include, for example, ion exchange materials, metal chelators (e.g., nitriloacetic acid or iminodiacetic acid), immobilized metal chelates, hydrophobic interaction adsorbents, hydrophilic interaction adsorbents, dyes, simple biomolecules (e.g., nucleotides, amino acids, simple sugars and fatty acids) and mixed mode adsorbents (e.g., hydrophobic attraction/electrostatic repulsion adsorbents). "Biospecific adsorbent" refers an adsorbent comprising a biomolecule, e.g., a nucleic acid molecule (e.g., an aptamer), a polypeptide, a polysaccharide, a lipid, a steroid or a conjugate of these (e.g., a glycoprotein, a lipoprotein, a glycolipid, a nucleic acid (e.g., DNA)-protein conjugate). In certain instances the biospecific adsorbent can be a macromolecular structure such as a multiprotein complex, a biological membrane or a virus. Examples of biospecific adsorbents are antibodies, receptor proteins and nucleic acids. Biospecific adsorbents typically have higher specificity for a target analyte than chromatographic adsorbents. Further examples of adsorbents for use in SELDI can be found in U.S. Patent No. 6,225,047; incorporated herein by reference in its entirety. In some embodiments, a SEAC probe is provided as a pre-activated surface which can be modified to provide an adsorbent of choice. For example, certain probes are provided with a reactive moiety that is capable of binding a biological molecule through a covalent bond. Epoxide and carbodiimidizole are useful reactive moieties to covalently bind biospecific adsorbents such as antibodies or cellular receptors.

As used therein, the term "adsorption" refers to detectable non-covalent binding of an analyte to an adsorbent or capture reagent.

As used therein, the term "Surface-Enhanced Neat Desorption" or "SEND" is a version of SELDI that involves the use of probes comprising energy absorbing molecules chemically bound to the probe surface. ("SEND probe.") "Energy absorbing molecules" ("EAM") refer to molecules that are capable of absorbing energy from a laser desorption/ionization source and thereafter contributing to desorption and ionization of analyte molecules in contact therewith. The phrase includes molecules used in MALDI, frequently referred to as "matrix", and explicitly includes cinnamic acid derivatives, sinapinic acid ("SPA"), cyano-hydroxy-cinnamic acid ("CHCA") and dihydroxybenzoic acid, ferulic acid, hydroxyacetophenone derivatives, as well as others. It also includes EAMs used in SELDI.

As used therein, the term "Surface-Enhanced Photolabile Attachment and Release" or "SEPAR" is a version of SELDI that involves the use of probes having moieties attached to

the surface that can covalently bind an analyte, and then release the analyte through breaking a photolabile bond in the moiety after exposure to light e.g., laser light. SEPAR is further described in U.S. Patent No. 5,719,060; incorporated by reference in its entirety.

As used therein, the term "eluant" or "wash solution" refers to an agent, typically a solution, which is used to affect or modify adsorption of an analyte to an adsorbent surface and/or remove unbound materials from the surface. The elution characteristics of an eluant can depend, for example, on pH, ionic strength, hydrophobicity, degree of chaotropism, detergent strength and temperature.

As used herein, the term "biochip" refers to a solid substrate having a generally planar surface to which an adsorbent is attached. Frequently, the surface of the biochip comprises a plurality of addressable locations, each of which location has the adsorbent bound there. Biochips can be adapted to engage a probe interface and, therefore, function as probes.

As used herein, the term "protein biochip" refers to a biochip adapted for the capture of polypeptides. Many protein biochips are described in the art. These include, for example, protein biochips produced by Ciphergen Biosystems (Fremont, Calif.), Packard BioScience Company (Meriden Conn.), Zyomyx (Hayward, Calif.) and Phylos (Lexington, Mass.). Examples of such protein biochips are described in the following patents or patent applications: U.S. Pat. Nos. 6,329,209 and 6,225,047; International publication Nos. WO 99/51773 and WO 00/56934; each incorporated herein by reference in its entirety.

DETAILED DESCRIPTION OF THE INVENTION

The molecular pathogenesis of mature T-cell leukemia (TCL) is poorly understood and current treatment options are limited to non-targeted cytotoxic agents. T-cell prolymphocytic leukemia (T-PLL) and Sezary syndrome (SS) are aggressive and chemotherapy-resistant neoplasia of mature T-lymphocytes characterized by a rapid clinical course and high mortality.

Experiments conducted during the course of developing embodiments for the present invention integrated mass spectrometry-driven phosphoproteomics with whole genome and exome sequencing to uncover oncogenic activation of the IL2R-JAK1/JAK3-STAT5B pathway in mature T-cell leukemias including T-PLL and SS. Phosphoproteomic and exome sequencing analysis of the SS-derived T-cell line HUT78 revealed activating phosphorylation of JAK3 and STAT5B and gain-of-function mutations in both *JAK1* and *JAK3*. Whole genome sequencing of 4 index T-PLL samples identified JAK1 mutations not previously associated with T-PLL while WES of 39 additional T-PLL samples uncovered recurrently

altered genes not previously implicated in T-PLL pathogenesis including *CHEK2*, *EZH2* and *FBXW10* in addition to frequent recurrent mutations targeting members of the JAK-STAT signaling pathway. Altogether, 38 of 50 T-PLL genomes (76.0%) harbored activating mutations in *IL2RG*, *JAK1*, *JAK3* and *STAT5B* genes. Additional mutations in *JAK1*, *JAK3* and *STAT5B* were also detected in SS (5/66, 7.6%). Functionally, expression of representative JAK1, JAK3 and STAT5b mutants exhibited STAT5 hyperactivation and/or IL-3 independent growth. Moreover, expression of recurrent STAT5B mutations exhibited enhanced colony formation. Importantly, such experiments further demonstrated constitutive activation of STAT5 in primary T-PLLs. Finally, targeted STAT5B inhibition reduced pSTAT5B levels in primary T-PLL cells with consequent apoptotic cell death. Altogether, these results implicate recurrent mutational activation of cytokine receptor-JAK-STAT signaling in the pathogenesis of mature T-cell leukemias and suggest opportunities for more effective and less toxic novel therapies targeting the JAK-STAT pathway in these aggressive cancers.

Accordingly, the present invention provides methods and biomarkers for detection and characterization of mature T-cell neoplasias / leukemias (e.g., T-cell prolymphocytic leukemia, Sezary syndrome) in biological samples (e.g., tissue samples, blood samples, plasma samples, cell samples, serum samples).

I. Diagnostic and Screening Applications

Embodiments of the present invention provide diagnostic, prognostic, and screening methods. In some embodiments, the methods characterize and diagnose mature T-cell leukemias (e.g., T-cell prolymphocytic leukemia (T-PLL), Sezary syndrome (SS), mycosis fungoides (MF)) through detection and/or characterization of aberrant JAK/STAT pathway activity within T-cells from a biological sample. Examples of mature T-cell leukemias include T-cell prolymphocytic leukemia (T-PLL), Sezary syndrome (SS), and mycosis fungoides (MF). Exemplary, non-limiting methods of identifying aberrant JAK/STAT pathway activity within T-cells from a biological are described below.

A. JAK/STAT Signaling Pathway Mutations

Embodiments of the present invention provide compositions and methods for detecting JAK/STAT pathway mutations (e.g., to identify or diagnose T-cell leukemias). The present invention is not limited to particular JAK/STAT pathway mutations. In some

embodiments, mutations are loss of function mutations (e.g., truncation, nonsense, missense, or frameshift mutations) and/or gain of function mutations.

Exemplary mutations include, but are not limited to, any nucleic acid and/or polypeptide mutation related to the JAK/STAT pathway (e.g., nucleic acid and/or polypeptide mutations related to JAK1, JAK3, STAT5B, IL2RG). Examples of JAK1 mutations include, for example, JAK1 polypeptides having an amino acid differing from its respective wild type sequence (and nucleic acid sequence encoding such amino acid sequence) (e.g., JAK1 p.F636L, JAK1 p.G646C, JAK1 p. Y654F, JAK1 p.V658F, JAK1 p.S703I, and JAK1 p.T901R). Examples of JAK3 mutations include, for example, JAK3 polypeptides having an amino acid differing from its respective wild type sequence (and nucleic acid sequence encoding such amino acid sequence) (e.g., JAK3 p.G662W, JAK3 p.P664T, JAK3 p.M511I, JAK3 p. A573V, JAK3 p.R657., JAK3 p.ΔKNC563, JAK3 p.Y980, JAK3 p. Y981, and JAK3 p.S989I). Examples of STAT5B mutations include, for example, STAT5B polypeptides having an amino acid differing from its respective wild type sequence (and nucleic acid sequence encoding such amino acid sequence) (e.g., STAT5B p.T628S, STAT5B p.N642H, STAT5B p.Y699, STAT5B p.R659C, STAT5B p.Q706L, and STAT5B p.Y665H). Examples of IL2RG mutations include, for example, IL2RG polypeptides having an amino acid differing from its respective wild type sequence (and nucleic acid sequence encoding such amino acid sequence) (e.g., IL2RG p.ΔGSM268, Il2RG p.Y325 and IL2RG p. K315E).

In some embodiments, detection of one or more of the following JAK/STAT pathway mutations (in comparison to wild type) is used to identify or diagnose T-cell prolymphocytic leukemia: JAK1 p.V658F, JAK1 p.S703I, JAK1 p.T901R, JAK3 p.ΔKNC563, JAK3 p.M511I, JAK3 p. A573V, JAK3 p.R657, STAT5B p.T628S, STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.N642H, STAT5B p.Y665H, IL2RG p.ΔGSM268, and IL2RG p. K315E.

In some embodiments, detection of one or more of the following JAK/STAT pathway mutations (in comparison to wild type) is used to identify or diagnose Sezary syndrome: JAK1 p. Y654F, JAK3 p. A573V, JAK3 p.Y980, JAK3 p. Y981, JAK3 p.S989I, STAT5B p.Y699, STAT5B p.N642H, and Il2RG p.Y325.

In some embodiments, detection of one or more of the following JAK/STAT pathway mutations (in comparison to wild type) is used to identify or diagnose mycosis fungoides: JAK1 p.F636L, JAK1 p.G646C, JAK3 p.G662W, and JAK3 p.P664T.

While the present invention exemplifies several markers specific for detecting mature T-cell leukemias (e.g., T-PLL, SS, MF), any marker that is correlated with the presence or absence or prognosis of mature T-cell leukemias may be used. A marker, as used herein, includes, for example, nucleic acid(s) whose production or mutation or lack of production is characteristic of a mature T-cell leukemia and mutations that cause the same effect (e.g., deletions, truncations, etc).

In some embodiments, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or more (e.g., all)) of the mutations are identified in order to diagnose or characterize a mature T-cell leukemia (e.g., T-PLL, SS, MF). In some embodiments, multiple markers are detected in a panel or multiplex format.

Particular combinations of markers may be used that show optimal function with different ethnic groups or sex, different geographic distributions, different stages of disease, different degrees of specificity or different degrees of sensitivity. Particular combinations may also be developed which are particularly sensitive to the effect of therapeutic regimens on disease progression. Subjects may be monitored after a therapy and/or course of action to determine the effectiveness of that specific therapy and/or course of action.

B. Detection of Alleles

In some embodiments, the present invention provides methods of detecting the presence of wild type or variant (*e.g.*, mutant or polymorphic) nucleic acids or polypeptides related to the JAK/STAT pathway (e.g., JAK1, JAK3, STAT5B, IL2RG). The detection of mutant nucleic acids or polypeptides related to the JAK/STAT pathway finds use in the diagnosis of disease (*e.g.*, mature T-cell leukemias), research, and selection of appropriate treatment and/or monitoring regimens.

Accordingly, the present invention provides methods for determining whether a subject (e.g., a human patient) has a JAK/STAT pathway related mutation profile associated with a mature T-cell leukemia (e.g., T-PLL, SS, MF).

A number of methods are available for analysis of variant (*e.g.*, mutant or polymorphic) nucleic acid sequences. Assays for detecting variants (*e.g.*, polymorphisms or mutations) fall into several categories, including, but not limited to direct sequencing assays, fragment polymorphism assays, hybridization assays, and computer based data analysis. Protocols and commercially available kits or services for performing multiple variations of these assays are available. In some embodiments, assays are performed in combination or in

hybrid (*e.g.*, different reagents or technologies from several assays are combined to yield one assay). The following assays are useful in the present invention.

Any patient sample containing JAK/STAT pathway nucleic acids or polypeptides (e.g., JAK1, JAK3, STAT5B, IL2RG) may be tested according to the methods of the present invention. By way of non-limiting examples, the sample may be tissue, blood, urine, semen, or a fraction thereof (e.g., plasma, serum, whole blood, spleen cells, etc.).

The patient sample may undergo preliminary processing designed to isolate or enrich the sample for the JAK/STAT pathway nucleic acids or polypeptides (e.g., JAK1, JAK3, STAT5B, IL2RG) or cells that contain such nucleic acids or polypeptides. A variety of techniques known to those of ordinary skill in the art may be used for this purpose, including but not limited: centrifugation; immunocapture; cell lysis; and, nucleic acid target capture (See, e.g., EP Pat. No. 1 409 727, herein incorporated by reference in its entirety).

i. DNA and RNA Detection

Variant JAK/STAT pathway nucleic acids or polypeptides (e.g., JAK1, JAK3, STAT5B, IL2RG) of the present invention may be detected as genomic DNA or mRNA using a variety of nucleic acid techniques known to those of ordinary skill in the art, including but not limited to: nucleic acid sequencing; nucleic acid hybridization; and, nucleic acid amplification.

1. Sequencing

Illustrative non-limiting examples of nucleic acid sequencing techniques include, but are not limited to, chain terminator (Sanger) sequencing and dye terminator sequencing. Those of ordinary skill in the art will recognize that because RNA is less stable in the cell and more prone to nuclease attack experimentally RNA is usually reverse transcribed to DNA before sequencing.

Chain terminator sequencing uses sequence-specific termination of a DNA synthesis reaction using modified nucleotide substrates. Extension is initiated at a specific site on the template DNA by using a short radioactive, fluorescent or other labeled, oligonucleotide primer complementary to the template at that region. The oligonucleotide primer is extended using a DNA polymerase, standard four deoxynucleotide bases, and a low concentration of one chain terminating nucleotide, most commonly a di-deoxynucleotide. This reaction is repeated in four separate tubes with each of the bases taking turns as the di-deoxynucleotide. Limited incorporation of the chain terminating nucleotide by the DNA polymerase results in

a series of related DNA fragments that are terminated only at positions where that particular di-deoxynucleotide is used. For each reaction tube, the fragments are size-separated by electrophoresis in a slab polyacrylamide gel or a capillary tube filled with a viscous polymer. The sequence is determined by reading which lane produces a visualized mark from the labeled primer as you scan from the top of the gel to the bottom.

Dye terminator sequencing alternatively labels the terminators. Complete sequencing can be performed in a single reaction by labeling each of the di-deoxynucleotide chain-terminators with a separate fluorescent dye, which fluoresces at a different wavelength.

Some embodiments of the present invention utilize next generation or high-throughput sequencing. A variety of nucleic acid sequencing methods are contemplated for use in the methods of the present disclosure including, for example, chain terminator (Sanger) sequencing, dye terminator sequencing, and high-throughput sequencing methods. Many of these sequencing methods are well known in the art. See, e.g., Sanger et al., Proc. Natl. Acad. Sci. USA 74:5463-5467 (1997); Maxam et al., Proc. Natl. Acad. Sci. USA 74:560-564 (1977); Drmanac, et al., Nat. Biotechnol. 16:54-58 (1998); Kato, Int. J. Clin. Exp. Med. 2:193-202 (2009); Ronaghi et al., Anal. Biochem. 242:84-89 (1996); Margulies et al., Nature 437:376-380 (2005); Ruparel et al., Proc. Natl. Acad. Sci. USA 102:5932-5937 (2005), and Harris et al., Science 320:106-109 (2008); Levene et al., Science 299:682-686 (2003); Korlach et al., Proc. Natl. Acad. Sci. USA 105:1176-1181 (2008); Branton et al., Nat. Biotechnol. 26(10):1146-53 (2008); Eid et al., Science 323:133-138 (2009); each of which is herein incorporated by reference in its entirety.

In some embodiments, sequencing technology including, but not limited to, pyrosequencing, sequencing-by-ligation, single molecule sequencing, sequence-by-synthesis (SBS), massive parallel clonal, massive parallel single molecule SBS, massive parallel single molecule real-time, massive parallel single molecule real-time nanopore technology, etc. Morozova and Marra provide a review of some such technologies in Genomics, 92: 255 (2008), herein incorporated by reference in its entirety. Those of ordinary skill in the art will recognize that because RNA is less stable in the cell and more prone to nuclease attack experimentally RNA is usually reverse transcribed to DNA before sequencing.

A number of DNA sequencing techniques are known in the art, including fluorescence-based sequencing methodologies (see, e.g., Birren et al., Genome Analysis: Analyzing DNA, 1, Cold Spring Harbor, N.Y.; herein incorporated by reference in its entirety). In some embodiments, the technology finds use in automated sequencing techniques understood in that art. In some embodiments, the present technology finds use in

parallel sequencing of partitioned amplicons (PCT Publication No: WO2006084132 to Kevin McKernan et al., herein incorporated by reference in its entirety). In some embodiments, the technology finds use in DNA sequencing by parallel oligonucleotide extension (See, e.g., U.S. Pat. No. 5,750,341 to Macevicz et al., and U.S. Pat. No. 6,306,597 to Macevicz et al., both of which are herein incorporated by reference in their entireties). Additional examples of sequencing techniques in which the technology finds use include the Church polony technology (Mitra et al., 2003, Analytical Biochemistry 320, 55-65; Shendure et al., 2005 Science 309, 1728-1732; U.S. Pat. No. 6,432,360, U.S. Pat. No. 6,485,944, U.S. Pat. No. 6,511,803; herein incorporated by reference in their entireties), the 454 picotiter pyrosequencing technology (Margulies et al., 2005 Nature 437, 376-380; US 20050130173; herein incorporated by reference in their entireties), the Solexa single base addition technology (Bennett et al., 2005, Pharmacogenomics, 6, 373-382; U.S. Pat. No. 6,787,308; U.S. Pat. No. 6,833,246; herein incorporated by reference in their entireties), the Lynx massively parallel signature sequencing technology (Brenner et al. (2000). Nat. Biotechnol. 18:630-634; U.S. Pat. No. 5,695,934; U.S. Pat. No. 5,714,330; herein incorporated by reference in their entireties), and the Adessi PCR colony technology (Adessi et al. (2000). Nucleic Acid Res. 28, E87; WO 00018957; herein incorporated by reference in its entirety).

Next-generation sequencing (NGS) methods share the common feature of massively parallel, high-throughput strategies, with the goal of lower costs in comparison to older sequencing methods (see, e.g., Voelkerding et al., Clinical Chem., 55: 641-658, 2009; MacLean et al., Nature Rev. Microbiol., 7: 287-296; each herein incorporated by reference in their entirety). NGS methods can be broadly divided into those that typically use template amplification and those that do not. Amplification-requiring methods include pyrosequencing commercialized by Roche as the 454 technology platforms (e.g., GS 20 and GS FLX), the Solexa platform commercialized by Illumina, and the Supported Oligonucleotide Ligation and Detection (SOLiD) platform commercialized by Applied Biosystems. Non-amplification approaches, also known as single-molecule sequencing, are exemplified by the HeliScope platform commercialized by Helicos BioSciences, and emerging platforms commercialized by VisiGen, Oxford Nanopore Technologies Ltd., Life Technologies/Ion Torrent, and Pacific Biosciences, respectively.

In pyrosequencing (Voelkerding et al., Clinical Chem., 55: 641-658, 2009; MacLean et al., Nature Rev. Microbiol., 7: 287-296; U.S. Pat. No. 6,210,891; U.S. Pat. No. 6,258,568; each herein incorporated by reference in its entirety), template DNA is fragmented, end-repaired, ligated to adaptors, and clonally amplified in-situ by capturing single template

molecules with beads bearing oligonucleotides complementary to the adaptors. Each bead bearing a single template type is compartmentalized into a water-in-oil microvesicle, and the template is clonally amplified using a technique referred to as emulsion PCR. The emulsion is disrupted after amplification and beads are deposited into individual wells of a picotitre plate functioning as a flow cell during the sequencing reactions. Ordered, iterative introduction of each of the four dNTP reagents occurs in the flow cell in the presence of sequencing enzymes and luminescent reporter such as luciferase. In the event that an appropriate dNTP is added to the 3′ end of the sequencing primer, the resulting production of ATP causes a burst of luminescence within the well, which is recorded using a CCD camera. It is possible to achieve read lengths greater than or equal to 400 bases, and 10^6 sequence reads can be achieved, resulting in up to 500 million base pairs (Mb) of sequence.

In the Solexa/Illumina platform (Voelkerding et al., Clinical Chem., 55: 641-658, 2009; MacLean et al., Nature Rev. Microbiol., 7: 287-296; U.S. Pat. No. 6,833,246; U.S. Pat. No. 7,115,400; U.S. Pat. No. 6,969,488; each herein incorporated by reference in its entirety), sequencing data are produced in the form of shorter-length reads. In this method, single-stranded fragmented DNA is end-repaired to generate 5′-phosphorylated blunt ends, followed by Klenow-mediated addition of a single A base to the 3′ end of the fragments. A-addition facilitates addition of T-overhang adaptor oligonucleotides, which are subsequently used to capture the template-adaptor molecules on the surface of a flow cell that is studded with oligonucleotide anchors. The anchor is used as a PCR primer, but because of the length of the template and its proximity to other nearby anchor oligonucleotides, extension by PCR results in the "arching over" of the molecule to hybridize with an adjacent anchor oligonucleotide to form a bridge structure on the surface of the flow cell. These loops of DNA are denatured and cleaved. Forward strands are then sequenced with reversible dye terminators. The sequence of incorporated nucleotides is determined by detection of post-incorporation fluorescence, with each fluor and block removed prior to the next cycle of dNTP addition. Sequence read length ranges from 36 nucleotides to over 50 nucleotides, with overall output exceeding 1 billion nucleotide pairs per analytical run.

Sequencing nucleic acid molecules using SOLiD technology (Voelkerding et al., Clinical Chem., 55: 641-658, 2009; MacLean et al., Nature Rev. Microbiol., 7: 287-296; U.S. Pat. No. 5,912,148; U.S. Pat. No. 6,130,073; each herein incorporated by reference in their entirety) also involves fragmentation of the template, ligation to oligonucleotide adaptors, attachment to beads, and clonal amplification by emulsion PCR. Following this, beads bearing template are immobilized on a derivatized surface of a glass flow-cell, and a primer

complementary to the adaptor oligonucleotide is annealed. However, rather than utilizing this primer for 3′ extension, it is instead used to provide a 5′ phosphate group for ligation to interrogation probes containing two probe-specific bases followed by 6 degenerate bases and one of four fluorescent labels. In the SOLiD system, interrogation probes have 16 possible combinations of the two bases at the 3′ end of each probe, and one of four fluors at the 5′ end. Fluor color, and thus identity of each probe, corresponds to specified color-space coding schemes. Multiple rounds (usually 7) of probe annealing, ligation, and fluor detection are followed by denaturation, and then a second round of sequencing using a primer that is offset by one base relative to the initial primer. In this manner, the template sequence can be computationally re-constructed, and template bases are interrogated twice, resulting in increased accuracy. Sequence read length averages 35 nucleotides, and overall output exceeds 4 billion bases per sequencing run.

In certain embodiments, the technology finds use in nanopore sequencing (see, e.g., Astier et al., J. Am. Chem. Soc. 2006 Feb 8; 128(5):1705–10, herein incorporated by reference). The theory behind nanopore sequencing has to do with what occurs when a nanopore is immersed in a conducting fluid and a potential (voltage) is applied across it. Under these conditions a slight electric current due to conduction of ions through the nanopore can be observed, and the amount of current is exceedingly sensitive to the size of the nanopore. As each base of a nucleic acid passes through the nanopore, this causes a change in the magnitude of the current through the nanopore that is distinct for each of the four bases, thereby allowing the sequence of the DNA molecule to be determined.

In certain embodiments, the technology finds use in HeliScope by Helicos BioSciences (Voelkerding et al., Clinical Chem., 55: 641-658, 2009; MacLean et al., Nature Rev. Microbiol., 7: 287-296; U.S. Pat. No. 7,169,560; U.S. Pat. No. 7,282,337; U.S. Pat. No. 7,482,120; U.S. Pat. No. 7,501,245; U.S. Pat. No. 6,818,395; U.S. Pat. No. 6,911,345; U.S. Pat. No. 7,501,245; each herein incorporated by reference in their entirety). Template DNA is fragmented and polyadenylated at the 3′ end, with the final adenosine bearing a fluorescent label. Denatured polyadenylated template fragments are ligated to poly(dT) oligonucleotides on the surface of a flow cell. Initial physical locations of captured template molecules are recorded by a CCD camera, and then label is cleaved and washed away. Sequencing is achieved by addition of polymerase and serial addition of fluorescently-labeled dNTP reagents. Incorporation events result in fluor signal corresponding to the dNTP, and signal is captured by a CCD camera before each round of dNTP addition. Sequence read length ranges

from 25–50 nucleotides, with overall output exceeding 1 billion nucleotide pairs per analytical run.

The Ion Torrent technology is a method of DNA sequencing based on the detection of hydrogen ions that are released during the polymerization of DNA (see, e.g., Science 327(5970): 1190 (2010); U.S. Pat. Appl. Pub. Nos. 20090026082, 20090127589, 20100301398, 20100197507, 20100188073, and 20100137143, incorporated by reference in their entireties for all purposes). A microwell contains a template DNA strand to be sequenced. Beneath the layer of microwells is a hypersensitive ISFET ion sensor. All layers are contained within a CMOS semiconductor chip, similar to that used in the electronics industry. When a dNTP is incorporated into the growing complementary strand a hydrogen ion is released, which triggers a hypersensitive ion sensor. If homopolymer repeats are present in the template sequence, multiple dNTP molecules will be incorporated in a single cycle. This leads to a corresponding number of released hydrogens and a proportionally higher electronic signal. This technology differs from other sequencing technologies in that no modified nucleotides or optics are used. The per-base accuracy of the Ion Torrent sequencer is ~99.6% for 50 base reads, with ~100 Mb generated per run. The read-length is 100 base pairs. The accuracy for homopolymer repeats of 5 repeats in length is ~98%. The benefits of ion semiconductor sequencing are rapid sequencing speed and low upfront and operating costs.

The technology finds use in another nucleic acid sequencing approach developed by Stratos Genomics, Inc. and involves the use of Xpandomers. This sequencing process typically includes providing a daughter strand produced by a template-directed synthesis. The daughter strand generally includes a plurality of subunits coupled in a sequence corresponding to a contiguous nucleotide sequence of all or a portion of a target nucleic acid in which the individual subunits comprise a tether, at least one probe or nucleobase residue, and at least one selectively cleavable bond. The selectively cleavable bond(s) is/are cleaved to yield an Xpandomer of a length longer than the plurality of the subunits of the daughter strand. The Xpandomer typically includes the tethers and reporter elements for parsing genetic information in a sequence corresponding to the contiguous nucleotide sequence of all or a portion of the target nucleic acid. Reporter elements of the Xpandomer are then detected. Additional details relating to Xpandomer-based approaches are described in, for example, U.S. Pat. Pub No. 20090035777, entitled "High Throughput Nucleic Acid Sequencing by Expansion," filed June 19, 2008, which is incorporated herein in its entirety.

Other emerging single molecule sequencing methods include real-time sequencing by synthesis using a VisiGen platform (Voelkerding et al., Clinical Chem., 55: 641–58, 2009; U.S. Pat. No. 7,329,492; U.S. Pat. App. Ser. No. 11/671956; U.S. Pat. App. Ser. No. 11/781166; each herein incorporated by reference in their entirety) in which immobilized, primed DNA template is subjected to strand extension using a fluorescently-modified polymerase and florescent acceptor molecules, resulting in detectible fluorescence resonance energy transfer (FRET) upon nucleotide addition.

In some embodiments, capillary electrophoresis (CE) is utilized to analyze amplification fragments. During capillary electrophoresis, nucleic acids (e.g., the products of a PCR reaction) are injected electrokinetically into capillaries filled with polymer. High voltage is applied so that the fluorescent DNA fragments are separated by size and are detected by a laser/camera system. In some embodiments, CE systems from Life Technogies (Grand Island, NY) are utilized for fragment sizing (see e.g., US 6706162, US8043493, each of which is herein incorporated by reference in its entirety).

2. Hybridization

Illustrative non-limiting examples of nucleic acid hybridization techniques include, but are not limited to, in situ hybridization (ISH), microarray, and Southern or Northern blot. In situ hybridization (ISH) is a type of hybridization that uses a labeled complementary DNA or RNA strand as a probe to localize a specific DNA or RNA sequence in a portion or section of tissue (in situ), or, if the tissue is small enough, the entire tissue (whole mount ISH). DNA ISH can be used to determine the structure of chromosomes. RNA ISH is used to measure and localize mRNAs and other transcripts within tissue sections or whole mounts. Sample cells and tissues are usually treated to fix the target transcripts in place and to increase access of the probe. The probe hybridizes to the target sequence at elevated temperature, and then the excess probe is washed away. The probe that was labeled with either radio-, fluorescent- or antigen-labeled bases is localized and quantitated in the tissue using either autoradiography, fluorescence microscopy or immunohistochemistry, respectively. ISH can also use two or more probes, labeled with radioactivity or the other non-radioactive labels, to simultaneously detect two or more transcripts.

In some embodiments, the present invention provides nucleic acid probes specific for a particular JAK / STAT pathway variant. For example, in some embodiments, separate nucleic acid probes are provided that are only specific for one JAK / STAT pathway variant as described herein (e.g., the nucleic acid encoding any JAK / STAT pathway variant

including, but not limited to, JAK1 p.F636L, JAK1 p.G646C, JAK1 p. Y654F, JAK1 p.V658F, JAK1 p.S703I, JAK1 p.T901R, JAK3 p.G662W, JAK3 p.P664T, JAK3 p.ΔKNC563, JAK3 p.M511I, JAK3 p. A573V, JAK3 p.R657. JAK3 p.Y980, JAK3 p. Y981, and JAK3 p.S989I, STAT5B p.T628S, STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.N642H, STAT5B p.Y699, and STAT5B p.Y665H, IL2RG p.Y325, IL2RG p.ΔGSM268, and IL2RG p. K315E). In some embodiments, such separate nucleic acid probes are specific for the entire JAK / STAT pathway variant. In some embodiments, such separate nucleic acid probes are specific for a nucleic acid fragment of such a JAK / STAT pathway variant. In some embodiments, such separate nucleic acid probes specific for a JAK / STAT pathway variant will not bind the respective wild type equivalent JAK / STAT pathway variant. In some embodiments, such separate nucleic acid probes specific for a JAK / STAT pathway variant will not bind different JAK / STAT pathway variants.

3. Microarrays

In some embodiments, microarrays are utilized for detection of JAK/STAT pathway nucleic acid (e.g., JAK1, JAK3, STAT5B, IL2RG) sequences. Examples of microarrays include, but not limited to: DNA microarrays (e.g., cDNA microarrays and oligonucleotide microarrays); protein microarrays; tissue microarrays; transfection or cell microarrays; chemical compound microarrays; and, antibody microarrays. A DNA microarray, commonly known as gene chip, DNA chip, or biochip, is a collection of microscopic DNA spots attached to a solid surface (e.g., glass, plastic or silicon chip) forming an array for the purpose of expression profiling or monitoring expression levels for thousands of genes simultaneously. The affixed DNA segments are known as probes, thousands of which can be used in a single DNA microarray. Microarrays can be used to identify disease genes by comparing gene expression in disease and normal cells. Microarrays can be fabricated using a variety of technologies, including but not limiting: printing with fine-pointed pins onto glass slides; photolithography using pre-made masks; photolithography using dynamic micromirror devices; ink-jet printing; or, electrochemistry on microelectrode arrays.

Arrays can also be used to detect copy number variations at a specific locus. These genomic micorarrys detect microscopic deletions or other variants that lead to disease causing alleles.

Southern and Northern blotting is used to detect specific DNA or RNA sequences, respectively. DNA or RNA extracted from a sample is fragmented, electrophoretically

separated on a matrix gel, and transferred to a membrane filter. The filter bound DNA or RNA is subject to hybridization with a labeled probe complementary to the sequence of interest. Hybridized probe bound to the filter is detected. A variant of the procedure is the reverse Northern blot, in which the substrate nucleic acid that is affixed to the membrane is a collection of isolated DNA fragments and the probe is RNA extracted from a tissue and labeled.

4. Amplification

JAK/STAT pathway (e.g., JAK1, JAK3, STAT5B, IL2RG) nucleic acid may be amplified prior to or simultaneous with detection. Illustrative non-limiting examples of nucleic acid amplification techniques include, but are not limited to, polymerase chain reaction (PCR), reverse transcription polymerase chain reaction (RT-PCR), transcription-mediated amplification (TMA), ligase chain reaction (LCR), strand displacement amplification (SDA), and nucleic acid sequence based amplification (NASBA). Those of ordinary skill in the art will recognize that certain amplification techniques (e.g., PCR) require that RNA be reversed transcribed to DNA prior to amplification (e.g., RT-PCR), whereas other amplification techniques directly amplify RNA (e.g., TMA and NASBA).

The polymerase chain reaction (U.S. Pat. Nos. 4,683,195, 4,683,202, 4,800,159 and 4,965,188, each of which is herein incorporated by reference in its entirety), commonly referred to as PCR, uses multiple cycles of denaturation, annealing of primer pairs to opposite strands, and primer extension to exponentially increase copy numbers of a target nucleic acid sequence. In a variation called RT-PCR, reverse transcriptase (RT) is used to make a complementary DNA (cDNA) from mRNA, and the cDNA is then amplified by PCR to produce multiple copies of DNA. For other various permutations of PCR see, e.g., U.S. Pat. Nos. 4,683,195, 4,683,202 and 4,800,159; Mullis et al., Meth. Enzymol. 155: 335 (1987); and, Murakawa et al., DNA 7: 287 (1988), each of which is herein incorporated by reference in its entirety.

Transcription mediated amplification (U.S. Pat. Nos. 5,480,784 and 5,399,491, each of which is herein incorporated by reference in its entirety), commonly referred to as TMA, synthesizes multiple copies of a target nucleic acid sequence autocatalytically under conditions of substantially constant temperature, ionic strength, and pH in which multiple RNA copies of the target sequence autocatalytically generate additional copies. See, e.g., U.S. Pat. Nos. 5,399,491 and 5,824,518, each of which is herein incorporated by reference in its entirety. In a variation described in U.S. Publ. No. 20060046265 (herein incorporated by

reference in its entirety), TMA optionally incorporates the use of blocking moieties, terminating moieties, and other modifying moieties to improve TMA process sensitivity and accuracy.

The ligase chain reaction (Weiss, R., Science 254: 1292 (1991), herein incorporated by reference in its entirety), commonly referred to as LCR, uses two sets of complementary DNA oligonucleotides that hybridize to adjacent regions of the target nucleic acid. The DNA oligonucleotides are covalently linked by a DNA ligase in repeated cycles of thermal denaturation, hybridization and ligation to produce a detectable double-stranded ligated oligonucleotide product.

Strand displacement amplification (Walker, G. et al., Proc. Natl. Acad. Sci. USA 89: 392-396 (1992); U.S. Pat. Nos. 5,270,184 and 5,455,166, each of which is herein incorporated by reference in its entirety), commonly referred to as SDA, uses cycles of annealing pairs of primer sequences to opposite strands of a target sequence, primer extension in the presence of a dNTPαS to produce a duplex hemiphosphorothioated primer extension product, endonuclease-mediated nicking of a hemimodified restriction endonuclease recognition site, and polymerase-mediated primer extension from the 3' end of the nick to displace an existing strand and produce a strand for the next round of primer annealing, nicking and strand displacement, resulting in geometric amplification of product. Thermophilic SDA (tSDA) uses thermophilic endonucleases and polymerases at higher temperatures in essentially the same method (EP Pat. No. 0 684 315).

Other amplification methods include, for example: nucleic acid sequence based amplification (U.S. Pat. No. 5,130,238, herein incorporated by reference in its entirety), commonly referred to as NASBA; one that uses an RNA replicase to amplify the probe molecule itself (Lizardi et al., BioTechnol. 6: 1197 (1988), herein incorporated by reference in its entirety), commonly referred to as Qβ replicase; a transcription based amplification method (Kwoh et al., Proc. Natl. Acad. Sci. USA 86:1173 (1989)); and, self-sustained sequence replication (Guatelli et al., Proc. Natl. Acad. Sci. USA 87: 1874 (1990), each of which is herein incorporated by reference in its entirety). For further discussion of known amplification methods see Persing, David H., "In Vitro Nucleic Acid Amplification Techniques" in Diagnostic Medical Microbiology: Principles and Applications (Persing et al., Eds.), pp. 51-87 (American Society for Microbiology, Washington, DC (1993)).

5. Detection Methods

Non-amplified or amplified JAK/STAT pathway (e.g., JAK1, JAK3, STAT5B, IL2RG) nucleic acids can be detected by any conventional means. For example, nucleic acid can be detected by hybridization with a detectably labeled probe and measurement of the resulting hybrids. Illustrative non-limiting examples of detection methods are described below.

One illustrative detection method, the Hybridization Protection Assay (HPA) involves hybridizing a chemiluminescent oligonucleotide probe (e.g., an acridinium ester-labeled (AE) probe) to the target sequence, selectively hydrolyzing the chemiluminescent label present on unhybridized probe, and measuring the chemiluminescence produced from the remaining probe in a luminometer. See, e.g., U.S. Pat. No. 5,283,174 and Norman C. Nelson et al., Nonisotopic Probing, Blotting, and Sequencing, ch. 17 (Larry J. Kricka ed., 2d ed. 1995, each of which is herein incorporated by reference in its entirety).

Another illustrative detection method provides for quantitative evaluation of the amplification process in real-time. Evaluation of an amplification process in "real-time" involves determining the amount of amplicon in the reaction mixture either continuously or periodically during the amplification reaction, and using the determined values to calculate the amount of target sequence initially present in the sample. A variety of methods for determining the amount of initial target sequence present in a sample based on real-time amplification are well known in the art. These include methods disclosed in U.S. Pat. Nos. 6,303,305 and 6,541,205, each of which is herein incorporated by reference in its entirety. Another method for determining the quantity of target sequence initially present in a sample, but which is not based on a real-time amplification, is disclosed in U.S. Pat. No. 5,710,029, herein incorporated by reference in its entirety.

Amplification products may be detected in real-time through the use of various self-hybridizing probes, most of which have a stem-loop structure. Such self-hybridizing probes are labeled so that they emit differently detectable signals, depending on whether the probes are in a self-hybridized state or an altered state through hybridization to a target sequence. By way of non-limiting example, "molecular torches" are a type of self-hybridizing probe that includes distinct regions of self-complementarity (referred to as "the target binding domain" and "the target closing domain") which are connected by a joining region (e.g., non-nucleotide linker) and which hybridize to each other under predetermined hybridization assay conditions. In a preferred embodiment, molecular torches contain single-stranded base regions in the target binding domain that are from 1 to about 20 bases in length and are

accessible for hybridization to a target sequence present in an amplification reaction under strand displacement conditions. Under strand displacement conditions, hybridization of the two complementary regions, which may be fully or partially complementary, of the molecular torch is favored, except in the presence of the target sequence, which will bind to the single-stranded region present in the target binding domain and displace all or a portion of the target closing domain. The target binding domain and the target closing domain of a molecular torch include a detectable label or a pair of interacting labels (e.g., luminescent/quencher) positioned so that a different signal is produced when the molecular torch is self-hybridized than when the molecular torch is hybridized to the target sequence, thereby permitting detection of probe:target duplexes in a test sample in the presence of unhybridized molecular torches. Molecular torches and a variety of types of interacting label pairs are disclosed in U.S. Pat. No. 6,534,274, herein incorporated by reference in its entirety.

Another example of a detection probe having self-complementarity is a "molecular beacon." Molecular beacons include nucleic acid molecules having a target complementary sequence, an affinity pair (or nucleic acid arms) holding the probe in a closed conformation in the absence of a target sequence present in an amplification reaction, and a label pair that interacts when the probe is in a closed conformation. Hybridization of the target sequence and the target complementary sequence separates the members of the affinity pair, thereby shifting the probe to an open conformation. The shift to the open conformation is detectable due to reduced interaction of the label pair, which may be, for example, a fluorophore and a quencher (e.g., DABCYL and EDANS). Molecular beacons are disclosed in U.S. Pat. Nos. 5,925,517 and 6,150,097, herein incorporated by reference in its entirety.

Other self-hybridizing probes are well known to those of ordinary skill in the art. By way of non-limiting example, probe binding pairs having interacting labels, such as those disclosed in U.S. Pat. No. 5,928,862 (herein incorporated by reference in its entirety) might be adapted for use in the present invention. Probe systems used to detect single nucleotide polymorphisms (SNPs) might also be utilized in the present invention. Additional detection systems include "molecular switches," as disclosed in U.S. Publ. No. 20050042638, herein incorporated by reference in its entirety. Other probes, such as those comprising intercalating dyes and/or fluorochromes, are also useful for detection of amplification products in the present invention. See, e.g., U.S. Pat. No. 5,814,447 (herein incorporated by reference in its entirety).

ii. Detection of Variant JAK/STAT Pathway Proteins

In other embodiments, variant JAK/STAT pathway (e.g., JAK1, JAK3, STAT5B, IL2RG) polypeptides are detected. Any suitable method may be used to detect truncated or mutant JAK/STAT pathway (e.g., JAK1, JAK3, STAT5B, IL2RG) polypeptides. For example, detection paradigms that can be employed to this end include optical methods, electrochemical methods (voltametry and amperometry techniques), atomic force microscopy, and radio frequency methods, e.g., multipolar resonance spectroscopy. Illustrative of optical methods, in addition to microscopy, both confocal and non-confocal, are detection of fluorescence, luminescence, chemiluminescence, absorbance, reflectance, transmittance, and birefringence or refractive index (e.g., surface plasmon resonance, ellipsometry, a resonant mirror method, a grating coupler waveguide method or interferometry).

1. Antibody Binding

In some embodiments, antibodies (see below for antibody production) are used to determine if an individual contains an allele encoding a variant JAK/STAT pathway (e.g., JAK1, JAK3, STAT5B, IL2RG) polypeptide. In preferred embodiments, antibodies are utilized that discriminate between variant (*i.e.*, truncated proteins); and wild-type proteins. In some embodiments, the antibodies are directed to the C-terminus of JAK/STAT pathway (e.g., JAK1, JAK3, STAT5B, IL2RG) proteins. Proteins that are recognized by the N-terminal, but not the C-terminal antibody are truncated. In some embodiments, quantitative immunoassays are used to determine the ratios of C-terminal to N-terminal antibody binding. In other embodiments, identification of variants of JAK/STAT pathway (e.g., JAK1, JAK3, STAT5B, IL2RG) polypeptides is accomplished through the use of antibodies that differentially bind to wild type or variant forms of JAK/STAT pathway (e.g., JAK1, JAK3, STAT5B, IL2RG) proteins. In some embodiments, the present invention provides antibodies specific for a particular JAK / STAT pathway variant. For example, in some embodiments, separate antibodies are provided that are only specific for one JAK / STAT pathway variant as described herein (e.g., JAK1 p.F636L, JAK1 p.G646C, JAK1 p. Y654F, JAK1 p.V658F, JAK1 p.S703I, JAK1 p.T901R, JAK3 p.ΔKNC563, JAK3 p.G662W, JAK3 p.P664T, JAK3 p.M511I, JAK3 p. A573V, JAK3 p.R657. JAK3 p.Y980, JAK3 p. Y981, and JAK3 p.S989I, STAT5B p.T628S, STAT5B p.N642H, STAT5B p.Y699, and STAT5B p.Y665H, IL2RG p.ΔGSM268, IL2RG p.Y325, and IL2RG p. K315E). In some embodiments, such separate

antibodies are specific for the entire JAK / STAT pathway variant. In some embodiments, such separate antibodies are specific for a fragment of such a JAK / STAT pathway variant. In some embodiments, such separate antibodies specific for a JAK / STAT pathway variant will not bind the respective wild type equivalent JAK / STAT pathway variant. In some embodiments, such separate antibodies specific for a JAK / STAT pathway variant will not bind different JAK / STAT pathway variants.

Antibody binding is detected by techniques known in the art (*e.g.*, radioimmunoassay, ELISA (enzyme-linked immunosorbant assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, *in situ* immunoassays (*e.g.*, using colloidal gold, enzyme or radioisotope labels, for example), Western blots, precipitation reactions, agglutination assays (*e.g.,* gel agglutination assays, hemagglutination assays, etc.), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc.

In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many methods are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In some embodiments, an automated detection assay is utilized. Methods for the automation of immunoassays include those described in U.S. Patents 5,885,530, 4,981,785, 6,159,750, and 5,358,691, each of which is herein incorporated by reference. In some embodiments, the analysis and presentation of results is also automated. For example, in some embodiments, software that generates a prognosis based on the result of the immunoassay is utilized. In other embodiments, the immunoassay described in U.S. Patents 5,599,677 and 5,672,480; each of which is herein incorporated by reference.

C. Kits for Detecting JAK/STAT Pathway Mutant or Variant Alleles

The present invention also provides kits for determining whether an individual contains a JAK/STAT pathway (e.g., JAK1, JAK3, STAT5B, IL2RG) wild-type or variant (*e.g.*, mutant or polymorphic) allele. In some embodiments, the kits are useful for determining whether the subject has a mature T-cell leukemia (e.g., T-PLL, SS, MF) or to provide a prognosis to an individual diagnosed with a mature T-cell leukemia (e.g., T-PLL, SS, MF). The diagnostic kits are produced in a variety of ways. In some embodiments, the kits contain at least one reagent useful, necessary, or sufficient for specifically detecting a

mutant or variant JAK/STAT pathway (e.g., JAK1, JAK3, STAT5B, IL2RG) allele or protein. In some embodiments, the kits contain reagents for detecting a truncation in a JAK/STAT pathway (e.g., JAK1, JAK3, STAT5B, IL2RG) polypeptide. In preferred embodiments, the reagent is a nucleic acid that hybridizes to nucleic acids containing the mutation and that does not bind to nucleic acids that do not contain the mutation. In other embodiments, the reagents are primers for amplifying the region of DNA containing the mutation. In still other embodiments, the reagents are antibodies that preferentially bind either the wild-type or truncated or variant JAK/STAT pathway (e.g., JAK1, JAK3, STAT5B, IL2RG) proteins.

In some embodiments, the kits include ancillary reagents such as buffering agents, nucleic acid stabilizing reagents, protein stabilizing reagents, and signal producing systems (*e.g.*, florescence generating systems as Fret systems), and software (e.g., data analysis software). The test kit may be packages in any suitable manner, typically with the elements in a single container or various containers as necessary along with a sheet of instructions for carrying out the test. In some embodiments, the kits also preferably include a positive control sample.

In some embodiments, markers (e.g., those described herein) are detected alone or in combination with other markers in a panel or multiplex format. For example, in some embodiments, a plurality of markers are simultaneously detected in an array or multiplex format (e.g., using the detection methods described herein).

D. Bioinformatics

In some embodiments, a computer-based analysis program is used to translate raw data generated by detection assay (*e.g.*, the presence, absence, or amount of a given JAK/STAT pathway (e.g., JAK1, JAK3, STAT5B, IL2RG) related allele or polypeptide) of the present invention into data of predictive value for a clinician. The clinician can access the predictive data using any suitable means. Thus, in some preferred embodiments, the present invention provides the further benefit that the clinician, who may not be trained in genetics or molecular biology, need not understand the raw data. The data is presented directly to the clinician in its most useful form. The clinician is then able to immediately utilize the information in order to optimize the care of the subject.

The present invention contemplates any method capable of receiving, processing, and transmitting the information to and from laboratories conducting the assays, information providers, medical personal, and subjects. For example, in some embodiments of the present

invention, a sample (*e.g.*, a biopsy or a blood or serum sample) is obtained from a subject and submitted to a profiling service (*e.g.*, clinical lab at a medical facility, genomic profiling business, etc.), located in any part of the world (*e.g.*, in a country different than the country where the subject resides or where the information is ultimately used) to generate raw data. Where the sample comprises a tissue or other biological sample, the subject may visit a medical center to have the sample obtained and sent to the profiling center, or subjects may collect the sample themselves (*e.g.*, a urine sample) and directly send it to a profiling center. Where the sample comprises previously determined biological information, the information may be directly sent to the profiling service by the subject (*e.g.*, an information card containing the information may be scanned by a computer and the data transmitted to a computer of the profiling center using an electronic communication systems). Once received by the profiling service, the sample is processed and a profile is produced (*i.e.*, presence of wild type or mutant JAK/STAT pathway (e.g., JAK1, JAK3, STAT5B, IL2RG) related allele or protein), specific for the screening, diagnostic or prognostic information desired for the subject.

The profile data is then prepared in a format suitable for interpretation by a treating clinician. For example, rather than providing raw data, the prepared format may represent a diagnosis or risk assessment (*e.g.*, diagnosis or prognosis of a mature T-cell leukemia) for the subject, along with recommendations for particular treatment options. The data may be displayed to the clinician by any suitable method. For example, in some embodiments, the profiling service generates a report that can be printed for the clinician (*e.g.*, at the point of care) or displayed to the clinician on a computer monitor.

In some embodiments, the information is first analyzed at the point of care or at a regional facility. The raw data is then sent to a central processing facility for further analysis and/or to convert the raw data to information useful for a clinician or patient. The central processing facility provides the advantage of privacy (all data is stored in a central facility with uniform security protocols), speed, and uniformity of data analysis. The central processing facility can then control the fate of the data following treatment of the subject. For example, using an electronic communication system, the central facility can provide data to the clinician, the subject, or researchers.

In some embodiments, the subject is able to directly access the data using the electronic communication system. The subject may chose further intervention or counseling based on the results. In some embodiments, the data is used for research use. For example,

the data may be used to further optimize the inclusion or elimination of markers as useful indicators of a particular condition or stage of disease.

In some embodiments, the methods disclosed herein are useful in monitoring the treatment of a mature T-cell leukemia (e.g., T-PLL, SS, MF). For example, in some embodiments, the methods may be performed immediately before, during and/or after a treatment to monitor treatment success. In some embodiments, the methods are performed at intervals on disease free patients to ensure treatment success.

The present invention also provides a variety of computer-related embodiments. Specifically, in some embodiments the invention provides computer programming for analyzing and comparing a pattern of a mature T-cell leukemia-specific marker detection results in a sample obtained from a subject to, for example, a library of such marker patterns known to be indicative of the presence or absence of a mature T-cell leukemia, or a particular stage or prognosis of a mature T-cell leukemia.

In some embodiments, the present invention provides computer programming for analyzing and comparing a first and a second pattern of a mature T-cell leukemia-specific marker detection results from a sample taken at least two different time points. In some embodiments, the first pattern may be indicative of a pre-cancerous condition and/or low risk condition for a mature T-cell leukemia and/or progression from a pre-cancerous condition to a cancerous condition. In such embodiments, the comparing provides for monitoring of the progression of the condition from the first time point to the second time point.

In yet another embodiment, the invention provides computer programming for analyzing and comparing a pattern of mature T-cell leukemia-specific marker detection results from a sample to a library of mature T-cell leukemia-specific marker patterns known to be indicative of the presence or absence of a mature T-cell leukemia (e.g., T-PLL, SS, MF), wherein the comparing provides, for example, a differential diagnosis between an aggressively malignant mature T-cell leukemia and a less aggressive mature T-cell leukemia (e.g., the marker pattern provides for staging and/or grading of the cancerous condition).

The methods and systems described herein can be implemented in numerous ways. In one embodiment, the methods involve use of a communications infrastructure, for example the internet. Several embodiments of the invention are discussed below. It is also to be understood that the present invention may be implemented in various forms of hardware, software, firmware, processors, distributed servers (e.g., as used in cloud computing) or a combination thereof. The methods and systems described herein can be implemented as a combination of hardware and software. The software can be implemented as an application

program tangibly embodied on a program storage device, or different portions of the software implemented in the user's computing environment (e.g., as an applet) and on the reviewer's computing environment, where the reviewer may be located at a remote site (e.g., at a service provider's facility).

For example, during or after data input by the user, portions of the data processing can be performed in the user-side computing environment. For example, the user-side computing environment can be programmed to provide for defined test codes to denote platform, carrier/diagnostic test, or both; processing of data using defined flags, and/or generation of flag configurations, where the responses are transmitted as processed or partially processed responses to the reviewer's computing environment in the form of test code and flag configurations for subsequent execution of one or more algorithms to provide a results and/or generate a report in the reviewer's computing environment.

The application program for executing the algorithms described herein may be uploaded to, and executed by, a machine comprising any suitable architecture. In general, the machine involves a computer platform having hardware such as one or more central processing units (CPU), a random access memory (RAM), and input/output (I/O) interface(s). The computer platform also includes an operating system and microinstruction code. The various processes and functions described herein may either be part of the microinstruction code or part of the application program (or a combination thereof) which is executed via the operating system. In addition, various other peripheral devices may be connected to the computer platform such as an additional data storage device and a printing device.

As a computer system, the system generally includes a processor unit. The processor unit operates to receive information, which generally includes test data (e.g., specific gene products assayed), and test result data (e.g., the pattern of gastrointestinal neoplasm-specific marker detection results from a sample). This information received can be stored at least temporarily in a database, and data analyzed in comparison to a library of marker patterns known to be indicative of the presence or absence of a pre-cancerous condition, or known to be indicative of a stage and/or grade of gastrointestinal cancer.

Part or all of the input and output data can also be sent electronically; certain output data (e.g., reports) can be sent electronically or telephonically (e.g., by facsimile, e.g., using devices such as fax back). Exemplary output receiving devices can include a display element, a printer, a facsimile device and the like. Electronic forms of transmission and/or display can include email, interactive television, and the like. In some embodiments, all or a portion of the input data and/or all or a portion of the output data (e.g., usually at least the library of the

pattern of gastrointestinal neoplasm-specific marker detection results known to be indicative of the presence or absence of a pre-cancerous condition) are maintained on a server for access, e.g., confidential access. The results may be accessed or sent to professionals as desired.

A system for use in the methods described herein generally includes at least one computer processor (e.g., where the method is carried out in its entirety at a single site) or at least two networked computer processors (e.g., where detected marker data for a sample obtained from a subject is to be input by a user (e.g., a technician or someone performing the assays)) and transmitted to a remote site to a second computer processor for analysis (e.g., where the pattern of mature T-cell leukemia-specific marker) detection results is compared to a library of patterns known to be indicative of the presence or absence of a pre-cancerous condition), where the first and second computer processors are connected by a network, e.g., via an intranet or internet). The system can also include a user component(s) for input; and a reviewer component(s) for review of data, and generation of reports, including detection of a pre-cancerous condition, staging and/or grading of a mature T-cell leukemia, or monitoring the progression of a pre-cancerous condition or mature T-cell leukemia. Additional components of the system can include a server component(s); and a database(s) for storing data (e.g., as in a database of report elements, e.g., a library of marker patterns known to be indicative of the presence or absence of a pre-cancerous condition and/or known to be indicative of a grade and/or a stage of a mature T-cell leukemia, or a relational database (RDB) which can include data input by the user and data output. The computer processors can be processors that are typically found in personal desktop computers (e.g., IBM, Dell, Macintosh), portable computers, mainframes, minicomputers, tablet computer, smart phone, or other computing devices.

The input components can be complete, stand-alone personal computers offering a full range of power and features to run applications. The user component usually operates under any desired operating system and includes a communication element (e.g., a modem or other hardware for connecting to a network using a cellular phone network, Wi-Fi, Bluetooth, Ethernet, etc.), one or more input devices (e.g., a keyboard, mouse, keypad, or other device used to transfer information or commands), a storage element (e.g., a hard drive or other computer-readable, computer-writable storage medium), and a display element (e.g., a monitor, television, LCD, LED, or other display device that conveys information to the user). The user enters input commands into the computer processor through an input device.

Generally, the user interface is a graphical user interface (GUI) written for web browser applications.

The server component(s) can be a personal computer, a minicomputer, or a mainframe, or distributed across multiple servers (e.g., as in cloud computing applications) and offers data management, information sharing between clients, network administration and security. The application and any databases used can be on the same or different servers. Other computing arrangements for the user and server(s), including processing on a single machine such as a mainframe, a collection of machines, or other suitable configuration are contemplated. In general, the user and server machines work together to accomplish the processing of the present invention.

Where used, the database(s) is usually connected to the database server component and can be any device which will hold data. For example, the database can be any magnetic or optical storing device for a computer (e.g., CDROM, internal hard drive, tape drive). The database can be located remote to the server component (with access via a network, modem, etc.) or locally to the server component.

Where used in the system and methods, the database can be a relational database that is organized and accessed according to relationships between data items. The relational database is generally composed of a plurality of tables (entities). The rows of a table represent records (collections of information about separate items) and the columns represent fields (particular attributes of a record). In its simplest conception, the relational database is a collection of data entries that "relate" to each other through at least one common field.

Additional workstations equipped with computers and printers may be used at point of service to enter data and, in some embodiments, generate appropriate reports, if desired. The computer(s) can have a shortcut (e.g., on the desktop) to launch the application to facilitate initiation of data entry, transmission, analysis, report receipt, etc. as desired.

In certain embodiments, the present invention provides methods for obtaining a subject's risk profile for developing a mature T-cell leukemia or having an aggressive form of a mature T-cell leukemia. In some embodiments, such methods involve obtaining a blood or blood product sample from a subject (e.g., a human at risk for developing a mature T-cell leukemia; a human undergoing a routine physical examination, or a human diagnosed with a mature T-cell leukemia), detecting the presence or absence of JAK/STAT pathway variants described herein (e.g., JAK1, JAK3, STAT5B, IL2RG) in the sample, and generating a risk profile for developing a mature T-cell leukemia (e.g., T-PLL, SS, MF) or progressing to a metastatic or aggressive form of such mature T-cell leukemia. For example, in some

embodiments, a generated profile will change depending upon specific markers and detected as present or absent or at defined threshold levels. The present invention is not limited to a particular manner of generating the risk profile. In some embodiments, a processor (e.g., computer) is used to generate such a risk profile. In some embodiments, the processor uses an algorithm (e.g., software) specific for interpreting the presence and absence of specific exfoliated epithelial markers as determined with the methods of the present invention. In some embodiments, the presence and absence of specific JAK/STAT pathway variants described herein (e.g., JAK1, JAK3, STAT5B, IL2RG) as determined with the methods of the present invention are imputed into such an algorithm, and the risk profile is reported based upon a comparison of such input with established norms (e.g., established norm for pre-cancerous condition, established norm for various risk levels for developing a mature T-cell leukemia, established norm for subjects diagnosed with various stages of a mature T-cell leukemia). In some embodiments, the risk profile indicates a subject's risk for developing a mature T-cell leukemia or a subject's risk for re-developing a mature T-cell leukemia. In some embodiments, the risk profile indicates a subject to be, for example, a very low, a low, a moderate, a high, and a very high chance of developing or re-developing a mature T-cell leukemia or having a poor prognosis (e.g., likelihood of long term survival) from a mature T-cell leukemia. In some embodiments, a health care provider (e.g., an oncologist) will use such a risk profile in determining a course of treatment or intervention (e.g., biopsy, wait and see, referral to an oncologist, referral to a surgeon, etc.).

D. Mass Spectrometry

Mass spectrometry is a particularly powerful methodology to resolve different forms of a protein because the different forms typically have different masses that can be resolved by mass spectrometry. Accordingly, if one form of a protein is a superior biomarker for a disease than another form of the biomarker, mass spectrometry may be able to specifically detect and measure the useful form where traditional immunoassay fails to distinguish the forms and fails to specifically detect to useful biomarker.

One useful methodology for detecting a specific JAK/STAT pathway variant described herein (e.g., JAK1, JAK3, STAT5B, IL2RG) combines mass spectrometry with immunoassay. First, a biospecific capture reagent (e.g., an antibody, aptamer or Affibody that recognizes the biomarker and other forms of it) is used to capture the biomarker of interest (e.g., a JAK1 variant, a JAK3 variant, a STAT5B variant, a IL2RG variant). Preferably, the biospecific capture reagent is bound to a solid phase, such as a bead, a plate, a membrane or

an array. After unbound materials are washed away, the captured analytes are detected and/or measured by mass spectrometry. In some embodiments, such methods also permit capture of protein interactors, if present, that are bound to the proteins or that are otherwise recognized by antibodies and that, themselves, can be biomarkers. Various forms of mass spectrometry are useful for detecting the protein forms, including laser desorption approaches, such as traditional MALDI or SELDI, and electrospray ionization.

In some embodiments, a biomarker of this invention (e.g., a JAK1 variant, a JAK3 variant, a STAT5B variant, a IL2RG variant) is detected by mass spectrometry, a method that employs a mass spectrometer to detect gas phase ions. Examples of mass spectrometers are time-of-flight, magnetic sector, quadrupole filter, ion trap, ion cyclotron resonance, electrostatic sector analyzer and hybrids of these.

In some embodiments, the mass spectrometer is a laser desorption/ionization mass spectrometer. In laser desorption/ionization mass spectrometry, the analytes are placed on the surface of a mass spectrometry probe, a device adapted to engage a probe interface of the mass spectrometer and to present an analyte to ionizing energy for ionization and introduction into a mass spectrometer. A laser desorption mass spectrometer employs laser energy, typically from an ultraviolet laser, but also from an infrared laser, to desorb analytes from a surface, to volatilize and ionize them and make them available to the ion optics of the mass spectrometer. (e.g., a JAK1 variant, a JAK3 variant, a STAT5B variant, a IL2RG variant)

In some embodiments, the mass spectrometric technique for use is "Surface Enhanced Laser Desorption and Ionization" or "SELDI," as described, for example, in U.S. Patent No. 5,719,060 and No. 6,225,047; each herein incorporated by reference in its entirety. This refers to a method of desorption/ionization gas phase ion spectrometry (e.g. mass spectrometry) in which an analyte (e.g., one or more of the biomarkers of the present invention) is captured on the surface of a SELDI mass spectrometry probe. There are several versions of SELDI.

One version of SELDI is called "affinity capture mass spectrometry." It also is called "Surface-Enhanced Affinity Capture" or "SEAC". This version involves the use of probes that have a material on the probe surface that captures analytes through a non-covalent affinity interaction (adsorption) between the material and the analyte. The material is variously called an "adsorbent," a "capture reagent," an "affinity reagent" or a "binding moiety." Such probes can be referred to as "affinity capture probes" and as having an "adsorbent surface." The capture reagent can be any material capable of binding an analyte.

The capture reagent is attached to the probe surface by physisorption or chemisorption. In certain embodiments the probes have the capture reagent already attached to the surface. In other embodiments, the probes are pre-activated and include a reactive moiety that is capable of binding the capture reagent, e.g., through a reaction forming a covalent or coordinate covalent bond. Epoxide and acyl-imidizole are useful reactive moieties to covalently bind polypeptide capture reagents such as antibodies or cellular receptors. Nitrilotriacetic acid and iminodiacetic acid are useful reactive moieties that function as chelating agents to bind metal ions that interact non-covalently with histidine containing peptides. Adsorbents are generally classified as chromatographic adsorbents and biospecific adsorbents.

"Chromatographic adsorbent" refers to an adsorbent material typically used in chromatography. Chromatographic adsorbents include, for example, ion exchange materials, metal chelators (e.g., nitrilotriacetic acid or iminodiacetic acid), immobilized metal chelates, hydrophobic interaction adsorbents, hydrophilic interaction adsorbents, dyes, simple biomolecules (e.g., nucleotides, amino acids, simple sugars and fatty acids) and mixed mode adsorbents (e.g., hydrophobic attraction/electrostatic repulsion adsorbents).

"Biospecific adsorbent" refers to an adsorbent comprising a biomolecule, e.g., a nucleic acid molecule (e.g., an aptamer), a polypeptide, a polysaccharide, a lipid, a steroid or a conjugate of these (e.g., a glycoprotein, a lipoprotein, a glycolipid, a nucleic acid (e.g., DNA)-protein conjugate). In certain instances, the biospecific adsorbent can be a macromolecular structure such as a multiprotein complex, a biological membrane or a virus. Examples of biospecific adsorbents are antibodies, receptor proteins and nucleic acids. Biospecific adsorbents typically have higher specificity for a target analyte than chromatographic adsorbents. Further examples of adsorbents for use in SELDI can be found in U.S. Patent No. 6,225,047; herein incorporated by reference in its entirety. A "bioselective adsorbent" refers to an adsorbent that binds to an analyte with an affinity of at least 10^{-8} M.

Protein biochips produced by Ciphergen Biosystems, Inc. comprise surfaces having chromatographic or biospecific adsorbents attached thereto at addressable locations. Ciphergen ProteinChip® arrays include NP20 (hydrophilic); H4 and H50 (hydrophobic); SAX-2, Q-10 and LSAX-30 (anion exchange); WCX-2, CM-10 and LWCX-30 (cation exchange); IMAC-3, MAC-30 and IMAC 40 (metal chelate); and PS-10, PS-20 (reactive surface with acyl-imidizole, epoxide) and PG-20 (protein G coupled through acyl-imidizole). Hydrophobic ProteinChip arrays have isopropyl or nonylphenoxy-poly(ethylene glycol)methacrylate functionalities. Anion exchange ProteinChip arrays have quaternary ammonium functionalities. Cation exchange ProteinChip arrays have carboxylate

functionalities. Immobilized metal chelate ProteinChip arrays have nitrilotriacetic acid functionalities that adsorb transition metal ions, such as copper, nickel, zinc, and gallium, by chelation. Preactivated ProteinChip arrays have acyl-imidizole or epoxide functional groups that can react with groups on proteins for covalent binding. Such biochips are further described in: U.S. Patent Nos. 7,045,366, 6,579,719; 6,897,072; 6,555,813; U.S. Patent Publication Nos. U.S. 2003-0032043; US 2003-0218130; and PCT International Publication No. WO 03/040700; each herein incorporated by reference in its entirety.

In general, a probe with an adsorbent surface is contacted with the sample for a period of time sufficient to allow the biomarker or biomarkers that may be present in the sample to bind to the adsorbent. After an incubation period, the substrate is washed to remove unbound material. Any suitable washing solutions can be used; preferably, aqueous solutions are employed. The extent to which molecules remain bound can be manipulated by adjusting the stringency of the wash. The elution characteristics of a wash solution can depend, for example, on pH, ionic strength, hydrophobicity, degree of chaotropism, detergent strength, and temperature.

The biomarkers bound to the substrates are detected in a gas phase ion spectrometer such as a time-of-flight mass spectrometer. The biomarkers are ionized by an ionization source such as a laser, the generated ions are collected by an ion optic assembly, and then a mass analyzer disperses and analyzes the passing ions. The detector then translates information of the detected ions into mass-to-charge ratios. Detection of a biomarker typically will involve detection of signal intensity. Thus, both the quantity and mass of the biomarker can be determined.

Another version of SELDI is Surface-Enhanced Neat Desorption (SEND), which involves the use of probes comprising energy absorbing molecules that are chemically bound to the probe surface ("SEND probe"). The phrase "energy absorbing molecules" (EAM) denotes molecules that are capable of absorbing energy from a laser desorption/ionization source and, thereafter, contribute to desorption and ionization of analyte molecules in contact therewith. The EAM category includes molecules used in MALDI, frequently referred to as "matrix," and is exemplified by cinnamic acid derivatives, sinapinic acid (SPA), cyano-hydroxy-cinnamic acid (CHCA) and dihydroxybenzoic acid, ferulic acid, and hydroxyaceto-phenone derivatives. In certain embodiments, the energy absorbing molecule is incorporated into a linear or cross-linked polymer, e.g., a polymethacrylate. For example, the composition can be a co-polymer of α-cyano-4-methacryloyloxycinnamic acid and acrylate. In another embodiment, the composition is a co-polymer of α-cyano-4-methacryloyloxycinnamic acid,

acrylate and 3-(tri-ethoxy)silyl propyl methacrylate. In another embodiment, the composition is a co-polymer of α-cyano-4-methacryloyloxycinnamic acid and octadecylmethacrylate "C18 SEND"). SEND is further described in U.S. Patent No. 6,124,137 and PCT International Publication No. WO 03/64594; each herein incorporated in its entirety.

SEAC/SEND is a version of SELDI in which both a capture reagent and an energy absorbing molecule are attached to the sample presenting surface. SEAC/SEND probes therefore allow the capture of analytes through affinity capture and ionization/desorption without the need to apply external matrix. The C18 SEND biochip is a version of SEAC/SEND, comprising a C18 moiety which functions as a capture reagent, and a CHCA moiety which functions as an energy absorbing moiety.

E. Biochip Detection

In some embodiments, a sample is analyzed by means of a biochip. Biochips generally comprise solid substrates and have a generally planar surface, to which a capture reagent (also called an adsorbent or affinity reagent) is attached. Frequently, the surface of a biochip comprises a plurality of addressable locations, each of which has the capture reagent bound there. For example, in some embodiments, the present invention provides biochips having attached thereon one or more capture reagents specific for a JAK / STAT variant of the present invention (e.g., a JAK1 variant, a JAK3 variant, a STAT5B variant, a IL2RG variant).

Protein biochips are biochips adapted for the capture of polypeptides (e.g., a JAK / STAT variant of the present invention (e.g., a JAK1 variant, a JAK3 variant, a STAT5B variant, a IL2RG variant)). Many protein biochips are described in the art. These include, for example, protein biochips produced by Ciphergen Biosystems, Inc. (Fremont, Calif.), Zyomyx (Hayward, Calif.), Invitrogen (Carlsbad, Calif.), Biacore (Uppsala, Sweden) and Procognia (Berkshire, UK). Examples of such protein biochips are described in the following patents or published patent applications: U.S. Patent Nos. 6,225,047, 6,537,749, 6,329,209, and 5,242,828, and PCT International Publication Nos. WO 00/56934, and WO 03/048768; each herein incorporated by reference in its entirety.

II. Therapeutic Management

In certain embodiments, the present invention provides methods for managing a subject's treatment based on the status (e.g., presence or absence of mature T-cell leukemia). Such management includes the actions of the physician or clinician subsequent to

determining mature T-cell leukemia status. For example, if a physician makes a diagnosis of a mature T-cell leukemia, then a certain regime of treatment, such as prescription or administration of therapeutic agent might follow. Alternatively, a diagnosis of non-mature T-cell leukemia might be followed with further testing to determine a specific disease that the patient might be suffering from. Also, if the diagnostic test gives an inconclusive result on mature T-cell leukemia status, further tests may be called for.

III. Determining Therapeutic Efficacy of Pharmaceutical Drug

In another embodiment, the present invention provides methods for determining the therapeutic efficacy of a pharmaceutical drug (e.g., a pharmaceutical drug for treating mature T-cell leukemia). These methods are useful in performing clinical trials of the drug, as well as monitoring the progress of a patient on the drug. Therapy or clinical trials involve administering the drug in a particular regimen. The regimen may involve a single dose of the drug or multiple doses of the drug over time. The doctor or clinical researcher monitors the effect of the drug on the patient or subject over the course of administration. If the drug has a pharmacological impact on the condition, the amounts or relative amounts (e.g., the pattern or profile) of the biomarkers (e.g., any of the variant forms of JAK1, JAK3, STAT5B, IL2RG described herein) of this invention changes toward a non-disease profile. Therefore, one can follow the course of the amounts of these biomarkers in the subject during the course of treatment. Accordingly, this method involves measuring one or more biomarkers in a subject receiving drug therapy, and correlating the amounts of the biomarkers with the disease status of the subject. One embodiment of this method involves determining the levels of the biomarkers at least two different time points during a course of drug therapy, e.g., a first time and a second time, and comparing the change in amounts of the biomarkers, if any. For example, the biomarkers can be measured before and after drug administration or at two different time points during drug administration. The effect of therapy is determined based on these comparisons. If a treatment is effective, then the biomarkers will trend toward normal, while if treatment is ineffective, the biomarkers will trend toward disease indications. If a treatment is effective, then the biomarkers will trend toward normal, while if treatment is ineffective, the biomarkers will trend toward disease indications.

IV. Compositions of Matter

In another aspect, the present invention provides compositions of matter based on the biomarkers of this invention. For example, in one embodiment, the present invention

provides a biomarker of this invention in purified form. Purified biomarkers have utility as antigens to raise antibodies. Purified biomarkers also have utility as standards in assay procedures. As used herein, a "purified biomarker" is a biomarker that has been isolated from other proteins and peptides, and/or other material from the biological sample in which the biomarker is found. For example, in some embodiments, the present invention provides compositions comprising a purified JAK / STAT variant (e.g., any of the JAK1 variants described herein, JAK3 variants described herein, STAT5B variants described herein, IL2RG variants described herein).

Biomarkers may be purified using any method known in the art, including, but not limited to, mechanical separation (e.g., centrifugation), ammonium sulphate precipitation, dialysis (including size-exclusion dialysis), size-exclusion chromatography, affinity chromatography, anion-exchange chromatography, cation-exchange chromatography, and methal-chelate chromatography. Such methods may be performed at any appropriate scale, for example, in a chromatography column, or on a biochip.

In another embodiment, the present invention provides a biospecific capture reagent, optionally in purified form, that specifically binds a biomarker of this invention. In one embodiment, the biospecific capture reagent is an antibody. Such compositions are useful for detecting the biomarker in a detection assay, e.g., for diagnostics.

In another embodiment, this invention provides an article comprising a biospecific capture reagent that binds a biomarker of this invention, wherein the reagent is bound to a solid phase. For example, this invention contemplates a device comprising bead, chip, membrane, monolith or microtiter plate derivatized with the biospecific capture reagent. Such articles are useful in biomarker detection assays.

In another aspect the present invention provides a composition comprising a biospecific capture reagent, such as an antibody, bound to a biomarker of this invention, the composition optionally being in purified form. Such compositions are useful for purifying the biomarker or in assays for detecting the biomarker.

In another embodiment, this invention provides an article comprising a solid substrate to which is attached an adsorbent, e.g., a chromatographic adsorbent or a biospecific capture reagent, to which is further bound a biomarker of this invention.

In another embodiment, the invention provides compositions comprising reaction mixtures formed through, for example, binding of a biomarker of the present invention with a detection marker (e.g., antibody, proble, biochip, etc.) (e.g., via a detection assay of the present invention). In some embodiments, "reaction mixture" comprises any material

sufficient, necessary, or useful for conducting any of the assays described herein. In some embodiments, the present invention provides compositions comprising reaction mixtures comprising extension products complementary to a specific mutation. In some embodiments, the present invention provides compositions comprising reaction mixtures comprising extension products complementary to a specific mutation and sequences immediately surrounding such a mutation. In some embodiments, the extension product has thereon an labeling agent (e.g., a fluorophore or other lable). In some embodiments, the present invention provides compositions comprising reaction mixtures comprising extension products complementary to a specific mutation bound with such a complementary sequence. In some embodiments, the present invention provides compositions comprising reaction mixtures comprising extension products complementary to a specific mutation bound with such a complementary sequence, wherein the binding is to a solid surface, a biochip (e.g., in single copy or multiple copies). In some embodiments, the present invention provides compositions comprising fragments of a peptide of interest. In some embodiments, the present invention provides compositions comprising a peptide of interest in a mass-spectrometry compatible buffer.

V. Kits for Detection of Mature T-cell Leukemia Biomarkers

In another aspect, the present invention provides kits for qualifying mature T-cell leukemia status, which kits are used to detect one or more of the biomarkers according to the invention. In one embodiment, the kit comprises a solid support, such as a chip, a microtiter plate or a bead or resin having a capture reagent attached thereon, wherein the capture reagent binds a biomarker of the invention. Thus, for example, the kits of the present invention can comprise mass spectrometry probes for SELDI, such as ProteinChip® arrays. In the case of biospecfic capture reagents, the kit can comprise a solid support with a reactive surface, and a container comprising the biospecific capture reagent.

In some embodiments, the kit can also comprise a washing solution or instructions for making a washing solution, in which the combination of the capture reagent and the washing solution allows capture of the biomarker or biomarkers on the solid support for subsequent detection by, e.g., mass spectrometry. The kit may include more than type of adsorbent, each present on a different solid support.

In some embodiments, such a kit can comprise instructions for suitable operational parameters in the form of a label or separate insert. For example, the instructions may inform

a consumer about how to collect the sample, how to wash the probe or the particular biomarkers to be detected.

In some embodiments, the kit can comprise one or more containers with biomarker samples, to be used as standard(s) for calibration.

In some embodiments, the kit can comprise software necessary for interpreting results and/or generating prospective therapeutic outcomes. In some embodiments, for example, the software provides mutation specific databases and comparison programs.

VI. Additional Embodiments

The following additional embodiments are contemplated.

1. A method for detecting one or more JAK/STAT pathway variants associated with a mature T-cell leukemia in a subject, comprising:

a) contacting a sample from a subject with a JAK/STAT pathway variant detection assay under conditions that the presence of a JAK/STAT pathway variant associated with a mature T-cell leukemia is determined; and

b) diagnosing said subject with a mature T-cell leukemia when one or more of said JAK/STAT pathway variants are present in said sample.

2. The method of claim 1, wherein said one or more JAK/STAT pathway variants encodes a loss of function mutation and/or a gain of function mutation.

3. The method of claim 1, wherein said subject is a human patient.

4. The method of claim 1, wherein said JAK/STAT pathway variant is one or more variants selected from JAK1, JAK3, STAT5B, and IL2RG.

5. The method of claim 4, wherein said JAK1 variant is one or more JAK1 mutations selected from the group consisting of JAK1 p.F636L, JAK1 p.G646C, JAK1 p. Y654F, JAK1 p.V658F, JAK1 p.S703I, and JAK1 p.T901R.

6. The method of claim 4, wherein said JAK3 variant is one or more JAK3 mutations selected from the group consisting of JAK3 p.M511I, JAK3 p.ΔKNC563, JAK3 p. A573V,

JAK3 p.R657, JAK3 p.G662W, JAK3 p.P664T, JAK3 p.Y980, JAK3 p. Y981, and JAK3 p.S989I.

7. The method of claim 4, wherein said STAT5B variant is one or more STAT5B mutations selected from the group consisting of STAT5B p.T628S, STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.N642H, STAT5B p.Y699, and STAT5B p.Y665H.

8. The method of claim 4, wherein said IL2RG variant is one or more IL2RG mutations selected from the group consisting of IL2RG p.ΔGSM268, IL2RG p.Y325, and IL2RG p. K315E.

9. The method of claim 1, wherein said mature T-cell leukemia is T-cell prolymphocytic leukemia.

10. The method of claim 9, wherein one or more JAK/STAT pathway variants are selected from the group consisting of JAK1 p.V658F, JAK1 p.S703I, JAK1 p.T901R, JAK3 p.ΔKNC563, JAK3 p.M511I, JAK3 p. A573V, JAK3 p.R657, STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.T628S, STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.N642H, STAT5B p.Y665H, IL2RG p.ΔGSM268, and IL2RG p. K315E.

11. The method of claim 1, wherein said mature T-cell leukemia is Sezary syndrome.

12. The method of claim 11, wherein one or more JAK/STAT pathway variants are selected from the group consisting of JAK1 p. Y654F, JAK3 p. A573V, JAK3 p.Y980, JAK3 p. Y981, JAK3 p.S989I, STAT5B p.Y699, STAT5B p.N642H, and Il2RG p.Y325.

13. The method of claim 1, wherein said determining comprises detecting variant JAK1, JAK3, STAT5B, and IL2RG nucleic acids or polypeptides.

14. The method of claim 13, wherein said detecting variant JAK1, JAK3, STAT5B, and IL2RG nucleic acids comprises one or more nucleic acid detection method selected from the group consisting of sequencing, amplification and hybridization.

15. The method of Claim 1, wherein said biological sample is selected from the group consisting of a tissue sample, a cell sample, and a blood sample.

16. The method of claim 1, wherein said determining comprises a computer implemented method.

17. The method of claim 16, wherein said computer implemented method comprises analyzing JAK1, JAK3, STAT5B, and IL2RG variant information and displaying said information to a user.

18. The method of claim 1, further comprising the step of treating said subject for a mature T-cell leukemia and monitoring said subject for the presence of JAK1, JAK3, STAT5B, and IL2RG variants associated with said mature T-cell leukemia.

19. The method of claim 1, further comprising the step of treating said subject for a mature T-cell leukemia under condition such that at least one symptom of said mature T-cell leukemia is diminished or eliminated.

20. The method of claim 19, wherein said treating comprises inhibiting JAK1, JAK3, STAT5B, and/or IL2RG expression and/or activity.

21. The method of claim 20, wherein said inhibiting STAT5B expression and/or activity is accomplished through administration of an agent configured to inhibit STAT5B expression pimozide.

22. The method of claim, wherein said inhibiting JAK1 and/or JAK3 expression and/or activity is accomplished through administration of an agent configured to inhibit JAK1 and/or JAK3 expression and/or activity, wherein said agent is selected from the group consisting of ruxolitinib, tofacitinib, baricitinib, CYT387, and lestaurtinib.

23. The method of claim 21, further comprising administering one or more agents for treating a mature T-cell leukemia.

24. The method of claim 23, wherein said one or more agents is selected from the group consisting of a purine analog (e.g., pentostatin, fludarabine, cladrbine), chlorambucil, cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP), cyclophosphamide, vincristine, prednisone (COP), and vincristine, doxorubicin, prednisone, etoposide, cyclophosphamide, bleomycin, alemtuzumab, and vorinostat.

25. The method of claim 1, further comprising the step of detecting a variant in one or more additional genes associated with a mature T-cell leukemia.

26. The method of claim 25, wherein said one or more genes are selected from the group consisting of CHEK2, EZH2, and FBXW10.

27. Use of a variant JAK/STAT pathway nucleic acid or polypeptide for detecting a mature T-cell leukemia in a subject.

28. The use of claim 27, wherein said JAK/STAT pathway variant encodes a loss of function mutation and/or a gain of function mutuation.

29. The use of claim 27, wherein said subject is a human subject.

30. The use of claim 27, wherein said JAK/STAT pathway variant is one or more variants selected from JAK1, JAK3, STAT5B, and IL2RG.

31. The use of claim 30, wherein said JAK1 variant is one or more JAK1 mutations selected from the group consisting of JAK1 p.F636L, JAK1 p.G646C, JAK1 p. Y654F, JAK1 p.V658F, JAK1 p.S703I, and JAK1 p.T901R.

32. The use of claim 30, wherein said JAK3 variant is one or more JAK3 mutations selected from the group consisting of JAK3 p.M511I, JAK3 p.ΔKNC563, JAK3 p. A573V, JAK3 p.R657., JAK3 p.G662W, JAK3 p.P664T, JAK3 p.Y980, JAK3 p. Y981, and JAK3 p.S989I.

33. The use of claim 30, wherein said STAT5B variant is one or more STAT5B mutations selected from the group consisting of STAT5B p.T628S, STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.N642H, STAT5B p.Y699, and STAT5B p.Y665H.

34. The use of claim 30, wherein said IL2RG variant is one or more IL2RG mutations selected from the group consisting of IL2RG p.ΔGSM268, IL2RG p.Y325 and IL2RG p. K315E.

35. The use of claim 27, wherein said mature T-cell leukemia is T-cell prolymphocytic leukemia.

36. The use of claim 35, wherein one or more JAK/STAT pathway variants are selected from the group consisting of JAK1 p.V658F, JAK1 p.S703I, JAK1 p.T901R, JAK3 p.ΔKNC563, JAK3 p.M511I, JAK3 p. A573V, JAK3 p.R657, STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.T628S, STAT5B p.N642H, STAT5B p.Y665H, and IL2RG p.ΔGSM268, IL2RG p. K315E.

37. The use of claim 27, wherein said mature T-cell leukemia is Sezary syndrome.

38. The use of claim 37, wherein one or more JAK/STAT pathway variants are selected from the group consisting of JAK1 p. Y654F, JAK3 p. A573V, JAK3 p.Y980, JAK3 p. Y981, JAK3 p.S989I, STAT5B p.Y699, STAT5B p.N642H, and Il2RG p.Y325.

39. The use of claim 27, wherein said determining comprises detecting variant JAK1, JAK3, STAT5B, and IL2RG nucleic acids or polypeptides.

40. The use of claim 39, wherein said detecting variant JAK1, JAK3, STAT5B, and IL2RG nucleic acids comprises one or more nucleic acid detection method selected from the group consisting of sequencing, amplification and hybridization.

41. A method of determining a decreased time to adverse outcome in a subject diagnosed with a mature T-cell leukemia, comprising:

a) contacting a sample from a subject with a JAK/STAT pathway variant detection assay under conditions that the presence of a JAK/STAT pathway variant associated with a mature T-cell leukemia is determined; and

c) detecting a decreased time to adverse outcome in said subject when said JAK/STAT pathway variants are present in said sample.

42. The method of claim 41, wherein said adverse outcome is selected from the group consisting of relapse of said mature T-cell leukemia, metastasis, or death.

43. The method of claim 41, wherein said JAK/STAT pathway variant encodes a loss of function mutation and/or a gain of function mutuation.

44. The method of claim 41, wherein said subject is a human subject.

45. The method of claim 41, wherein said JAK/STAT pathway variant is one or more variants selected from JAK1, JAK3, STAT5B, and IL2RG.

46. The method of claim 45, wherein said JAK1 variant is one or more JAK1 mutations selected from the group consisting of JAK1 p.F636L, JAK1 p.G646C, JAK1 p. Y654F, JAK1 p.V658F, JAK1 p.S703I, and JAK1 p.T901R.

47. The method of claim 45, wherein said JAK3 variant is one or more JAK3 mutations selected from the group consisting of JAK3 p.ΔKNC563, JAK3 p.M511I, JAK3 p. A573V, JAK3 p.R657, JAK3 p.G662W, JAK3 p.P664T, JAK3 p.Y980, JAK3 p. Y981, and JAK3 p.S989I.

48. The method of claim 45, wherein said STAT5B variant is one or more STAT5B mutations selected from the group consisting of STAT5B p.T628S, STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.N642H, STAT5B p.Y699, and STAT5B p.Y665H.

49. The method of claim 45, wherein said IL2RG variant is one or more IL2RG mutations selected from the group consisting of IL2RG p.ΔGSM268, IL2RG p.Y325, and IL2RG p. K315E.

50. The method of claim 41, wherein said mature T-cell leukemia is T-cell prolymphocytic leukemia.

51. The method of claim 50, wherein one or more JAK/STAT pathway variants are selected from the group consisting of JAK1 p.V658F, JAK1 p.S703I, JAK1 p.T901R, JAK3 p.ΔKNC563, JAK3 p.M511I, JAK3 p. A573V, JAK3 p.R657, STAT5B p.T628S, STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.N642H, STAT5B p.Y665H, IL2RG p.ΔGSM268, and IL2RG p. K315E.

52. The method of claim 41, wherein said mature T-cell leukemia is Sezary syndrome.

53. The method of claim 52, wherein one or more JAK/STAT pathway variants are selected from the group consisting of JAK1 p. Y654F, JAK3 p. A573V, JAK3 p.Y980, JAK3 p. Y981, JAK3 p.S989I, STAT5B p.Y699, STAT5B p.N642H, and Il2RG p.Y325.

54. The method of claim 41, wherein said determining comprises detecting variant JAK1, JAK3, STAT5B, and IL2RG nucleic acids or polypeptides.

55. The method of claim 54, wherein said detecting variant JAK1, JAK3, STAT5B, and IL2RG nucleic acids comprises one or more nucleic acid detection method selected from the group consisting of sequencing, amplification and hybridization.

EXPERIMENTAL

The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

Example I.

This example demonstrates that constitutive activation of the JAK-STAT pathway could play a role in the pathogenesis of mature T-cell leukemia.

Unbiased tandem-mass spectrometry phosphoproteomic analysis (see, e.g., Walters, D. K. et al. 2005 Cancer Cell 10, 65-75; Rush, J. et al. 2006 Nat Biotechnol 23, 94-101; each

herein incorporated by reference in its entirety) (Figure 1) of the SS-derived mature T-cell leukemia cell line, HUT78, revealed numerous phosphorylated peptides corresponding to proteins whose phosphorylation has not previously been reported in T-cell leukemias. Among 59 proteins with tyrosine-phosphorylated peptides in HUT78 cells, the IL2RG p.Y325, JAK3 p.Y980/p.Y981 and the STAT5B p.Y699 residues were identified with high spectral counts suggesting constitutive activation of the JAK-STAT pathway (Figure 2a-d). To identify possible mutational mechanisms underlying the constitutive JAK-STAT activation detected by phosphoproteomic screening, WES was performed on HUT78 cells (Figure 2d, right). This analysis revealed mutations in both *JAK1* (p.Y654F) and *JAK3* (p.A573V; Figure 2e-g). While the *JAK3* p.A573V mutation had previously been reported to be associated with leukemic progression in acute lymphoblastic T-cell leukemia (see, e.g., Bains, T. et al. 2005 Leukemia 26, 2144-2146; Zhang, J. et al. 2012 Nature 481, 157-163; each herein incorporated by reference in its entirety), NK/T-cell leukemias (see, e.g., Koo, G. C. et al. 2012 Cancer Discov 2, 591-597; herein incorporated by reference in its entirety) and megakaryoblastic leukemias (see, e.g., Kiyoi, H., 2007 Leukemia 21, 574-576; De Vita, S. et al. 2007 Br J Haematol 137, 337-341; Malinge, S. et al. 2008 Blood 112, 4220-4226; each herein incorporated by reference in its entirety) it had not previously been reported in SS or in other mature T-cell leukemias. Indeed, the *JAK1* p.Y654F mutation has not previously been reported in any human cancer. Altogether, such phosphoproteomic analysis and exome sequencing suggested that constitutive activation of the JAK-STAT pathway could play a role in the pathogenesis of mature T-cell leukemia.

Example II.

To gain further insight into the structural alterations driving JAK-STAT activation in mature T-cell leukemias, whole genome sequencing (WGS) of 4 well-characterized index cases of T-PLL selected based on fulfillment of established diagnostic criteria including possession of characteristic cytologic, immunophenotypic and karyotypic features was performed (Figure 3). WGS confirmed presence of structural alterations involving the *TCL1B* locus or its analogue *MTCP* in all 4 cases (Figure 4). This analysis also revealed mutations in 2 of 4 samples in *JAK1* not previously been associated with T-PLL (p.V658F (see, e.g., Zhang, J. et al. 2008 Blood 118, 3080-3087; Jeong, E. G. et al. 2008 Clin Cancer Res 14, 3716-3721; each herein incorporated by reference in its entirety) and p.S703I (see, e.g., Zhang, J. et al. 2012 Nature 481, 157-163; herein incorporated by reference in its entirety), both of which were confirmed to be somatic (for p.S703I see Figure 5b). One *JAK1*

mutation (p.V658F) is homologous to the *JAK2* p.V617F mutation characteristic of myeloproliferative neoplasms (MPNs) and both have been reported in isolated cases of lymphoid malignancy in the COSMIC database of somatic cancer mutations. The integrated phosphoproteomic and mutational screen highlighted alterations in the JAK-STAT pathway in these samples, raising the possibility that additional genetic defects other than those previously known to characterize T-PLL might play an important role in disease pathogenesis.

Example III.

To better understand the molecular genetic basis of T-PLL, whole exome sequencing (WES) and high-resolution copy number variant (CNV; array comparative genomic hybridization, aCGH) analysis was next performed on an additional 36 and 39 T-PLL cases, respectively. Consistent with previous reports (see, e.g., Yuille, M. A. et al. 1998 Oncogene 16, 789-796, doi:10.1038/sj.onc.1201603 (1998); Pekarsky, Y., et al. 1999 Proc Natl Acad Sci U S A 96, 2949-2951 (1999); each herein incorporated by reference in its entirety), aCGH confirmed loss of the *ATM* locus at chromosome 11q in 65.1% (28/39) of all cases (Figure 6, arrow) and isochromosome 8 or large-scale gains of chromosome 8q in 76.9% (30/39) of the T-PLL samples analyzed (Figure 6, arrowhead). Additionally, 65.1% (28/43) of samples analyzed harbored somatic mutations in the tumor suppressor *ATM* (Figure 7) predicted to be deleterious to protein function including frameshift and nonsense mutations as well as missense mutations clustered in the FAT and PI3K domains similar to previous reports (see, e.g., Stilgenbauer, S. et al. 1997 Nat Med 3, 1155-1159; Vorechovsky, I. et al. 1997 Nat Genet 17, 96-99; each herein incorporated by reference in its entirety). Using this approach, a number of genetic alterations not previously associated with T-PLL were identified including mutations in *CHEK2*, a gene encoding a protein kinase activated in response to DNA damage (see, e.g., Antoni, L., et al. 2007 Nat Rev Cancer 7, 925-936; herein incorporated by reference in its entirety) (2/43, 4.7%) and novel mutations in *EZH2*, a member of the PcG-family of transcriptional repressors (5/43, 11.6%) frequently mutated in myeloid (see, e.g., Khan, S. N. et al. 2013 Leukemia 27, 1301-1309; herein incorporated by reference in its entirety) and lymphoid (see, e.g., Morin, R. D. et al. 2010 Nat Genet 42, 181-185; herein incorporated by reference in its entirety) malignancy and *FBWX10*, a member of the F-box protein family of ubiquitin ligases (3/43, 7.0%; Figure 12). Mutations in these proteins included several deleterious frameshift and nonsense mutations raising the possibility that *ATM, CHEK2, EZH2* and *FBWX10* might contribute to T-PLL pathogenesis

via their roles in DNA repair, epigenetic transcriptional regulation and proteosomal degradation pathways, respectively.

Strikingly, WES analysis also identified numerous mutations in *JAK1, JAK3* and *STAT5B* including recurrent lesions not previously associated with T-PLL (Figure 12 and Figure 5e-h and l circles). The Janus kinase/signal transducers and activators of transcription (JAK/STAT) family mediates cytokine signaling in lymphocytes and has been implicated in the pathogenesis of a number of other hematopoietic malignancies (see, e.g., Chen, E., et al. 2012 Immunity 36, 529-541; herein incorporated by reference in its entirety). Altogether, WGS, WES and targeted Sanger sequencing analysis identified mutations in *JAK1* (10.0%, 5/50) and *JAK3* (30.0%, 15/50; Figure 5f-g and 5i) that were clustered in the auto-inhibitory-pseudokinase and included variants previously detected in leukemias other than mature T-cell neoplasia or shown to lead to constitutive activation of JAK-STAT signaling (see, e.g., Chen, E., et al. 2012 Immunity 36, 529-541; Knoops, L., et al. 2008 Oncogene 27, 1511-1519; each herein incorporated by reference in its entirety) (including JAK1 p.V658F (see, e.g.,Zhang, J. et al. 2011 Blood 118, 3080-3087; Jeong, E. G. et al. 2008 Clin Cancer Res 14, 3716-3721; each herein incorporated by reference in its entirety) and p.S703I (see, e.g., Zhang, J. et al. 2012 Nature 481, 157-163; herein incorporated by reference in its entirety); JAK3 p.M511I (see, e.g., Walters, D. K. et al. 2006 Cancer Cell 10, 65-75; Zhang, J. et al. 2012 Nature 481, 157-163; Yamashita, Y. et al. 2010 Oncogene 29, 3723-3731; each herein incorporated by reference in its entirety), p.A573V (see, e.g., Koo, G. C. et al. 2012 Cancer Discov 2, 591-597; herein incorporated by reference in its entirety) and p.R657 (see, e.g., Yamashita, Y. et al. 2010 Oncogene 29, 3723-3731; herein incorporated by reference in its entirety). Mutations in *STAT5B* (34.0%, 17/50) were clustered in the SH2 domain (p.T628S, p.N642H, p.R659C, and p.Y665H; Figure 5h and 5l) that mediates the interaction between JAK and STAT proteins. Prior to the experiments of the present invention, high frequency *STAT5B* mutations have not been reported in any human cancer (see, e.g., Rajala, H. L. et al. 2013 Blood 121, 4541-4550; herein incorporated by reference in its entirety). The high homology between STAT5A and STAT5B (Figure 7a) permitted localization of the STAT5A residues homologous to the mutated STAT5B residues (Figure 5j) and revealed a very close 3-D proximity for these three residues in the SH2 domain to the predicted phosphotyrosine-binding loop. A novel mutation in the *IL2RG* transmembrane domain (p.ΔGSM268 and p.K315E; 2.0%, 1/50; Figure 5a and e) was also identified in a single T-PLL case similar to the mutations described in *IL7R* in T-acute lymphoblastic leukemia (see, e.g., Zhang, J. et al.

2012 Nature 481, 157-163; herein incorporated by reference in its entirety). The experiments conducted during the course of developing embodiments for the present invention, as such, represents the first report of a somatic mutation in *IL2RG* in human malignancy. Additionally, a novel JAK1 mutation (p.T901R; 2.0%, 1/50; Figure 5f) in the kinase domain was also identified. All of the mutations identified in these genes were confirmed by Sanger sequencing and where normal matched DNA was available were confirmed to be somatic (Figure 12; Figure 5e-h and l, closed circles).

An additional p.Q706L mutation in STAT5B was also identified in a sample with a JAK1 p.V658F mutation) that mediates the interaction between JAK and STAT proteins. Significantly, mutations in STAT5B have never been previously reported in T-PLL. In the cohort, STAT5B mutations actually represented the highest frequency of all JAK-STAT family members (36.0%, Fig. 12 and Fig. 13). Notably, the WGS and WES studies of 50 cases of T-PLL revealed no evidence of activating STAT3 mutations. In total, 38 out of 50 T-PLL genomes harbored somatic mutations in IL2RG, JAK1, JAK3 or STAT5B (76.0%, Fig. 12). With one exception (JAK1 p.V658F and STAT5B p.Q706L; see Fig. 13), all cases harbored mutually exclusive mutations in genes comprising the IL2RG-JAK1-JAK3-STAT5B pathway. In the majority of patients with mutations in the JAK-STAT pathway (32/38, 84.2%), matched constitutional normal tissue (e.g. CD4- leukocytes from peripheral blood) was available to confirm the somatic status of the mutations (Fig. 12 and Fig. 13). Of the 18 distinct JAK-STAT pathway mutations identified, all but one (JAK3 p.R657W) were confirmed to be somatic (Fig. 12; 17/18, 94.4%). These data indicate a significant role for mutational activation of the IL2R-JAK1-JAK3-STAT5 axis in T-PLL.

Example IV.

To investigate whether alterations in the IL2R/JAK1/JAK3/STAT5B pathway occurred in other mature T-cell diseases, targeted Sanger sequencing of regions of recurrent mutation in *IL2RG, JAK1, JAK3* and *STAT5B* was performed in 66 well-characterized cases of SS and 27 cases of Mycosis Fungoides (MF), the most common mature T-cell neoplasm of the skin with periodic extra-cutaneous and leukemic dissemination. This analysis detected 2 mutations in the pseudokinase domain of *JAK1* (the previously identified p.Y654F mutation and the novel p.L710V mutation, Figure 5d) and 1 additional mutation in the kinase domain of *JAK3* (p.S989I, Figure 5g) among SS samples (4.5%, 3/66; Figure 5i) and 4 novel mutations in the JAK1 or JAK3 pseudokinase domains (JAK1 p.F636L, JAK3 p.G646C, JAK3 p.G662W, and JAK3 p.P664T; note that the p.G646C, p.G662W and p.P664T

mutations are *JAK3* mutations and were inadvertently identified in Fig. 5 as *JAK1* mutations in the first version of Figure 5f; Figure 5f) in 2/27 MF samples (6.9%, Figure 5i). An additional 2 mutations in *STAT5B* were also identified in 2 SS samples (p.N642H; 3.0%, 2/66; note that these mutations were inadvertently not illustrated in the first version of Figure 5h; Figure 5h and l; Figure 5h-i, l). Mutations in *IL2RG*, *JAK1, JAK3* or *STAT5B* were not identified in 16 cases of reactive lymphoid hyperplasia (not shown) nor in 12 cases of Peripheral T-cell Leukemia (PTCL, Figure 5i). Altogether, these data indicate a high prevalence of altered JAK-STAT5 signaling in mature T-cell leukemias.

Example V.

The clustering of the JAK and STAT5B mutations strongly suggested a gain-of-function mechanism of pathogenesis. This raised the possibility that mutations identified in *JAK1* and *JAK3* were similar to the *JAK2* p.V617F mutation. To test this, the sequence of *JAK2* and *JAK3*, the most recurrently altered protein in the dataset, was analyzed. The crystal structure of JAK3 has not been reported however, the high degree of homology between JAK2 and JAK3 (Figure 7c-d) prompted localization in the JAK2 3-dimensional structure the JAK2 residues analogous to the recurrent *JAK3* mutations, p.M511I and p.A573V and the adjacent p.ΔKNC563 mutation. Plotting these residues onto the 3-dimensional structure of JAK2 revealed the close proximity of the p.V617 residue and residues analogous to the recurrent JAK3 mutations (Figure 5k). These results strongly suggest that the *JAK3* p.M511I mutation in T-PLL is acting similar to the *JAK2* p.V617F mutation in MPN. Indeed, the p.M511I mutation has been detected in acute megakaryoblastic leukemia supporting its pathogenic role in T-PLL (see, e.g., Walters, D. K. et al. 2006 Cancer Cell 10, 65-75; herein incorporated by reference in its entirety).

Example VI.

To investigate the functional effect of representative novel mutations on JAK-STAT5 activation, HeLa cells were engineered to express mutant IL2RG (p.ΔGMS628), JAK1 (p.S703I), JAK3 (p.Q705P) and STAT5B (p.T628S) proteins (Figure 8a-b). In each case, expression of mutant IL2RG, JAK1, JAK3 and STAT5B protein led to elevated levels of STAT5 activation as determined by transcriptional reporter assays (1.4-5.7 fold increase) and pSTAT5 expression as determined by Western blot analysis (Figure 8a-b). Expression of the previously described p.N642H mutant STAT5B protein also led to an increase in STAT5B

activation as expected (Figure 8a-b). Moreover, expression of the novel STAT5B p.T628S mutant protein lead to increased cell proliferation in Ba/F3 cells (Figure 8c). In addition, STAT5B p.N642H significantly increased colony-forming capacity in the Jurkat T-cell line (Figure 8d). Immunofluorescence microscopy on primary T-PLL cases harboring *IL2RG*, *JAK3* or *STAT5B* mutations showed elevated total cellular levels and nuclear localization of pSTAT5B protein (data shown for representative *IL2RG* and *JAK3* mutated primary cells; Figure 8e) illustrating a common mechanism of pathogenesis in both *IL2RG*-, *JAK*- and *STAT5B*-mutated T-PLL cases.

Example VII.

Given the convergence of common gamma chain (IL2RG), JAK1 and JAK3 signaling on STAT5B activation and the established role of STAT5 in cytokine-induced peripheral T-cell proliferation (see, e.g., Constantinescu, S. N., et al. 2008 Trends Biochem Sci 33, 122-131; herein incorporated by reference in its entirety), the effect of specific STAT5 inhibition on primary T-PLL and SS cells harboring *IL2RG*, *JAK1/3* or *STAT5B* mutations was determined. Treatment of cultured primary T-PLL cells with a selective STAT5 inhibitor (Pimozide) previously shown to reduce STAT5 levels in myeloid leukemia (see, e.g., Nelson, E. A. et al. 2011 Blood 117, 3421-3429; herein incorporated by reference in its entirety) resulted in significant and specific reduction in leukemia cell proliferation (Figure 8f) and viability (Figure 8g-h; arrow in Figure 8h indicates PARP-mediated apoptotic activation) and lead to a dose- and time-dependent reduction in pSTAT5B levels (Figure 8h).

Example VIII.

This example provide the materials and methods for Examples I – VII.

DNA from primary T-PLL samples was subjected to WGS, WES and 270K feature aCGH. HUT78 cells were subjected to WES and phosphoproteomic analyses. SS, MF, PTCL and reactive lymphoid hyperplasia samples were subjected to targeted Sanger sequencing of selected regions of *IL2RG*, *JAK1*, *JAK3* and *STAT5B*. Confirmation of selected mutations identified by WGS and WES was performed by Sanger sequencing of tumor DNA as well as any available DNA from constitutional normal samples. Primary T-PLL and HUT78 cells were cultured in the presence of IL2 with the addition of the specific STAT5 inhibitor Pimozide. Mutational analysis was performed in HeLa and Jurkat cell lines.

Patients samples and DNA extraction All T-PLL cases fulfilled pathologic criteria for diagnosis of T-PLL according to World Health Organization classification criteria without knowledge of JAK/STAT mutational status. For a given patient, samples represented either formalin-fixed paraffin embedded tissue (FFPE), cryopreserved peripheral blood leukocytes or both. Where applicable, constitutional normal tissue represented either tumor-free FFPE tissue derived from the same patient or otherwise tumor depleted peripheral blood leukocytes generated using EasySep column enrichment and B220 and/or Mac-1 positive cell selection (Stem Cell Technologies, Inc.). Relative tumor-depletion of resultant cell suspensions was determined by flow cytometry using antibodies directed against CD4 (BD Pharmingen). DNA was extracted from both FFPE and frozen samples using QIAGEN DNA extraction kits according to manufacturer's instructions.

Protein extraction and digestion Approximately sixty million cells were lysed in buffer containing 9 M urea/20 mM HEPES pH8.0/0.1% SDS and a cocktail of phosphatase inhibitors. For each sample, 6 mg of protein were reduced with 4.5 mM DTT and then alkylated with 10 mM iodoacetamide. Samples were diluted 5-fold with 20 mM HEPES and then digested with trypsin overnight at 37°C using an enzyme-to-protein ratio of 1/50 (w/w). Samples were desalted on a C18 cartridge (Sep-Pak plus C18 cartridge, Waters), then purified peptides were dried before further processing.

Phosphopeptide enrichment Metal oxide affinity chromatography (MOAC) was performed to enrich phosphorylated peptides and reduce the sample complexity prior to tyrosine-phosphorylated peptide immunopurification (pY-IP). Titanium dioxide (TiO2) microparticles (Titansphere® Phos-TiO, GL Sciences Inc.) applying a TiO2 microparticles-to-protein ratio of 6/1 (w/w) was used. Briefly TiO2 microparticles were conditioned with the buffer A (80% ACN/0.4% TFA), then equilibrated with the buffer B (75% buffer A/25% lactic acid). Peptides were solubilized with 200 µl buffer A and mixed with 400 µl buffer B then loaded twice on TiO2 microparticles. Microparticles were washed 2 times with buffer B and 3 times with buffer A. Hydrophilic phosphopeptides were eluted with 5% ammonium hydroxide solution and hydrophobic phosphopeptides were eluted with 5% pyrrolidine solution. After elution, peptides were dried using a SpeedVac. The equivalent of 5 mg of protein was further enriched for phosphorylated tyrosine peptides by overnight immunoprecipitation (pY-IP) using a cocktail of anti-phosphotyrosine antibodies (4G10,

Millipore/PT-66, Sigma/p-Tyr-100, Cell Signaling Technology). After elution, phosphotyrosine peptides were dried.

Mass spectrometry Ammonium hydroxide and pyrrolidine eluents were dried and reconstituted in 25 µl loading buffer (0.1% TFA/2% acetonitrile). Eluents from pY-IP were dried and reconstituted in 35 µl loading buffer. An LTQ Orbitrap XL (ThermoFisher) in-line with a Paradigm MS2 HPLC (Michrom bioresources) was employed for acquiring high-resolution MS and MS/MS data. Ten microliters of the phospho-enriched peptides were loaded onto a sample trap (Captrap, Bruker-Michrom) in-line with a nano-capillary column (Picofrit, 75 µm i.d.x 15 µm tip, New Objective) packed in-house with 10 cm of MAGIC AQ C18 reverse phase material (Michrom Bioresource). Two different gradient programs, one each for MOAC and pY-IP samples, were used for peptide elution. For MOAC samples, a gradient of 5-40% buffer B (95% acetonitrile/1% acetic acid) in 135 min and 5 min wash with 100% buffer B followed by 30 min of re-equilibration with buffer A (2% acetonitrile/1% acetic acid) was used. For pY-IP samples a 5-40% gradient with buffer B was achieved in 75 min followed by 5 min wash with buffer B and 30 min re-equilibration. Flow rate was ~0.3 µl/min. Peptides were directly introduced into the mass spectrometer using a nano-spray source. Orbitrap was set to collect 1 MS scan between 400-2000 m/z (resolution of 30,000 @ 400 m/z) in orbitrap followed by data dependent CID spectra on top 9 ions in LTQ (normalized collision energy ~35%). Dynamic exclusion was set to 2 MS/MS acquisitions followed by exclusion of the same precursor ion for 2 min. Maximum ion injection times were set to 300 ms for MS and 100 ms for MS/MS. Automatic Gain Control (AGC) was set to 1xe6 for MS and 5000 for MS/MS. Charge state screening was enabled to discard +1 and unassigned charge states. Technical duplicate data for each of the MOAC eluents (ammonium hydroxide and pyrrolidine) and triplicate data for the pY-IP eluents were acquired.

Bioinformatics analysis RAW files were converted to mzXML using msconvert and searched against the Swissprot Human taxonomic protein database (2013Jan09 release) appended with common proteomics contaminants and reverse sequences as decoys. Searches were performed with X!Tandem (version 2010.10.01.1) using the k-score plugin. For all searches the following parameters were used: A parent monoisotopic mass error window of 50 ppm was used. The fragment ion error window was 0.8 Da. Searches were performed allowing for up to 2 missed tryptic cleavages. Potential modifications of oxidation of

Methionine (+15.9949@M), carbamidomethylation of Cysteine (+57.0214@C), and phosphorylation of Serine, Threonine, and Tyrosine (+79.9663@[STY]) residues were allowed. The search results were then post-processed using PeptideProphet and ProteinProphet.

Mass spectral counts delivered through the Trans-Proteomic Pipeline (TPP) phosphoproteomic analyses for each cell line using the spectral counting software ABACUS. Tyrosine-enrichment data were processed through ABACUS separately from the Serine and Threonine data. ABACUS results were filtered to only retain those proteins with a ProteinProphet probability greater than 0.7. Only peptides with a probability greater than 0.7 and containing a phosphorylation on a serine, threonine or tyrosine were considered for spectral counting. For the tyrosine enrichment data no decoy proteins were reported when using these ABACUS parameters. Label-free spectral counting was used from the ABACUS output in all further analysis to quantify the relative abundance of phosphorylated peptides/proteins. The proteins reported by ABACUS were then manually curated to select the best representative protein identifier from among ambiguous cases. Phospho-site localization was performed with an in-house reimplementation of the Ascore algorithm as described (see, e.g., Beausoleil, S. A., et al. 2006 Nat Biotechnol 24, 1285-1292; herein incorporated by reference in its entirety). Recurrent phosphorylation site motifs were extracted with the Motif-X algorithm. Sequences were uploaded with the phosphorylated residue at the central position and six amino acids on each side. The minimum motif occurrences was set to 20, with a $p< 10\text{-}5$ required to be rated as significant.

High-throughput and Sanger DNA sequencing For WGS, 7-10 μg of high-molecular-weight genomic DNA was extracted from fresh frozen tumor tissue and subjected to WGS by Complete Genomics, Inc. (CGI; Mountain View, CA). CGI performs massively parallel short-read sequencing using a combinatorial probe-anchor ligation (cPAL) chemistry coupled with a patterned nanoarray-based platform of self-assembling DNA nanoballs (Drmanac et al., 2010). Library generation, read-mapping to the NCBI reference genome (Build 37, RefSeq Accession nos. CM000663-CM00686), local *de novo* assembly and variant-calling protocols were performed as previously described (Drmanac et al., 2010; Roach et al., 2010). Initial read mapping and variant calling were performed using CGAtools v1.3.0 (http://cgatools.sourceforge.net/docs/1.3.0/). Additional downstream bioinformatic analyses of WGS data were performed using custom designed processing routines. WGS yielded a mean of 351±13Gb mapped per sample with 97.4-97.8% fully called genome

fraction and 97.1-97.7% fully called exome fraction. The median genomic sequencing depth exceeded 60x in all samples normalized across the entire genome. Three of the 4 index T-PLL cases harbored the characteristic inv(14) alteration as anticipated based on clinical karyotyping and confirmatory FISH results and the fourth harbored the less common t(X;14) lesion (Figures 2 and 3).

For WES, genomic DNA samples were fragmented using a Covaris S2 fragmentation system to a target size of 400bp. The samples were end-repaired, a-tailed, and custom adapters were ligated using the NEBNext ® DNA Library Prep kit according to the manufacturers recommended protocols. The custom adapters included 6bp barcodes and synthesized by Integrated DNA Technologies (IDT). After ligation, the samples were size selected to 400bp on a 2% agarose gel and 1mm gel slices were retained. Samples were isolated from the gel using the Qiagen QIAquick gel extraction system. Seven microliters of each ligation product was enriched using the Phusion master mix kit and custom PCR primers with a total of 14 cycles of PCR amplification. Two PCR reactions were performed for each sample. The PCR products were pooled and purified using AmpureXP® beads.

Library QC was performed using the Agilent Tapestation and qPCR. Based on qPCR concentrations, 200ng of each of five samples were pooled for a total of three pools. Each pool was captured using the Nimblegen SeqCap EZ V3 Exome Enrichment Kit according to the manufacturer's recommended protocols. The capture pools were combined and sequenced on the Illumina HiSeq 2000 platform across four lanes with paired-end 100bp reads. The average depth of coverage for exome sequencing was 30.4±11.9x with greater than 95% of coding exons sequenced to a depth of 30 or more.

The analysis focused on variations that were not in the Database of SNPs (dbSNP) and/or those that had previously been associated with cancer based on information in the Catalogue of Online Somatic Mutations in Cancer (COSMIC) database. After identifying candidate mutations of interest, 10-50ng of genomic DNA from tumor and, where available, matched constitutional tissue or otherwise tumor-depleted, matched normal DNA was subjected to Sanger sequencing. Where available, tumor-depleted matched normal DNA was also subjected to WES to identify somatic alterations in T-PLL. For all such sequencing reactions, PCR amplification was performed using Phusion DNA polymerase (New England Biolabs) followed by conventional Sanger sequencing technology using BigDye version 3.1 chemistry run on an Applied Biosystems 3730xl DNA Sequencer at the University of Michigan DNA sequencing Core. All sequencing reactions were performed using nested sequencing primers. Sequencing trace analysis was performed using Mutation Surveyor

software. All primers were designed using a custom-developed program and purchased from IDT lyophilized in 96-well plates.

Bioinformatic processing of WES data WES FASTQ sequencing data files were aligned to human NCBI Build 36 reference sequence using BWA 0.6.2. Merging and deduplication was performed using Picard 1.79. The DepthOfCoverage, CountReads, RealignerTargetCreator, IndelRealigner, BaseRecalibrator, PrintReads and UnifiedGenotyper functions within GenomeAnalysisTK-1.6-9 were used to ascertain coverage and for variant calling. Resultant VCF files were annotated using snpEff v3.1. Variants previously identified as somatic as presented in COSMIC were highlighted using a custom-designed algorithm.

Array CGH Copy-number variants (CNVs) were detected using Nimblegen whole genome arrays containing 270,000 features (Roche Applied Science) sufficient to detect CNVs of 50kb or greater. Arrays were prepared according to manufacturer's protocol and analyzed on NimbleGen MS 200 Scanner followed by data extraction, normalization and processing for segMNT analysis using NimbleScan software according to manufacturer's instructions. Downstream data processing and interpretation was performed using custom designed processing algorithms solely reliant on the data comprising output segMNT data files. Circos plots were generated to display the segMNT data.

Mutation Analysis JAK1 and STAT5B mutants were made using site-directed mutagenesis PCR. Wild-type and mutant genes were cloned into the pCAGGS mammalian expression vector. The mutations were confirmed by Sanger sequencing of the plasmid DNA. For STAT5B reporter assays, HeLa cells were plated in 24 well plates and transiently transfected with either of pCAGGS STAT5B or JAK1 plasmids (400 ng/well) along with pGL4.52 (Luc2P/STAT5RE/Hygro) (Promega, 400 ng/well) using PolyJet (Signagen, 2.4 µl/well). After 36 hours, the cells were lysed and One-Glo luciferase detection reagent (Promega) was used to determine luciferase activity according to the manufacturer's recommendations. The lysates from the assay were used for determining pSTAT5B levels by Western blotting. STAT5B wild-type and mutants were subcloned in pLVX Ac GFP1 lentiviral mammalian expression vector (Clontech). Viral particles were generated by co-transfecting PMD2.G, psPAX2 packaging plasmids. Targeted cells were transduced with virus for 48hrs, followed by selection with puromycin (2 g/mL) for 2 days. The selected

cells were then tested for the expression of genes by Western blotting. These cells were then used for WST (Roche) and colony forming cells assay using MethoCult soft agar (StemCell Technologies) as per manufacturer's protocol.

Immunocytochemistry Cultured suspension cells were deposited onto glass slides by cytocentrifugation. Cells were fixed and permeabilized with methanol. After fixation, cells were first incubated with Y694/Y699 phospho-STAT5 rabbit monoclonal antibody (D47E7, Cell Signaling) followed by Alexa-conjugated donkey anti-rabbit IgG (Alexa594, Life Technologies). Coverslips were mounted on standard slides with mounting media supplemented with 4', 6'-diamidino-2-phenylindole (DAPI). The images were captured and recorded using an Olympus BX-51 upright light microscope equipped with an Olympus DP-70 camera.

Primary Cell Culture and Pimozide Treatment Cell lines and primary cells were thawed and incubated overnight at 37°C in cell culture medium (RPMI 1640 with 20% heat-inactivated fetal bovine serum, glutamine and antibiotics) followed by treatment with Pimozide (BioVision, diluted in DMSO vehicle), a specific STAT5 inhibitor, at a final concentration of 10-20 μM. Cells were harvested at different time points (4-12h), lysed in Laemmli buffer and incubated at 95°C for 10 min. Protein lysates (20μg of protein) were separated by SDS-PAGE electrophoresis, transferred to a PVDF membrane and probed with specific primary antibodies including The anti-pSTAT5 (1:1000 dilution), anti-STAT5 (1:1000 dilution) and anti-PARP (1:1000 dilution) rabbit antibodies (Cell Signaling Technology). Human leukemic T-cell line HH and Mac-2a were used as negative and positive controls, respectively. A trypan blue exclusion assay was also carried out in triplicate to monitor the cell viability over time.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the relevant fields are intended to be within the scope of the present invention.

INCORPORATION BY REFERENCE

The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

CLAIMS

1. A method for detecting one or more JAK/STAT pathway variants associated with a mature T-cell leukemia in a subject, comprising:

a) contacting a sample from a subject with a JAK/STAT pathway variant detection assay under conditions that the presence of a JAK/STAT pathway variant associated with a mature T-cell leukemia is determined; and

b) diagnosing said subject with a mature T-cell leukemia when one or more of said JAK/STAT pathway variants are present in said sample.

2. The method of claim 1, wherein said one or more JAK/STAT pathway variants encodes a loss of function mutation and/or a gain of function mutation.

3. The method of claim 1, wherein said subject is a human patient.

4. The method of claim 1, wherein said JAK/STAT pathway variant is one or more variants selected from JAK1, JAK3, STAT5B, and IL2RG.

5. The method of claim 4, wherein said JAK1 variant is one or more JAK1 mutations selected from the group consisting of JAK1 p.F636L, JAK1 p.G646C, JAK1 p. Y654F, JAK1 p.V658F, JAK1 p.S703I, and JAK1 p.T901R.

6. The method of claim 4, wherein said JAK3 variant is one or more JAK3 mutations selected from the group consisting of JAK3 p.ΔKNC563, JAK3 p.M511I, JAK3 p. A573V, JAK3 p.R657, JAK3 p.G662W, JAK3 p.P664T, JAK3 p.Y980, JAK3 p. Y981, and JAK3 p.S989I.

7. The method of claim 4, wherein said STAT5B variant is one or more STAT5B mutations selected from the group consisting of STAT5B p.T628S, STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.N642H, STAT5B p.Y699, and STAT5B p.Y665H.

8. The method of claim 4, wherein said IL2RG variant is one or more IL2RG mutations selected from the group consisting of IL2RG p.ΔGSM268, IL2RG p.Y325, and IL2RG p. K315E.

9. The method of claim 1, wherein said mature T-cell leukemia is T-cell prolymphocytic leukemia.

10. The method of claim 9, wherein one or more JAK/STAT pathway variants are selected from the group consisting of JAK1 p.V658F, JAK1 p.S703I, JAK1 p.T901R, JAK3 p.ΔKNC563, JAK3 p.M511I, JAK3 p. A573V, JAK3 p.R657, STAT5B p.T628S, STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.N642H, STAT5B p.Y665H, IL2RG p.ΔGSM268, and IL2RG p. K315E.

11. The method of claim 1, wherein said mature T-cell leukemia is Sezary syndrome.

12. The method of claim 11, wherein one or more JAK/STAT pathway variants are selected from the group consisting of JAK1 p. Y654F, JAK3 p. A573V, JAK3 p.Y980, JAK3 p. Y981, JAK3 p.S989I, STAT5B p.Y699, STAT5B p.N642H, and Il2RG p.Y325.

13. The method of claim 1, wherein said determining comprises detecting variant JAK1, JAK3, STAT5B, and IL2RG nucleic acids or polypeptides.

14. The method of claim 13, wherein said detecting variant JAK1, JAK3, STAT5B, and IL2RG nucleic acids comprises one or more nucleic acid detection method selected from the group consisting of sequencing, amplification and hybridization.

15. The method of Claim 1, wherein said biological sample is selected from the group consisting of a tissue sample, a cell sample, and a blood sample.

16. The method of claim 1, wherein said determining comprises a computer implemented method.

17. The method of claim 16, wherein said computer implemented method comprises analyzing JAK1, JAK3, STAT5B, and IL2RG variant information and displaying said information to a user.

18. The method of claim 1, further comprising the step of treating said subject for a mature T-cell leukemia and monitoring said subject for the presence of JAK1, JAK3, STAT5B, and IL2RG variants associated with said mature T-cell leukemia.

19. The method of claim 1, further comprising the step of treating said subject for a mature T-cell leukemia under condition such that at least one symptom of said mature T-cell leukemia is diminished or eliminated.

20. The method of claim 19, wherein said treating comprises inhibiting JAK1, JAK3, STAT5B, and/or IL2RG expression and/or activity.

21. The method of claim 20, wherein said inhibiting STAT5B expression and/or activity is accomplished through administration of an agent configured to inhibit STAT5B expression, wherein the agent configured to inhibit STAT5B expression is pimozide.

22. The method of claim, wherein said inhibiting JAK1 and/or JAK3 expression and/or activity is accomplished through administration of an agent configured to inhibit JAK1 and/or JAK3 expression and/or activity, wherein said agent is selected from the group consisting of ruxolitinib, tofacitinib, baricitinib, CYT387, and lestaurtinib.

23. The method of claim 21, further comprising administering one or more agents for treating a mature T-cell leukemia.

24. The method of claim 23, wherein said one or more agents is selected from the group consisting of a purine analog (e.g., pentostatin, fludarabine, cladrbine), chlorambucil, cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP), cyclophosphamide, vincristine, prednisone (COP), and vincristine, doxorubicin, prednisone, etoposide, cyclophosphamide, bleomycin, alemtuzumab, and vorinostat.

25. The method of claim 1, further comprising the step of detecting a variant in one or more additional genes associated with a mature T-cell leukemia.

26. The method of claim 25, wherein said one or more genes are selected from the group consisting of CHEK2, EZH2, and FBXW10.

27. Use of a variant JAK/STAT pathway nucleic acid or polypeptide for detecting a mature T-cell leukemia in a subject.

28. The use of claim 27, wherein said JAK/STAT pathway variant encodes a loss of function mutation and/or a gain of function mutuation.

29. The use of claim 27, wherein said subject is a human subject.

30. The use of claim 27, wherein said JAK/STAT pathway variant is one or more variants selected from JAK1, JAK3, STAT5B, and IL2RG.

31. The use of claim 30, wherein said JAK1 variant is one or more JAK1 mutations selected from the group consisting of JAK1 p.F636L, JAK1 p.G646C, JAK1 p. Y654F, JAK1 p.V658F, JAK1 p.S703I, and JAK1 p.T901R.

32. The use of claim 30, wherein said JAK3 variant is one or more JAK3 mutations selected from the group consisting of JAK3 p.ΔKNC563, JAK3 p.M511I, JAK3 p. A573V, JAK3 p.R657. JAK3 p.Y980, JAK3 p.G662W, JAK3 p.P664T, JAK3 p. Y981, and JAK3 p.S989I.

33. The use of claim 30, wherein said STAT5B variant is one or more STAT5B mutations selected from the group consisting of STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.T628S, STAT5B p.N642H, STAT5B p.Y699, and STAT5B p.Y665H.

34. The use of claim 30, wherein said IL2RG variant is one or more IL2RG mutations selected from the group consisting of IL2RG p.ΔGSM268, IL2RG p.Y325, and IL2RG p. K315E.

35. The use of claim 27, wherein said mature T-cell leukemia is T-cell prolymphocytic leukemia.

36. The use of claim 35, wherein one or more JAK/STAT pathway variants are selected from the group consisting of JAK1 p.V658F, JAK1 p.S703I, JAK1 p.T901R, JAK3 p.ΔKNC563, JAK3 p.M511I, JAK3 p. A573V, JAK3 p.R657, STAT5B p.T628S, STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.N642H, STAT5B p.Y665H, IL2RG p.ΔGSM268, and IL2RG p. K315E.

37. The use of claim 27, wherein said mature T-cell leukemia is Sezary syndrome.

38. The use of claim 37, wherein one or more JAK/STAT pathway variants are selected from the group consisting of JAK1 p. Y654F, JAK3 p. A573V, JAK3 p.Y980, JAK3 p. Y981, JAK3 p.S989I, STAT5B p.Y699, STAT5B p.N642H, and Il2RG p.Y325.

39. The use of claim 27, wherein said determining comprises detecting variant JAK1, JAK3, STAT5B, and IL2RG nucleic acids or polypeptides.

40. The use of claim 39, wherein said detecting variant JAK1, JAK3, STAT5B, and IL2RG nucleic acids comprises one or more nucleic acid detection method selected from the group consisting of sequencing, amplification and hybridization.

41. A method of determining a decreased time to adverse outcome in a subject diagnosed with a mature T-cell leukemia, comprising:

a) contacting a sample from a subject with a JAK/STAT pathway variant detection assay under conditions that the presence of a JAK/STAT pathway variant associated with a mature T-cell leukemia is determined; and

b) detecting a decreased time to adverse outcome in said subject when said JAK/STAT pathway variants are present in said sample.

42. The method of claim 41, wherein said adverse outcome is selected from the group consisting of relapse of said mature T-cell leukemia, metastasis, or death.

43. The method of claim 41, wherein said JAK/STAT pathway variant encodes a loss of function mutation and/or a gain of function mutuation.

44. The method of claim 41, wherein said subject is a human subject.

45. The method of claim 41, wherein said JAK/STAT pathway variant is one or more variants selected from JAK1, JAK3, STAT5B, and IL2RG.

46. The method of claim 45, wherein said JAK1 variant is one or more JAK1 mutations selected from the group consisting of JAK1 p.F636L, JAK1 p.G646C, JAK1 p. Y654F, JAK1 p.V658F, JAK1 p.G662W, JAK1 p.P664T, JAK1 p.S703I, and JAK1 p.T901R.

47. The method of claim 45, wherein said JAK3 variant is one or more JAK3 mutations selected from the group consisting of JAK3 p.ΔKNC563, JAK3 p.M511I, JAK3 p. A573V, JAK3 p.R657. JAK3 p.Y980, JAK3 p. Y981, and JAK3 p.S989I.

48. The method of claim 45, wherein said STAT5B variant is one or more STAT5B mutations selected from the group consisting of STAT5B p.T628S, STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.N642H, STAT5B p.Y699, and STAT5B p.Y665H.

49. The method of claim 45, wherein said IL2RG variant is one or more IL2RG mutations selected from the group consisting of IL2RG p.ΔGSM268, IL2RG p.Y325, and IL2RG p. K315E.

50. The method of claim 41, wherein said mature T-cell leukemia is T-cell prolymphocytic leukemia.

51. The method of claim 50, wherein one or more JAK/STAT pathway variants are selected from the group consisting of JAK1 p.V658F, JAK1 p.S703I, JAK1 p.T901R, JAK3 p.ΔKNC563, JAK3 p.M511I, JAK3 p. A573V, JAK3 p.R657, STAT5B p.T628S, STAT5B p.R659C, STAT5B p.Q706L, STAT5B p.N642H, STAT5B p.Y665H, IL2RG p.ΔGSM268, and IL2RG p. K315E.

52. The method of claim 41, wherein said mature T-cell leukemia is Sezary syndrome.

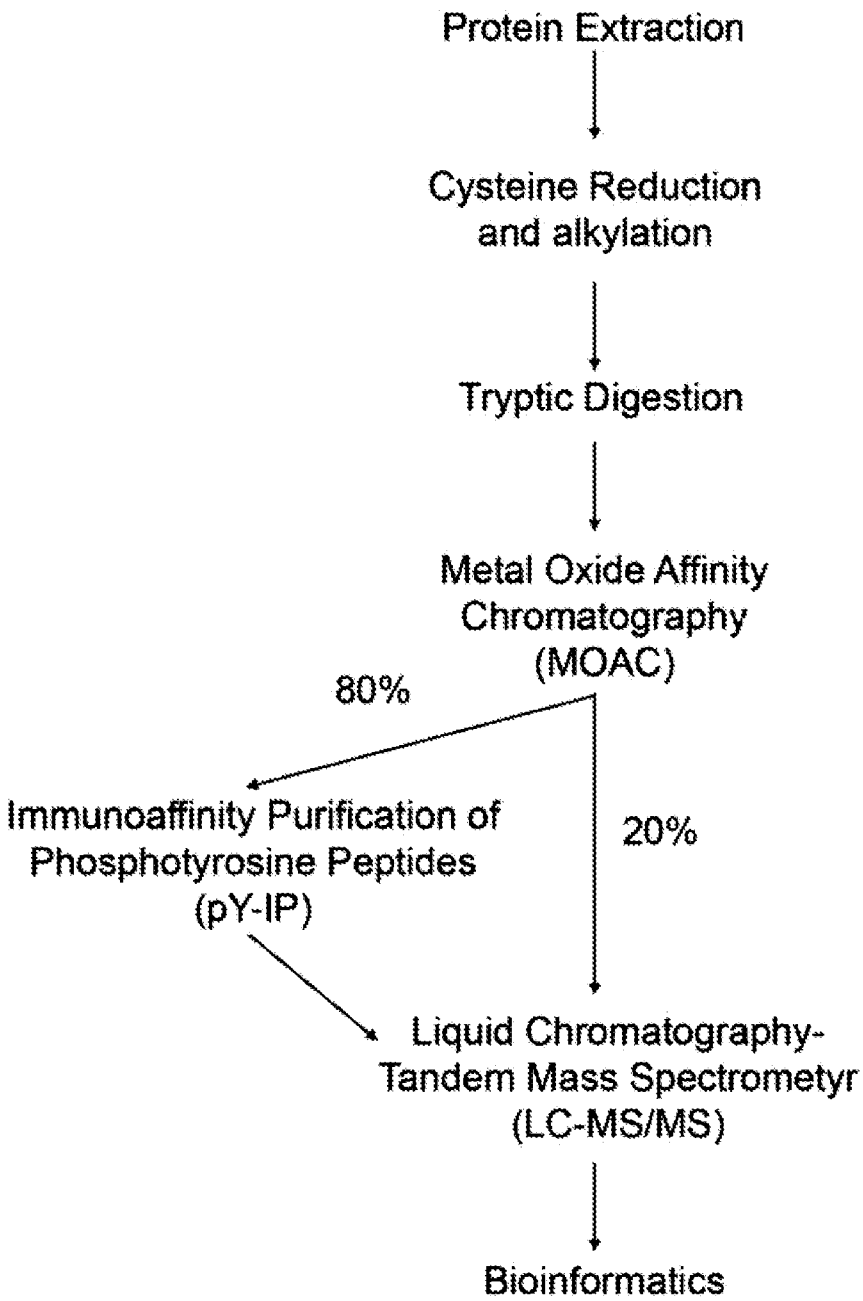
53. The method of claim 52, wherein one or more JAK/STAT pathway variants are selected from the group consisting of JAK1 p. Y654F, JAK3 p. A573V, JAK3 p.Y980, JAK3 p. Y981, JAK3 p.S989I, STAT5B p.Y699, STAT5B p.N642H, and Il2RG p.Y325.

54. The method of claim 41, wherein said determining comprises detecting variant JAK1, JAK3, STAT5B, and IL2RG nucleic acids or polypeptides.

55. The method of claim 54, wherein said detecting variant JAK1, JAK3, STAT5B, and IL2RG nucleic acids comprises one or more nucleic acid detection method selected from the group consisting of sequencing, amplification and hybridization.

FIG. 1

a


Protein Extraction
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Tryptic Digestion
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80%
20%
Immunoaffinity Purification of Phosphotyrosine Peptides (pY-IP)
Liquid Chromatography-Tandem Mass Spectrometyr (LC-MS/MS)
Bioinformatics

FIG. 1 (continued)

b

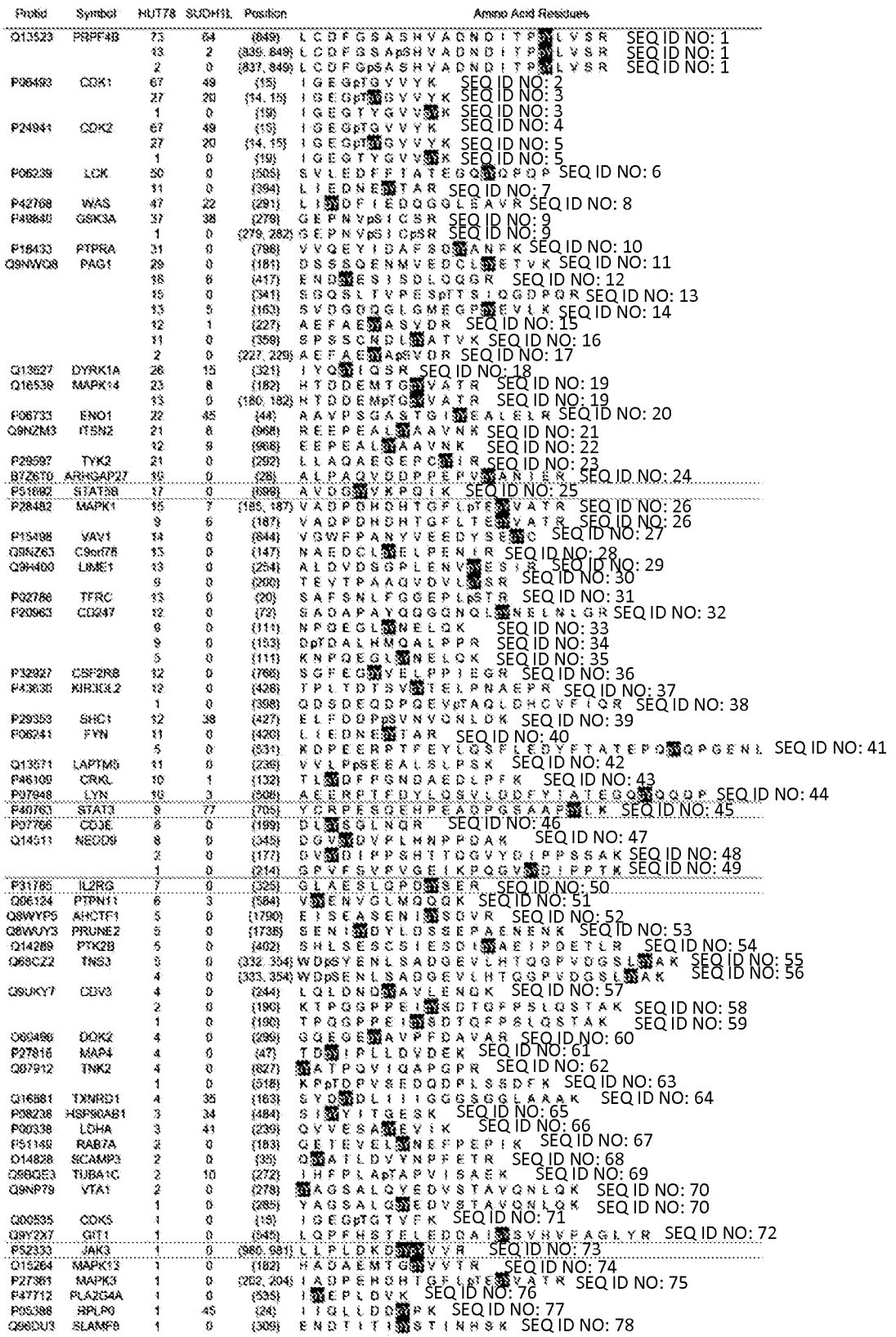

FIG. 2

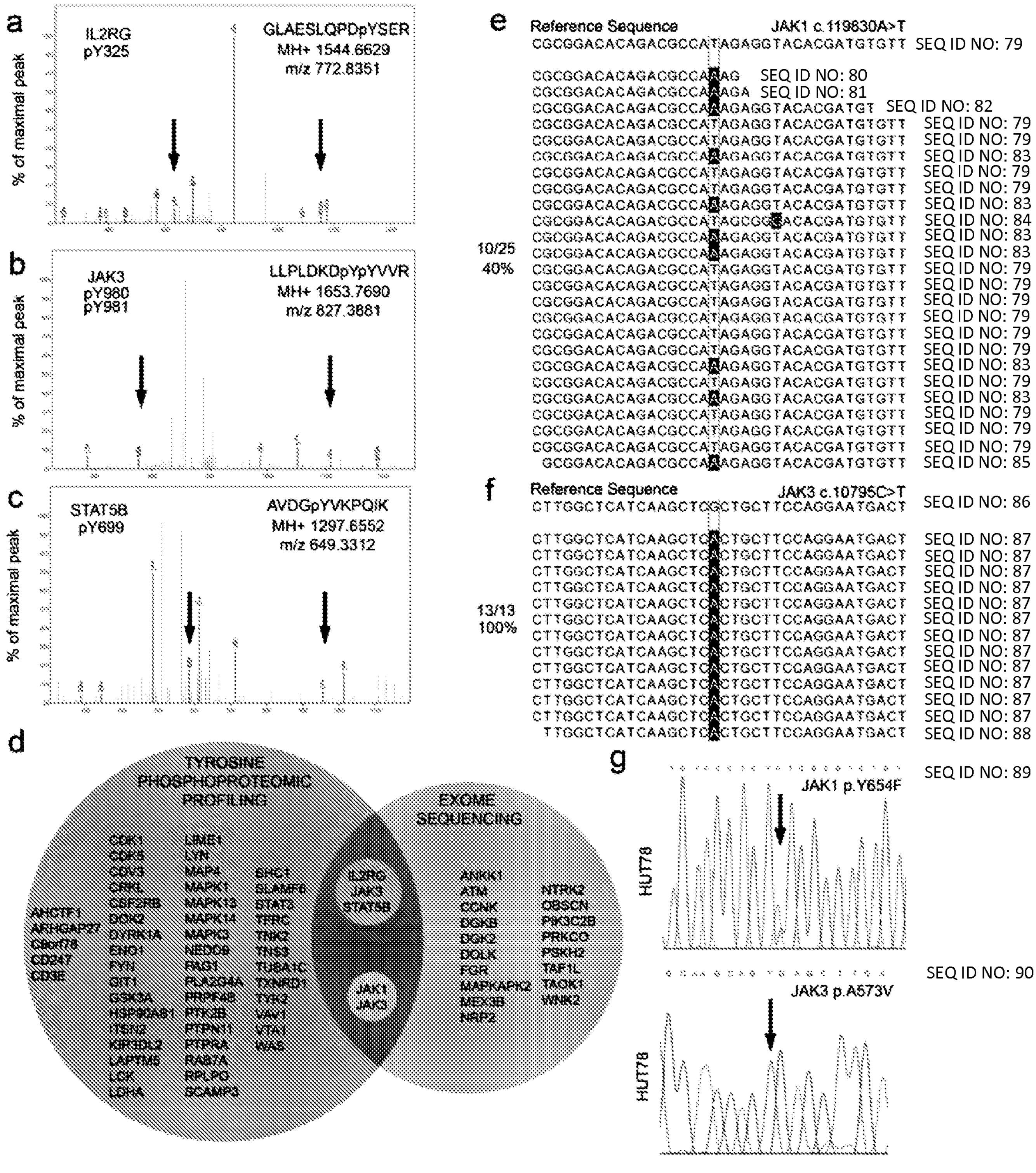

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FIG. 3

a

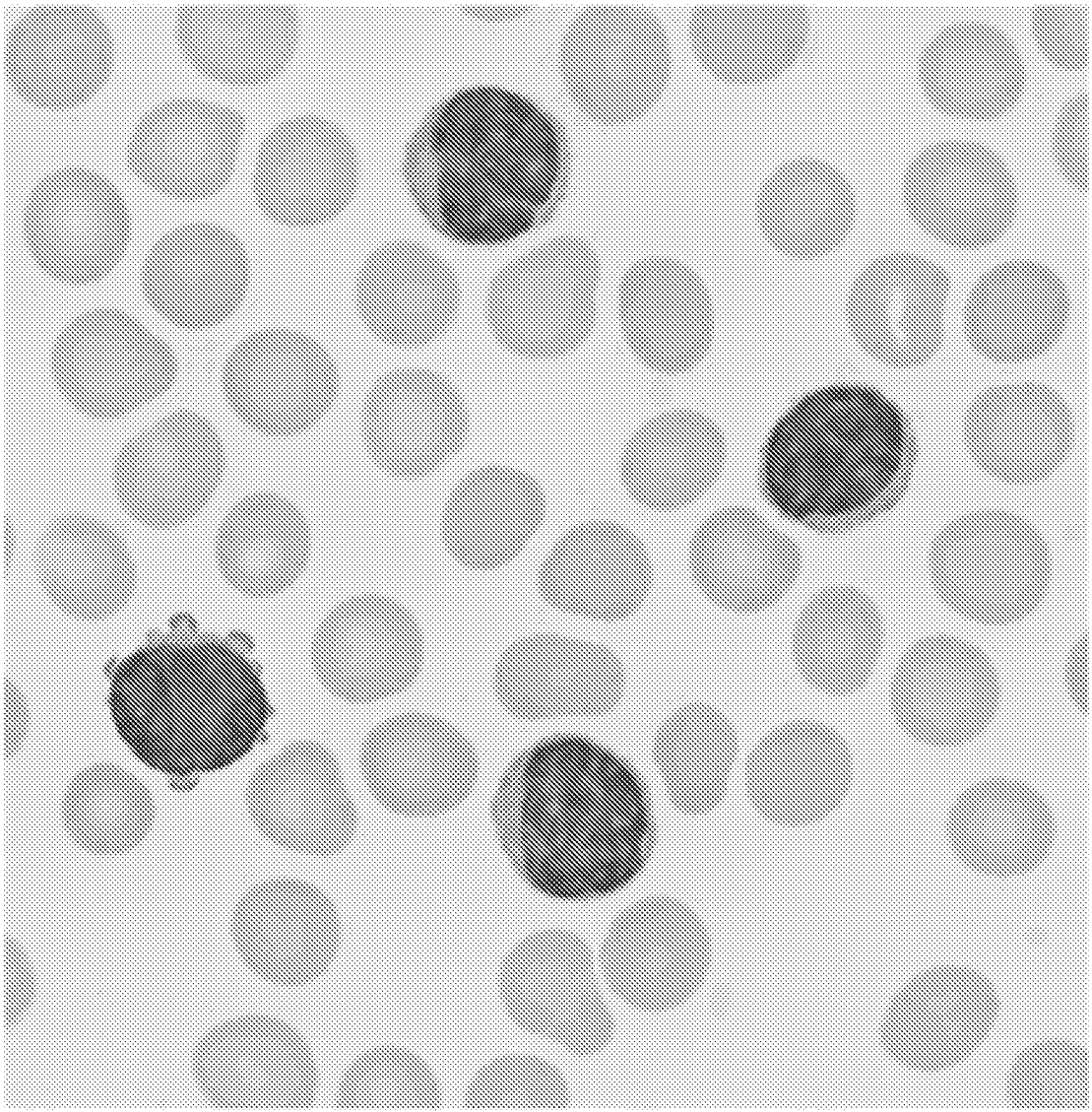


FIG. 3 (continued)

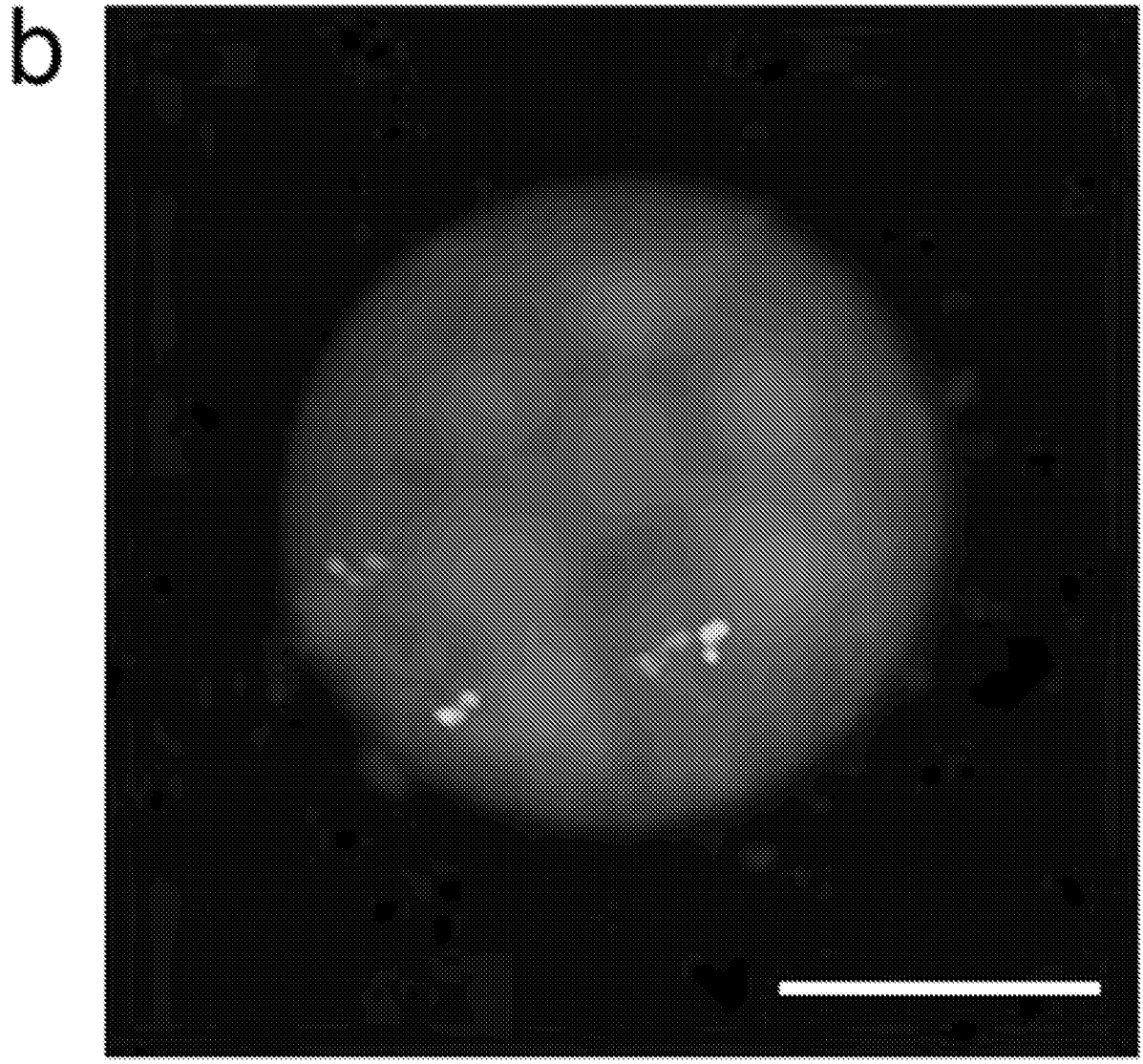
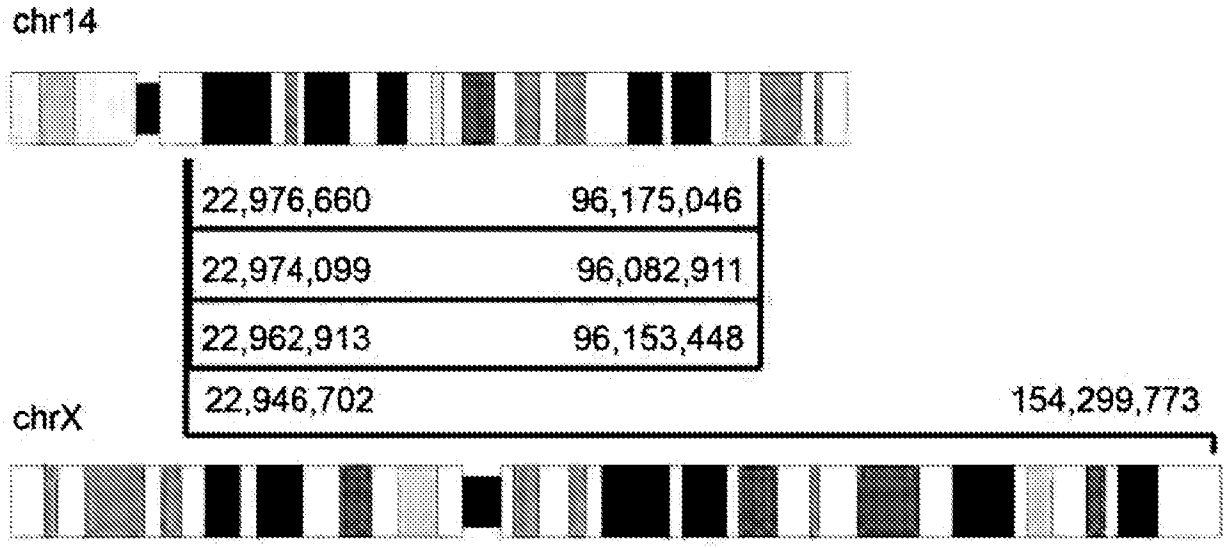


FIG. 4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chrX

FIG. 5

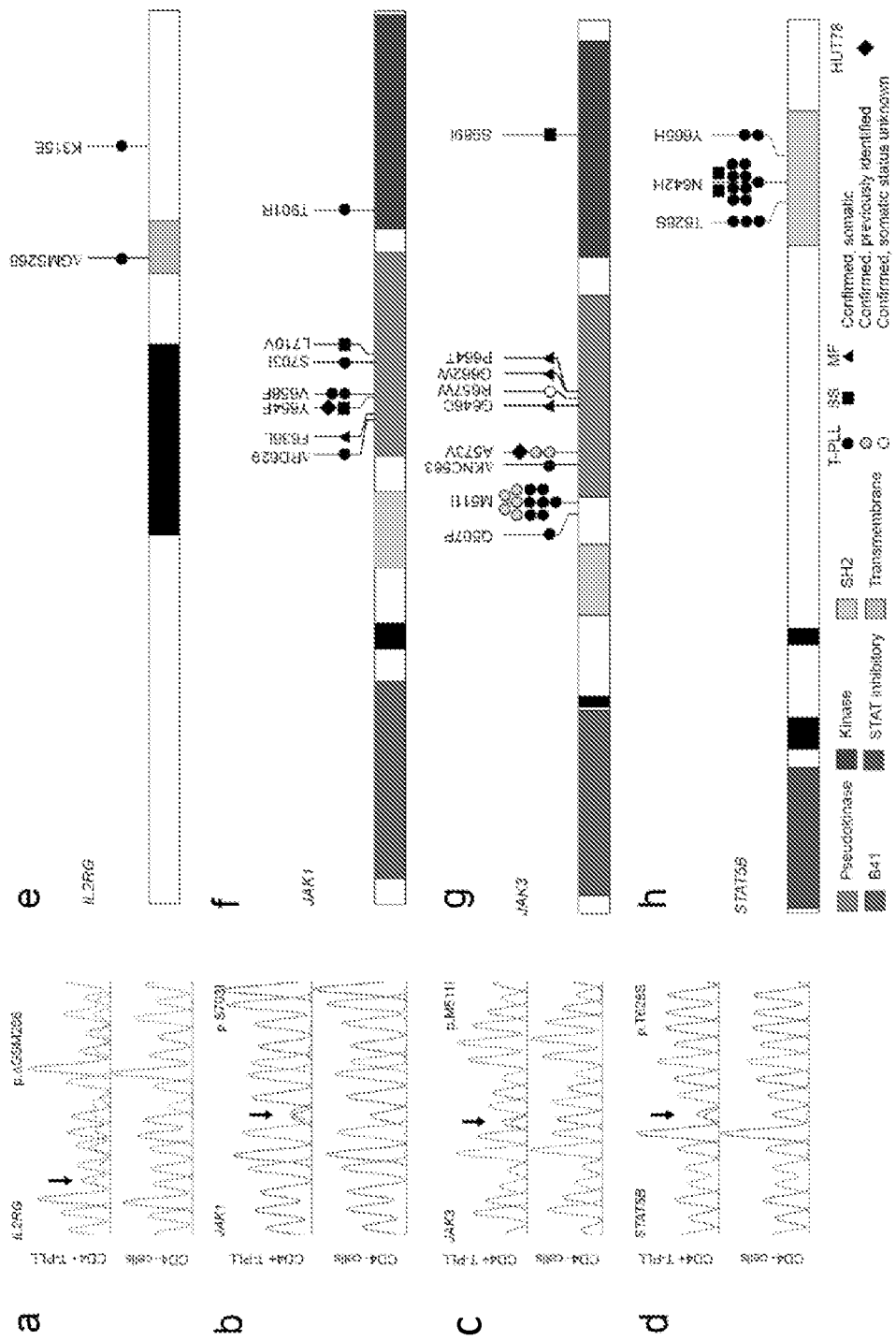
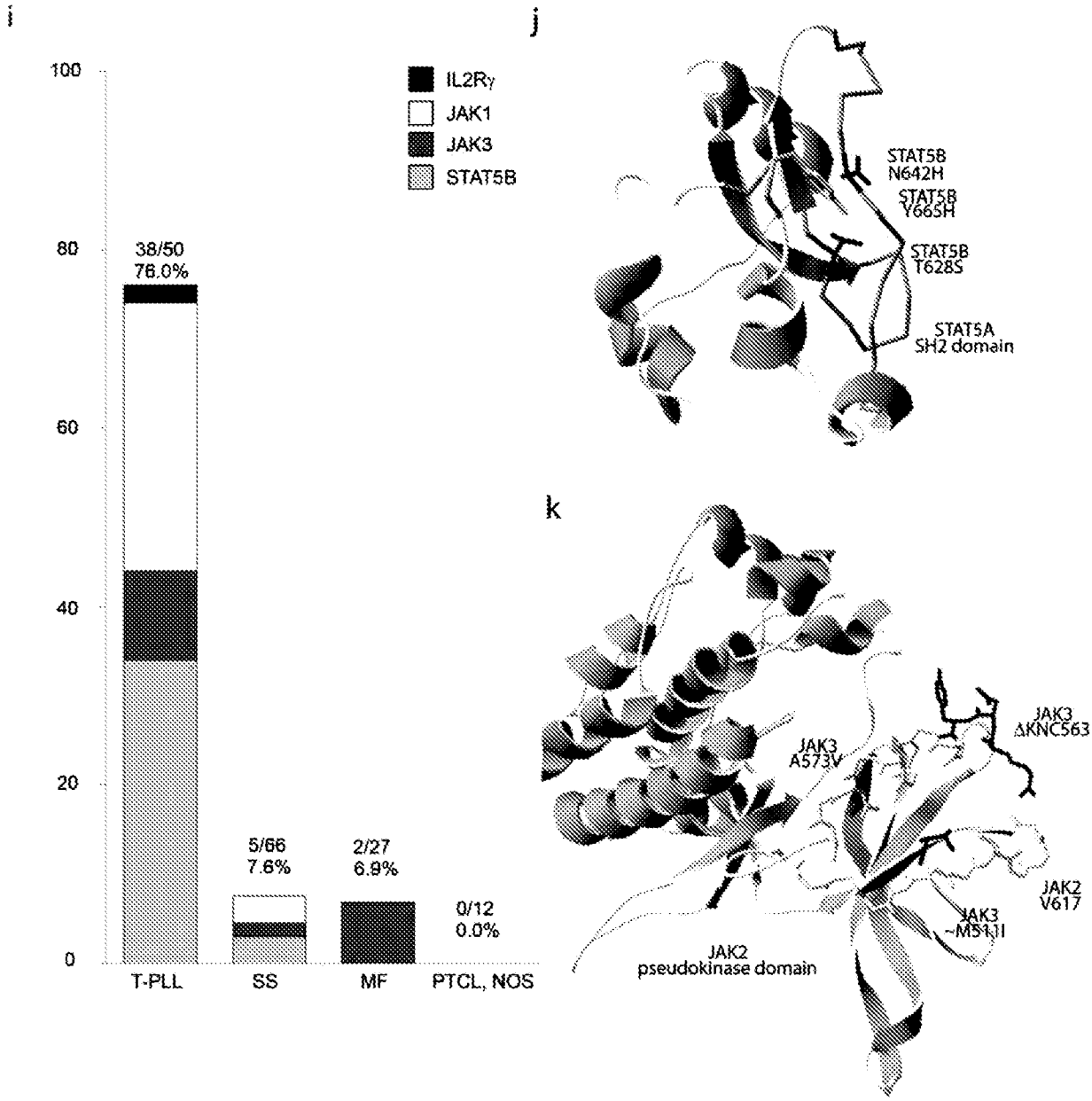

a
b
c
d
e
f
g
h
IL2RG
JAK1
JAK3
STAT5B
CD4+ TPLL
CD4+ cells
K315E
ACGM5266
T901R
L710V
S703I
V564F
V658F
F633L
M511I
A572V
S888I
Y665H
N642H
T628S
HU178
Pseudokinase
Kinase
SH2
Transmembrane
B41
STAT inhibitory
T-PLL
MF
Confirmed, somatic
Confirmed, previously identified
Confirmed, somatic status unknown

FIG. 5 (continued)



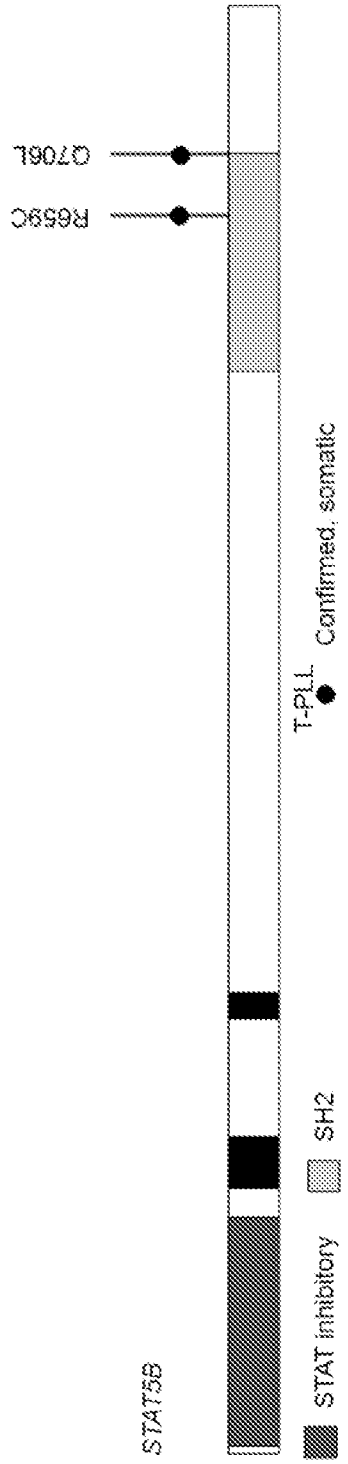
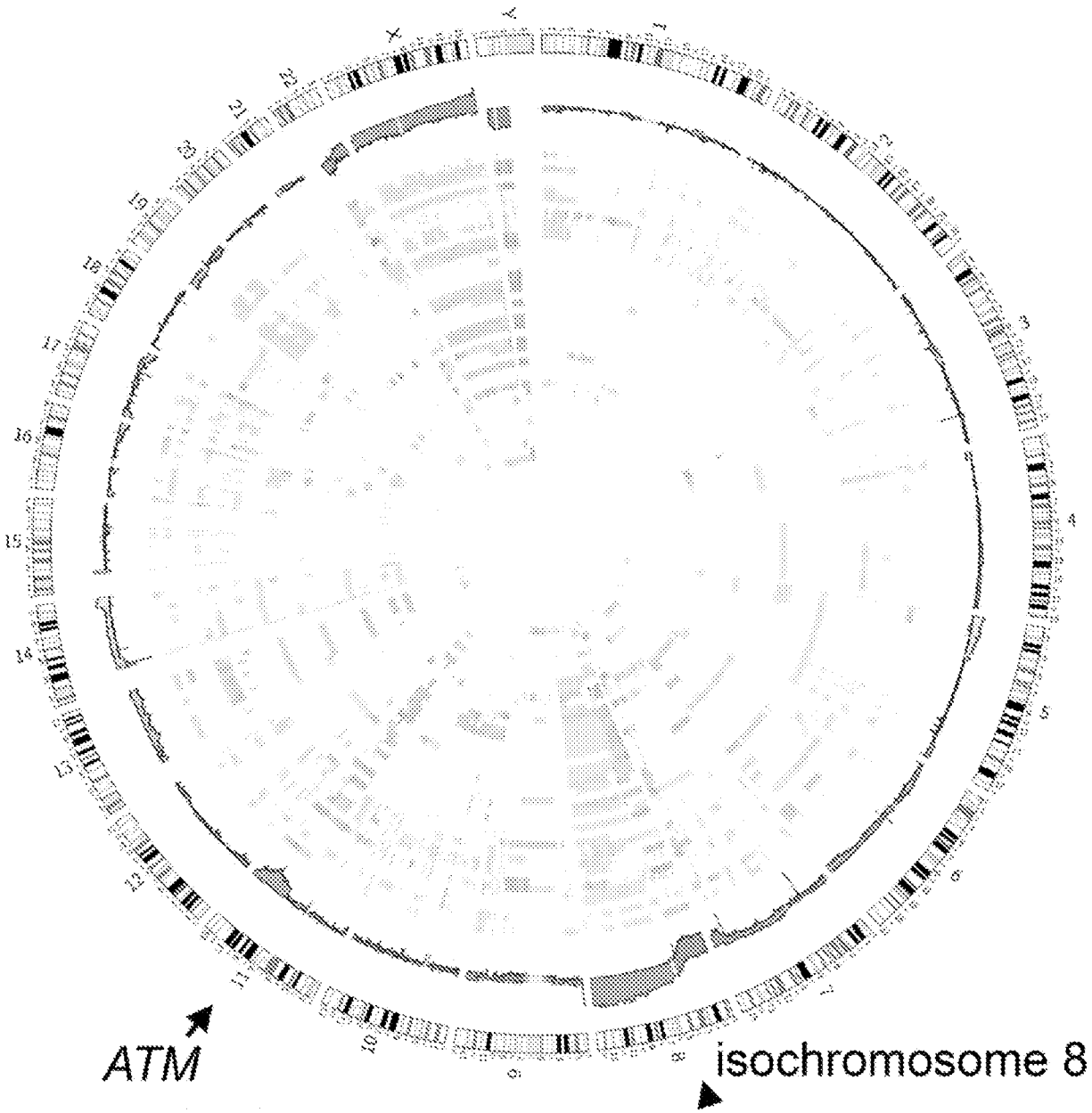
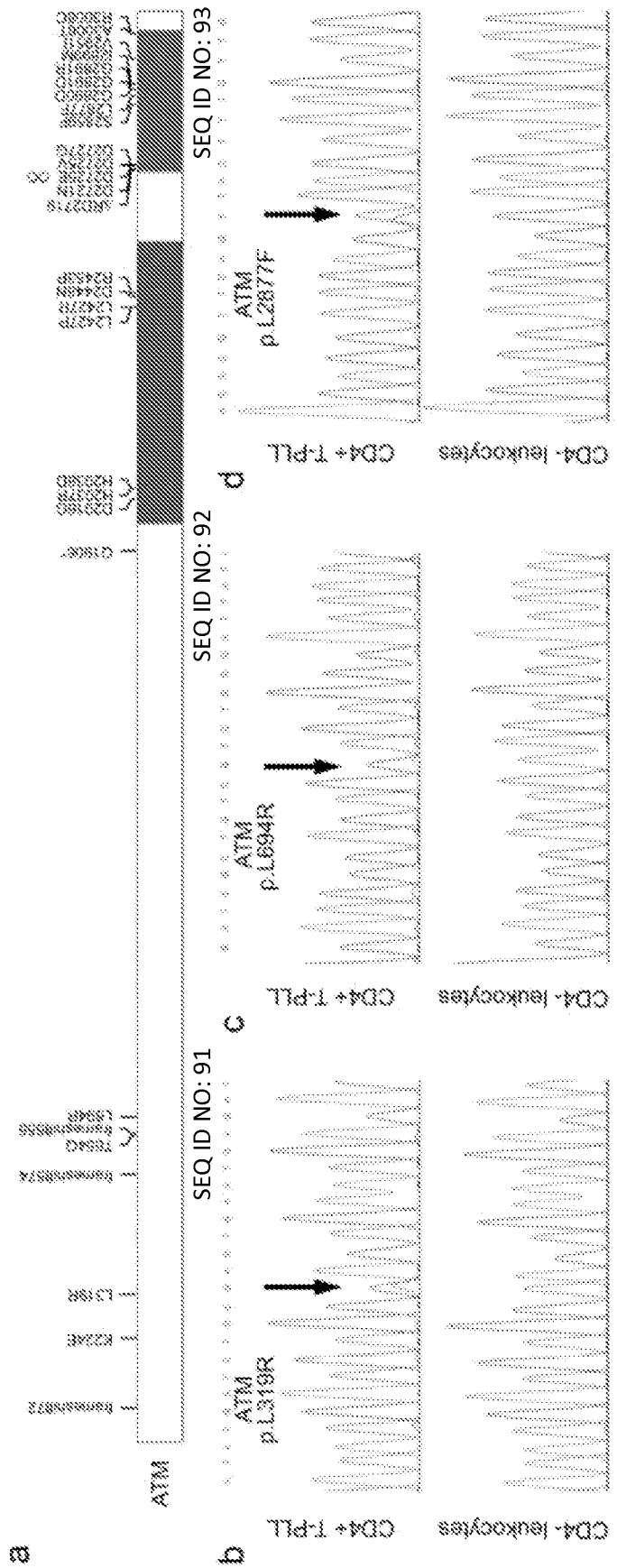

Q706L
R659C
T-PLL
Confirmed, somatic
SH2
STAT inhibitory
STAT5B

FIG. 5L

FIG. 6

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ATM
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SEQ ID NO: 92
SEQ ID NO: 93
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p.L319R
CD4+ T-PLL
CD4- leukocytes
c
ATM
p.L694R
CD4+ T-PLL
CD4- leukocytes
d
ATM
p.L2877F
CD4+ T-PLL
CD4- leukocytes

FIG. 7

FIG. 8

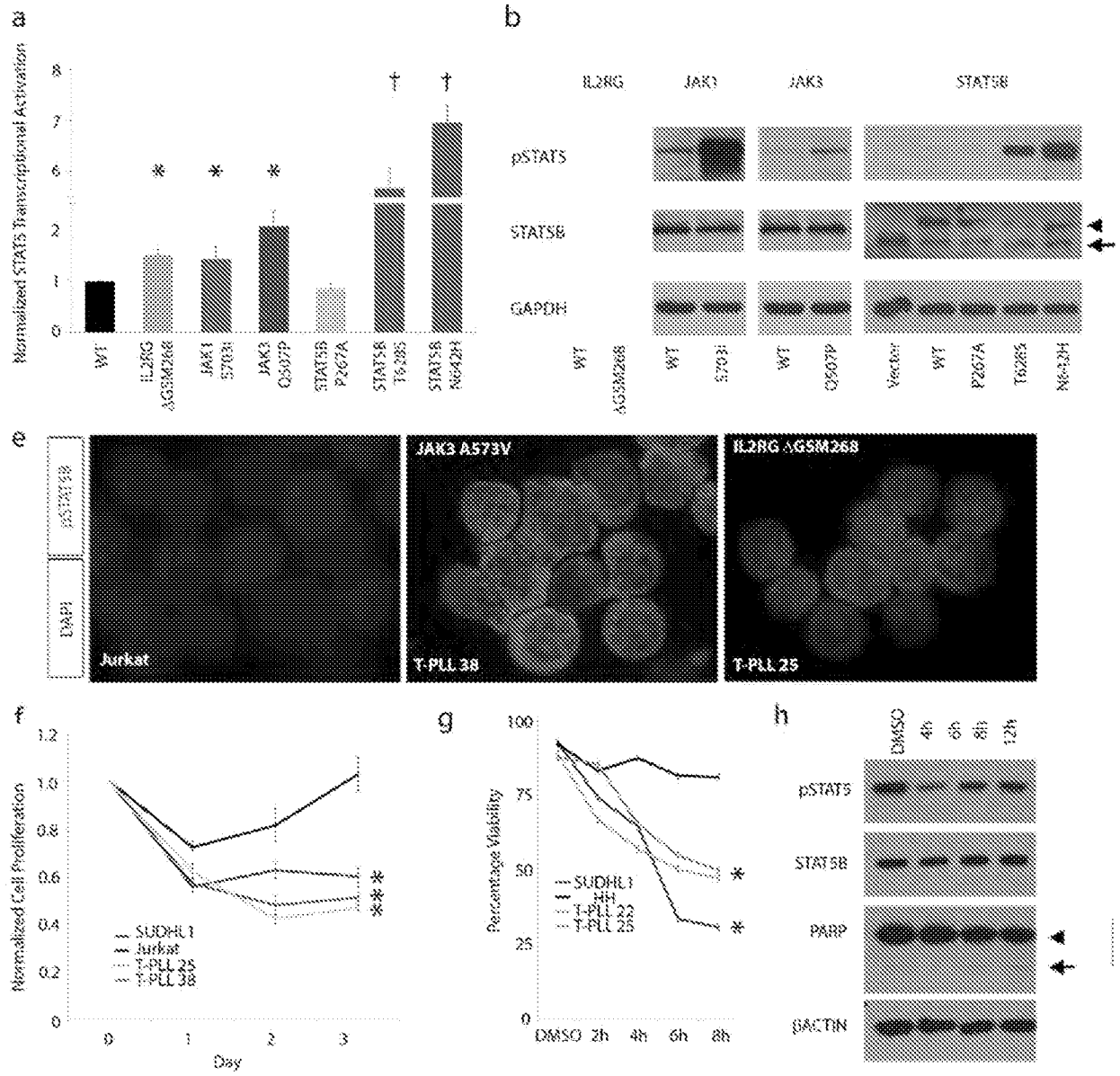
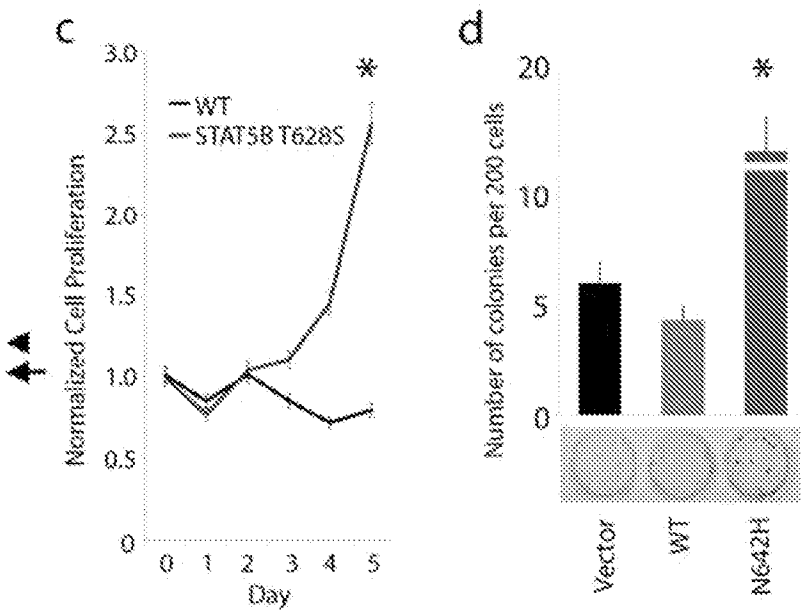

a
Normalized STAT5 Transcriptional Activation
WT
IL2RG ΔG5M268
JAK1 S703I
JAK3 Q507P
STAT5B P267A
STAT5B T628S
STAT5B N642H
b
IL2RG
JAK1
JAK3
STAT5B
pSTAT5
STAT5B
GAPDH
WT
ΔG5M268
S703I
Q507P
Vector
P267A
T628S
N642H
c
pSTAT5B
DAPI
Jurkat
JAK3 A573V
T-PLL 38
IL2RG ΔG5M268
T-PLL 25
f
Normalized Cell Proliferation
SUDHL1
Jurkat
T-PLL 25
T-PLL 38
Day
g
Percentage Viability
SUDHL1
HH
T-PLL 22
T-PLL 25
DMSO 2h 4h 6h 8h
h
DMSO
4h
6h
8h
12h
pSTAT5
STAT5B
PARP
βACTIN

FIG. 8 (continued)



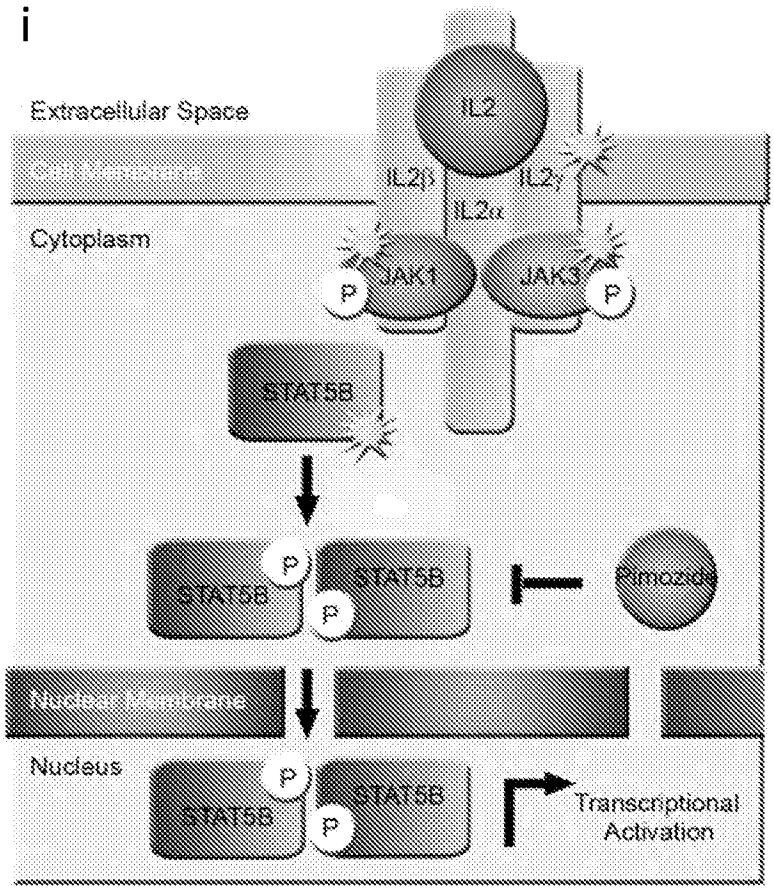


FIG. 9

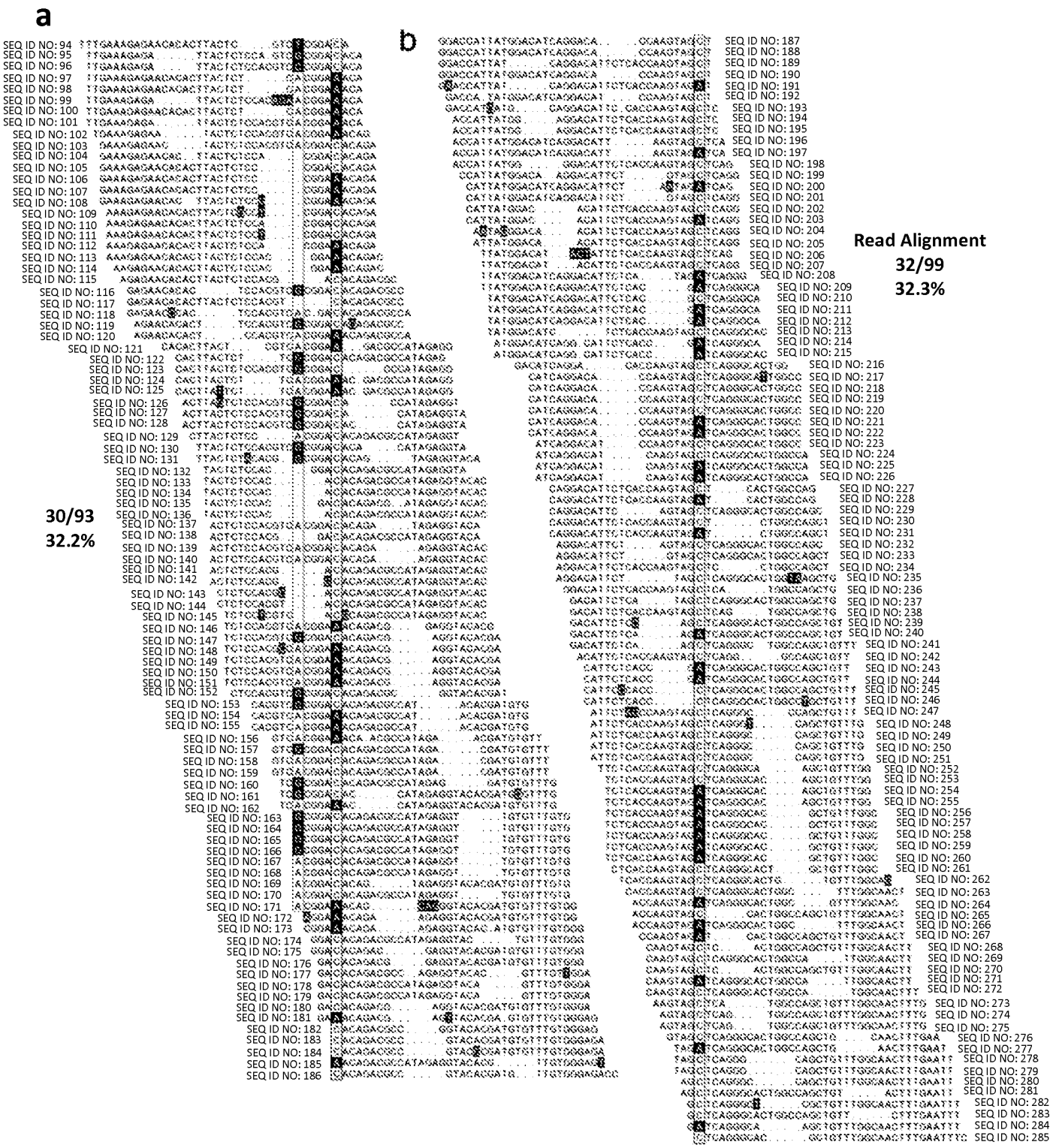


FIG. 10

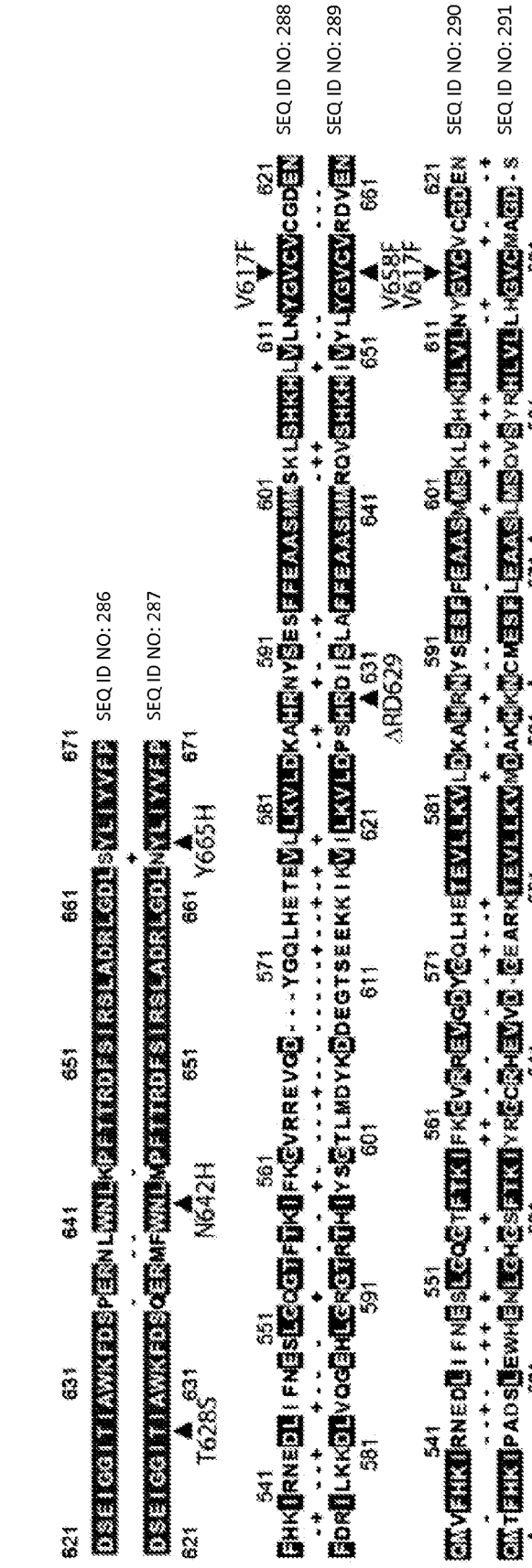

a
STAT5a
STAT5b
SEQ ID NO: 286
SEQ ID NO: 287
T628S
N642H
Y665H
b
JAK2
JAK1
SEQ ID NO: 288
SEQ ID NO: 289
ΔRD629
V617F
V658F
c
JAK2
JAK3
SEQ ID NO: 290
SEQ ID NO: 291
M511I
ΔKNC563
A573V

FIG. 11A

Wild type nucleic acid sequence (homo sapiens) for JAK1

```
   1 tgcagacagt gcgggcctgc gcccagtccc ggctgtcctc gccgcgaccc ctcctcagcc
  61 ctgggcgcgc gcacgctggg gccccgcggg gctggccgcc tagcgagcct gccggtcgac
 121 cccagccagc gcagcgacgg ggcgctgcct ggcccaggcg cacacggaag tgcgcttctc
 181 tgaagtagct ttggaaagta gagaagaaaa tccagtttgc ttcttggaga acactggaca
 241 gctgaataaa tgcagtatct aaatataaaa gaggactgca atgccatggc tttctgtgct
 301 aaaatgagga gctccaagaa gactgaggtg aacctggagg cccctgagcc aggggtggaa
 361 gtgatcttct atctgtcgga cagggagccc ctccggctgg gcagtggaga gtacacagca
 421 gaggaactgt gcatcagggc tgcacaggca tgccgtatct ctcctctttg tcacaacctc
 481 tttgccctgt atgacgagaa caccaagctc tggtatgctc caaatcgcac catcaccgtt
 541 gatgacaaga tgtccctccg gctccactac cggatgaggt tctatttcac caattggcat
 601 ggaaccaacg acaatgagca gtcagtgtgg cgtcattctc caaagaagca gaaaaatggc
 661 tacgagaaaa aaaagattcc agatgcaacc cctctccttg atgccagctc actggagtat
 721 ctgtttgctc agggacagta tgatttggtg aaatgcctgg ctcctattcg agaccccaag
 781 accgagcagg atggacatga tattgagaac gagtgtctag ggatggctgt cctggccatc
 841 tcacactatg ccatgatgaa gaagatgcag ttgccagaac tgcccaagga catcagctac
 901 aagcgatata ttccagaaac attgaataag tccatcagac agaggaacct tctcaccagg
 961 atgcggataa ataatgtttt caaggatttc ctaaaggaat ttaacaacaa gaccatttgt
1021 gacagcagcg tgtccacgca tgacctgaag gtgaaatact tggctacctt ggaaactttg
1081 acaaaacatt acggtgctga aatatttgag acttccatgt tactgatttc atcagaaaat
1141 gagatgaatt ggtttcattc gaatgacggt ggaaacgttc tctactacga agtgatggtg
1201 actgggaatc ttggaatcca gtggaggcat aaaccaaatg ttgtttctgt tgaaaaggaa
1261 aaaaataaac tgaagcggaa aaaactggaa aataaacaca agaaggatga ggagaaaaac
1321 aagatccggg aagagtggaa caatttttct tacttccctg aaatcactca cattgtaata
1381 aaggagtctg tggtcagcat taacaagcag gacaacaaga aaatggaact gaagctctct
1441 tcccacgagg aggccttgtc ctttgtgtcc ctggtagatg gctacttccg gctcacagca
1501 gatgcccatc attacctctg caccgacgtg gccccccgt tgatcgtcca caacatacag
1561 aatggctgtc atggtccaat ctgtacagaa tacgccatca ataaattgcg gcaagaagga
1621 agcgaggagg ggatgtacgt gctgaggtgg agctgcaccg actttgacaa catcctcatg
1681 accgtcacct gctttgagaa gtctgagcag gtgcagggtg cccagaagca gttcaagaac
1741 tttcagatcg aggtgcagaa gggccgctac agtctgcacg gttcggaccg cagcttcccc
1801 agcttgggag acctcatgag ccacctcaag aagcagatcc tgcgcacgga taacatcagc
1861 ttcatgctaa aacgctgctg ccagcccaag ccccgagaaa tctccaacct gctggtggct
1921 actaagaaag cccaggagtg gcagcccgtc taccccatga gccagctgag tttcgatcgg
1981 atcctcaaga aggatctggt gcagggcgag caccttggga gaggcacgag aacacacatc
2041 tattctggga ccctgatgga ttacaaggat gacgaaggaa cttctgaaga gaagaagata
2101 aaagtgatcc tcaaagtctt agaccccagc cacagggata tttccctggc cttcttcgag
2161 gcagccagca tgatgagaca ggtctcccac aaacacatcg tgtacctcta tggcgtctgt
2221 gtccgcgacg tggagaatat catggtggaa gagtttgtgg aaggggtcc tctggatctc
2281 ttcatgcacc ggaaaagcga tgtccttacc acaccatgga aattcaaagt tgccaaacag
2341 ctggccagtg ccctgagcta cttggaggat aaagacctgg tccatggaaa tgtgtgtact
2401 aaaaacctcc tcctggcccg tgagggcatc gacagtgagt gtggcccatt catcaagctc
2461 agtgaccccg gcatccccat tacggtgctg tctaggcaag aatgcattga acgaatccca
2521 tggattgctc ctgagtgtgt tgaggactcc aagaacctga gtgtggctgc tgacaagtgg
2581 agctttggaa ccacgctctg ggaaatctgc tacaatggcg agatcccctt gaaagacaag
2641 acgctgattg agaaagagag attctatgaa agccggtgca ggccagtgac accatcatgt
2701 aaggagctgg ctgacctcat gacccgctgc atgaactatg accccaatca gaggcctttc
2761 ttccgagcca tcatgagaga cattaataag cttgaagagc agaatccaga tattgtttca
2821 gaaaaaaaac cagcaactga agtggacccc acacattttg aaaagcgctt cctaaagagg
2881 atccgtgact tgggagaggg ccactttggg aaggttgagc tctgcaggta tgaccccgaa
2941 ggggacaata caggggagca ggtggctgtt aaatctctga agcctgagag tggaggtaac
3001 cacatagctg atctgaaaaa ggaaatcgag atcttaagga acctctatca tgagaacatt
3061 gtgaagtaca aaggaatctg cacagaagac ggaggaaatg gtattaagct catcatggaa
3121 tttctgcctt cgggaagcct taaggaatat cttccaaaga ataagaacaa aataaacctc
3181 aaacagcagc taaaatatgc cgttcagatt tgtaagggga tggactattt gggttctcgg
3241 caatacgttc accgggactt ggcagcaaga aatgtccttg ttgagagtga acaccaagtg
```

3301 aaaattggag acttcggttt aaccaaagca attgaaaccg ataaggagta ttacaccgtc
3361 aaggatgacc gggacagccc tgtgttttgg tatgctccag aatgtttaat gcaatctaaa
3421 ttttatattg cctctgacgt ctggtctttt ggagtcactc tgcatgagct gctgacttac
3481 tgtgattcag attctagtcc catggctttg ttcctgaaaa tgataggccc aacccatggc
3541 cagatgacag tcacaagact tgtgaatacg ttaaaagaag gaaaacgcct gccgtgccca
3601 cctaactgtc cagatgaggt ttatcaactt atgaggaaat gctgggaatt ccaaccatcc
3661 aatcggacaa gctttcagaa ccttattgaa ggatttgaag cacttttaaa ataagaagca
3721 tgaataacat ttaaattcca cagattatca agtccttctc ctgcaacaaa tgcccaagtc
3781 atttttaaa aatttctaat gaaagaagtt tgtgttctgt ccaaaaagtc actgaactca
3841 tacttcagta catatacatg tataaggcac actgtagtgc ttaatatgtg taaggacttc
3901 ctctttaaat ttggtaccag taacttagtg acacataatg acaaccaaaa tatttgaaag
3961 cacttaagca ctcctccttg tggaaagaat ataccaccat ttcatctggc tagttcacca
4021 tcacaactgc attaccaaaa ggggattttt gaaaacgagg agttgaccaa aataatatct
4081 gaagatgatt gcttttccct gctgccagct gatctgaaat gttttgctgg cacattaatc
4141 atagataaag aaagattgat ggacttagcc ctcaaatttc agtatctata cagtactaga
4201 ccatgcattc ttaaaatatt agataccagg tagtatatat tgtttctgta caaaaatgac
4261 tgtattctct caccagtagg acttaaactt tgtttctcca gtggcttagc tcctgttcct
4321 ttgggtgatc actagcaccc atttttgaga aagctggttc tacatggggg gatagctgtg
4381 gaatagataa tttgctgcat gttaattctc aagaactaag cctgtgccag tgctttccta
4441 agcagtatac ctttaatcag aactcattcc cagaacctgg atgctattac acatgctttt
4501 aagaaacgtc aatgtatatc cttttataac tctaccactt tggggcaagc tattccagca
4561 ctggttttga atgctgtatg caaccagtct gaataccaca tacgctgcac tgttcttaga
4621 gggtttccat acttaccacc gatctacaag ggttgatccc tgtttttacc atcaatcatc
4681 accctgtggt gcaacacttg aaagacccgg ctagaggcac tatggacttc aggatccact
4741 agacagtttt cagtttgctt ggaggtagct gggtaatcaa aaatgtttag tcattgattc
4801 aatgtgaacg attacggtct ttatgaccaa gagtctgaaa atctttttgt tatgctgttt
4861 agtattcgtt tgatattgtt acttttcacc tgttgagccc aaattcagga ttggttcagt
4921 ggcagcaatg aagttgccat ttaaatttgt tcatagccta catcaccaag gtctctgtgt
4981 caaacctgtg gccactctat atgcactttg tttactcttt atacaaataa atatactaaa
5041 gactttacat gca

FIG. 11B

Wild type amino acid sequence (homo sapiens) for JAK1

```
        10         20         30         40         50         60
MQYLNIKEDC NAMAFCAKMR SSKKTEVNLE APEPGVEVIF YLSDREPLRL GSGEYTAEEL

        70         80         90        100        110        120
CIRAAQACRI SPLCHNLFAL YDENTKLWYA PNRTITVDDK MSLRLHYRMR FYFTNWHGTN

       130        140        150        160        170        180
DNEQSVWRHS PKKQKNGYEK KKIPDATPLL DASSLEYLFA QGQYDLVKCL APIRDPKTEQ

       190        200        210        220        230        240
DGHDIENECL GMAVLAISHY AMMKKMQLPE LPKDISYKRY IPETLNKSIR QRNLLTRMRI

       250        260        270        280        290        300
NNVFKDFLKE FNNKTICDSS VSTHDLKVKY LATLETLTKH YGAEIFETSM LLISSENEMN

       310        320        330        340        350        360
WFHSNDGGNV LYYEVMVTGN LGIQWRHKPN VVSVEKEKNK LKRKKLENKH KKDEEKNKIR

       370        380        390        400        410        420
EEWNNFSYFP EITHIVIKES VVSINKQDNK KMELKLSSHE EALSFVSLVD GYFRLTADAH

       430        440        450        460        470        480
HYLCTDVAPP LIVHNIQNGC HGPICTEYAI NKLRQEGSEE GMYVLRWSCT DFDNILMTVT

       490        500        510        520        530        540
CFEKSEQVQG AQKQFKNFQI EVQKGRYSLH GSDRSFPSLG DLMSHLKKQI LRTDNISFML

       550        560        570        580        590        600
KRCCQPKPRE ISNLLVATKK AQEWQPVYPM SQLSFDRILK KDLVQGEHLG RGTRTHIYSG

       610        620        630        640        650        660
TLMDYKDDEG TSEEKKIKVI LKVLDPSHRD ISLAFFEAAS MMRQVSHKHI VYLYGVCVRD

       670        680        690        700        710        720
VENIMVEEFV EGGPLDLFMH RKSDVLTTPW KFKVAKQLAS ALSYLEDKDL VHGNVCTKNL

       730        740        750        760        770        780
LLAREGIDSE CGPFIKLSDP GIPITVLSRQ ECIERIPWIA PECVEDSKNL SVAADKWSFG

       790        800        810        820        830        840
TTLWEICYNG EIPLKDKTLI EKERFYESRC RPVTPSCKEL ADLMTRCMNY DPNQRPFFRA

       850        860        870        880        890        900
IMRDINKLEE QNPDIVSEKK PATEVDPTHF EKRFLKRIRD LGEGHFGKVE LCRYDPEGDN

       910        920        930        940        950        960
TGEQVAVKSL KPESGGNHIA DLKKEIEILR NLYHENIVKY KGICTEDGGN GIKLIMEFLP

       970        980        990       1000       1010       1020
SGSLKEYLPK NKNKINLKQQ LKYAVQICKG MDYLGSRQYV HRDLAARNVL VESEHQVKIG

      1030       1040       1050       1060       1070       1080
DFGLTKAIET DKEYYTVKDD RDSPVFWYAP ECLMQSKFYI ASDVWSFGVT LHELLTYCDS

      1090       1100       1110       1120       1130       1140
DSSPMALFLK MIGPTHGQMT VTRLVNTLKE GKRLPCPPNC PDEVYQLMRK CWEFQPSNRT
```

1150
SFQNLIEGFE ALLK

FIG. 11C

Wild type nucleic acid sequence (homo sapiens) for JAK3

```
   1 cacacaggaa ggagccgagt gggactttcc tctcgctgcc tcccggctct gcccgccctt
  61 cgaaagtcca gggtccctgc ccgctaggca agttgcactc atggcacctc caagtgaaga
 121 gacgcccctg atccctcagc gttcatgcag cctcttgtcc acggaggctg gtgccctgca
 181 tgtgctgctg cccgctcggg gccccgggcc cccccagcgc ctatctttct cctttgggga
 241 ccacttggct gaggacctgt gcgtgcaggc tgccaaggcc agcggcatcc tgcctgtgta
 301 ccactccctc tttgctctgg ccacggagga cctgtcctgc tggttccccc cgagccacat
 361 cttctccgtg gaggatgcca gcacccaagt cctgctgtac aggattcgct tttacttccc
 421 caattggttt gggctggaga agtgccaccg cttcgggcta cgcaaggatt tggccagtgc
 481 tatccttgac ctgccagtcc tggagcacct ctttgcccag caccgcagtg acctggtgag
 541 tgggcgcctc cccgtgggcc tcagtctcaa ggagcagggt gagtgtctca gcctggccgt
 601 gttggacctg gcccggatgg cgcgagagca ggcccagcgg ccgggagagc tgctgaagac
 661 tgtcagctac aaggcctgcc tacccccaag cctgcgcgac ctgatccagg gcctgagctt
 721 cgtgacgcgg aggcgtattc ggaggacggt gcgcagagcc ctgcgccgcg tggccgcctg
 781 ccaggcagac cggcactcgc tcatggccaa gtacatcatg gacctggagc ggctggatcc
 841 agccggggcc gccgagacct tccacgtggg cctccctggg gcccttggtg gccacgacgg
 901 gctggggctg ctccgcgtgg ctggtgacgg cggcatcgcc tggacccagg gagaacagga
 961 ggtcctccag cccttctgcg actttccaga aatcgtagac attagcatca agcaggcccc
1021 gcgcgttggc ccggccggag agcaccgcct ggtcactgtt accaggacag acaaccagat
1081 tttagaggcc gagttcccag ggctgcccga ggctctgtcg ttcgtggcgc tcgtggacgg
1141 ctacttccgg ctgaccacgg actcccagca cttcttctgc aaggaggtgg caccgccgag
1201 gctgctggag gaagtggccg agcagtgcca cggccccatc actctggact ttgccatcaa
1261 caagctcaag actgggggct cacgtcctgg ctcctatgtt ctccgccgca gcccccagga
1321 ctttgacagc ttcctcctca ctgtctgtgt ccagaacccc cttggtcctg attataaggg
1381 ctgcctcatc cggcgcagcc ccacaggaac cttccttctg gttggcctca gccgacccca
1441 cagcagtctt cgagagctcc tggcaacctg ctgggatggg gggctgcacg tagatggggt
1501 ggcagtgacc ctcacttcct gctgtatccc cagacccaaa gaaaagtcca acctgatcgt
1561 ggtccagaga ggtcacagcc cacccacatc atccttggtt cagccccaat cccaatacca
1621 gctgagtcag atgacatttc acaagatccc tgctgacagc ctggagtggc atgagaacct
1681 gggccatggg tccttcacca agatttaccg gggctgtcgc catgaggtgg tggatgggga
1741 ggcccgaaag acagaggtgc tgctgaaggt catggatgcc aagcacaaga actgcatgga
1801 gtcattcctg gaagcagcga gcttgatgag ccaagtgtcg taccggcatc tcgtgctgct
1861 ccacggcgtg tgcatggctg gagacagcac catggtgcag gaatttgtac acctgggggc
1921 catagacatg tatctgcgaa aacgtggcca cctggtgcca gccagctgga agctgcaggt
1981 ggtcaaacag ctggcctacg ccctcaacta tctggaggac aaaggcctgc cccatggcaa
2041 tgtctctgcc cggaaggtgc tcctggctcg ggaggggggct gatgggagcc cgcccttcat
2101 caagctgagt gaccctgggg tcagccccgc tgtgttaagc ctggagatgc tcaccgacag
2161 gatcccctgg gtggcccccg agtgtctccg ggaggcgcag acacttagct tggaagctga
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2281 tgccctggat cctgctaaga aactccaatt ttatgaggac cggcagcagc tgccggcccc
2341 caagtggaca gagctggccc tgctgattca acagtgcatg gcctatgagc cggtccagag
2401 gccctccttc cgagccgtca ttcgtgacct caatagcctc atctcttcag actatgagct
2461 cctctcagac cccacacctg gtgccctggc acctcgtgat gggctgtgga atggtgccca
2521 gctctatgcc tgccaagacc ccacgatctt cgaggagaga cacctcaagt acatctcaca
2581 gctgggcaag ggcaactttg gcagcgtgga gctgtgccgc tatgacccgc taggcgacaa
2641 tacaggtgcc ctggtggccg tgaaacagct gcagcacagc gggccagacc agcagaggga
2701 ctttcagcgg gagattcaga tcctcaaagc actgcacagt gatttcattg tcaagtatcg
2761 tggtgtcagc tatggcccgg gccgccagag cctgcggctg gtcatggagt acctgcccag
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2881 tctctattcc tcgcagatct gcaagggcat ggagtacctg ggctcccgcc gctgcgtgca
2941 ccgcgacctg gccgcccgaa acatcctcgt ggagagcgag gcacacgtca agatcgctga
3001 cttcggccta gctaagctgc tgccgcttga caaagactac tacgtggtcc gcgagccagg
3061 ccagagcccc attttctggt atgcccccga atccctctcg gacaacatct tctctcgcca
3121 gtcagacgtc tggagcttcg gggtcgtcct gtacgagctc ttcacctact gcgacaaaag
3181 ctgcagcccc tcggccgagt tcctgcggat gatgggatgt gagcgggatg tccccgccct
3241 ctgccgcctc ttggaactgc tggaggaggg ccagaggctg ccggcgcctc ctgcctgccc
```

```
3301 tgctgaggtt cacgagctca tgaagctgtg ctgggcccct agcccacagg accggccatc
3361 attcagcgcc ctgggccccc agctggacat gctgtggagc ggaagccggg ggtgtgagac
3421 tcatgccttc actgctcacc cagagggcaa acaccactcc ctgtcctttt catagctcct
3481 gcccgcagac ctctggatta ggtctctgtt gactggctgt gtgaccttag gcccggagct
3541 gcccctctct gggcctcaga ggccttatga gggtcctcta cttcaggaac acccccatga
3601 cattgcattt gggggggctc ccgtggcctg tagaatagcc tgtggccttt gcaatttgtt
3661 aaggttcaag acagatgggc atatgtgtca gtggggctct ctgagtcctg gcccaaagaa
3721 gcaaggaacc aaatttaaga ctctcgcatc ttcccaaccc cttaagccct ggccccctga
3781 gtttcctttt ctgtctctct ctttttattt tttttatttt tatttttatt tttgagacag
3841 agcctcgctc tgttacccag ggtggagtgc agtggtgcga tctcggctca gtgcaacctc
3901 tgcttcccag gttcaagcga ttctcctgcc tcagcctccc gagtagctgg gattacaggt
3961 gtgcaccacc acacccggct aatttttttt atttttaata gagatgaggt ttcaccatga
4021 tggccaggct gatctcgaac tcctaacctc aagtgatcct cccacctcag cctcccaaag
4081 tgttggaata ataggcatga gccactgcac ccaggctttt ttttttttaa atttattatt
4141 attattttta agagacagga tcttgctacg ttgcccaggc tggtcttgaa ctcctgggct
4201 acagtgatcc tcctgcctta tcctcctaaa tagctgggac tacagcacct agttttgagt
4261 ttcctgtctt atttccaatg gggacattca tgtagctttt ttttttttt ttttttgag
4321 acggagtctc gctctgtcgc ccaggctgga gtacagtggc gcaatctagg ctcactgcaa
4381 gctccgcctc ctgggttcac accattctct cgcctcagcc tcccaagtag ctgggactac
4441 aggcgcccgc caccacaccc ggctaatttt ttgtattttt agtagagacg gggtttcacc
4501 ttgttagcca ggatggtttc catctcctga cctcgtgatc tgcccgtctc ggcctcccaa
4561 agtgctggga ttacaggcat gagccactgc gcccggccct catgtagctt taaatgtatg
4621 atctgacttc tgctccccga tctctgtttc tctggaggaa gccaaggaca agagcagttg
4681 ctgtggctgg gactctgcct tttaggggag cccgtgtatc tctttgggat cctgaaaggg
4741 ggcaggaaag gctggggtcc cagtccaccc taatggtatc tgagtgtcct agggcttcag
4801 ttttcccacc tgtccaatgg gacccttttct gtcctcaccc tacaaggggc acaaagggat
4861 gacaccaaac ctggcaggaa cttttcacgc aatcaaggga aggaaaggca ttcctggcag
4921 agggaacagc atgccaagcg tgagaaggct cagagtaagg aggttaagag cccaagtatt
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5101 aactttctga ctggtgagag ggattctcat gcaggttaag cttctgctgc tcctcctcac
5161 ctgcaaagct tttctgccac ttttgcctcc ttggaaaact cttatccatc tctcaaaact
5221 ccagctacca catccttgca gccttccctc atataccccc actactactg tagccctgtc
5281 cttccctcca gccccactct ggccctgggg ctggggaagt gtctgtgtcc agctgtctcc
5341 cctgacctca gggttccttg ggggctgggc tgaggcctca gtacagaggg ggctctggaa
5401 atgtttgttg actgaataaa ggaattcagt ggaaaaaaaa aaaaaaaaa
```

FIG. 11D

Wild type amino acid sequence (homo sapiens) for JAK3

```
        10         20         30         40         50         60
MAPPSEETPL IPQRSCSLLS TEAGALHVLL PARGPGPPQR LSFSFGDHLA EDLCVQAAKA

        70         80         90        100        110        120
SGILPVYHSL FALATEDLSC WFPPSHIFSV EDASTQVLLY RIRFYFPNWF GLEKCHRFGL

       130        140        150        160        170        180
RKDLASAILD LPVLEHLFAQ HRSDLVSGRL PVGLSLKEQG ECLSLAVLDL ARMAREQAQR

       190        200        210        220        230        240
PGELLKTVSY KACLPPSLRD LIQGLSFVTR RRIRRTVRRA LRRVAACQAD RHSLMAKYIM

       250        260        270        280        290        300
DLERLDPAGA AETFHVGLPG ALGGHDGLGL LRVAGDGGIA WTQGEQEVLQ PFCDFPEIVD

       310        320        330        340        350        360
ISIKQAPRVG PAGEHRLVTV TRTDNQILEA EFPGLPEALS FVALVDGYFR LTTDSQHFFC

       370        380        390        400        410        420
KEVAPPRLLE EVAEQCHGPI TLDFAINKLK TGGSRPGSYV LRRSPQDFDS FLLTVCVQNP

       430        440        450        460        470        480
LGPDYKGCLI RRSPTGTFLL VGLSRPHSSL RELLATCWDG GLHVDGVAVT LTSCCIPRPK

       490        500        510        520        530        540
EKSNLIVVQR GHSPPTSSLV QPQSQYQLSQ MTFHKIPADS LEWHENLGHG SFTKIYRGCR

       550        560        570        580        590        600
HEVVDGEARK TEVLLKVMDA KHKNCMESFL EAASLMSQVS YRHLVLLHGV CMAGDSTMVQ

       610        620        630        640        650        660
EFVHLGAIDM YLRKRGHLVP ASWKLQVVKQ LAYALNYLED KGLPHGNVSA RKVLLAREGA

       670        680        690        700        710        720
DGSPPFIKLS DPGVSPAVLS LEMLTDRIPW VAPECLREAQ TLSLEADKWG FGATVWEVFS

       730        740        750        760        770        780
GVTMPISALD PAKKLQFYED RQQLPAPKWT ELALLIQQCM AYEPVQRPSF RAVIRDLNSL

       790        800        810        820        830        840
ISSDYELLSD PTPGALAPRD GLWNGAQLYA CQDPTIFEER HLKYISQLGK GNFGSVELCR

       850        860        870        880        890        900
YDPLGDNTGA LVAVKQLQHS GPDQQRDFQR EIQILKALHS DFIVKYRGVS YGPGRQSLRL

       910        920        930        940        950        960
VMEYLPSGCL RDFLQRHRAR LDASRLLLYS SQICKGMEYL GSRRCVHRDL AARNILVESE

       970        980        990       1000       1010       1020
AHVKIADFGL AKLLPLDKDY YVVREPGQSP IFWYAPESLS DNIFSRQSDV WSFGVVLYEL

      1030       1040       1050       1060       1070       1080
FTYCDKSCSP SAEFLRMMGC ERDVPALCRL LELLEEGQRL PAPPACPAEV HELMKLCWAP

      1090       1100       1110       1120
SPQDRPSFSA LGPQLDMLWS GSRGCETHAF TAHPEGKHHS LSFS
```

FIG. 11E

Wild type nucleic acid sequence (homo sapiens) for STAT5B

```
   1 ggcgggagga gagtcggcgg ccggagccgt caccccgggc ggggacccag cgcaggcaac
  61 tccgcgcggc ggcccggccg agggagggag cgagcgggcg ggcgggcaag ccagacagct
 121 gggccggagc agccgcgggc gcccgagggg ccgagcgaga ttgtaaacca tggctgtgtg
 181 gatacaagct cagcagctcc aaggagaagc ccttcatcag atgcaagcgt tatatggcca
 241 gcatttccc attgaggtgc ggcattattt atcccagtgg attgaaagcc aagcatggga
 301 ctcagtagat cttgataatc cacaggagaa cattaaggcc acccagctcc tggagggcct
 361 ggtgcaggag ctgcagaaga aggcagagca ccaggtgggg gaagatgggt ttttactgaa
 421 gatcaagctg gggcactatg ccacacagct ccagaacacg tatgaccgct gccccatgga
 481 gctggtccgc tgcatccgcc atatattgta caatgaacag aggttggtcc gagaagccaa
 541 caatggtagc tctccagctg gaagccttgc tgatgccatg tcccagaaac acctccagat
 601 caaccagacg tttgaggagc tgcgactggt cacgcaggac acagagaatg agttaaaaaa
 661 gctgcagcag actcaggagt acttcatcat ccagtaccag gagagcctga ggatccaagc
 721 tcagtttggc ccgctggccc agctgagccc ccaggagcgt ctgagccggg agacggccct
 781 ccagcagaag caggtgtctc tggaggcctg gttgcagcgt gaggcacaga cactgcagca
 841 gtaccgcgtg gagctggccg agaagcacca gaagaccctg cagctgctgc ggaagcagca
 901 gaccatcatc ctggatgacg agctgatcca gtggaagcgg cggcagcagc tggccgggaa
 961 cggcgggccc cccgagggca gcctggacgt gctacagtcc tggtgtgaga agttggccga
1021 gatcatctgg cagaaccggc agcagatccg cagggctgag cacctctgcc agcagctgcc
1081 catccccggc ccagtggagg agatgctggc cgaggtcaac gccaccatca cggacattat
1141 ctcagccctg gtgaccagca cgttcatcat tgagaagcag cctcctcagg tcctgaagac
1201 ccagaccaag tttgcagcca ctgtgcgcct gctggtgggc gggaagctga acgtgcacat
1261 gaaccccccc caggtgaagg ccaccatcat cagtgagcag caggccaagt ctctgctcaa
1321 gaacgagaac acccgcaatg attacagtgg cgagatcttg aacaactgct gcgtcatgga
1381 gtaccaccaa gccacaggca cccttagtgc ccacttcagg aatatgtccc tgaaacgaat
1441 taagaggtca gaccgtcgtg gggcagagtc ggtgacagaa gaaaaattta caatcctgtt
1501 tgaatcccag ttcagtgttg gtggaaatga gctggttttt caagtcaaga ccctgtccct
1561 gccagtggtg gtgatcgttc atggcagcca ggacaacaat gcgacggcca ctgttctctg
1621 ggacaatgct tttgcagagc ctggcagggt gccatttgcc gtgcctgaca aagtgctgtg
1681 gccacagctg tgtgaggcgc tcaacatgaa attcaaggcc gaagtgcaga gcaaccgggg
1741 cctgaccaag gagaacctcg tgttcctggc gcagaaactg ttcaacaaca gcagcagcca
1801 cctggaggac tacagtggcc tgtctgtgtc ctggtcccag ttcaacaggg agaatttacc
1861 aggacggaat tacactttct ggcaatggtt tgacggtgtg atggaagtgt taaaaaaaca
1921 tctcaagcct cattggaatg atggggccat tttggggttt gtaaacaagc aacaggccca
1981 tgacctactc attaacaagc cagatgggac cttcctcctg agattcagtg actcagaaat
2041 tggcggcatc accattgctt ggaagtttga ttctcaggaa agaatgtttt ggaatctgat
2101 gccttttacc accagagact tctccattcg gtccctagcc gaccgcttgg gagacttgaa
2161 ttaccttatc tacgtgtttc ctgatcggcc aaaagatgaa gtatactcca aatactacac
2221 accagttccc tgcgagtctg ctactgctaa agctgttgat ggatacgtga agccacagat
2281 caagcaagtg gtccctgagt ttgtgaacgc atctgcagat gccgggggcg gcagcgccac
2341 gtacatggac caggcccccc ccccagctgt gtgtccccag gctcactata acatgtaccc
2401 acagaaccct gactcagtcc ttgacaccga tggggacttc gatctggagg acacaatgga
2461 cgtagcgcgg cgtgtggagg agctcctggg ccggccaatg gacagtcagt ggatcccgca
2521 cgcacaatcg tgaccccgcg acctctccat cttcagcttc ttcatcttca ccagaggaat
2581 cactcttgtg gatgttttaa ttccatgaat cgcttctctt ttgaaacaat actcataatg
2641 tgaagtgtta atactagttg tgaccttagt gtttctgtgc atggtggcac cagcgaaggg
2701 agtgcgagta tgtgtttgtg tgtgtgtgtg tgtgtgtgtg tgtgtgcgtg tttgcacgtt
2761 atggtgtttc tccctctcac tgtctgagag tttagttgta gcagaggggc cacagacaga
2821 agctgtggtg gtttttactt tgtgcaaaaa ggcagtgagt ttcgtgaagc ctggaagttg
2881 gccatgtgtc ttaagagtgg ctggactttg acatgtggct gtttgaataa gagaaggaca
2941 aagggaggag aaagcacatg tgctccagtg agtcttcgtc actctgtctg ccaagcaatt
3001 gatatataac cgtgattgtc tctgcttttc ttctgaaatg tagataactg ctttttgaca
3061 aagagagcct tccctctccc ccacccctgt gttcttgggt aggaatggga aaaggggcaa
3121 cctacaaaga ttgttggggc aagggaagtc acaagctttc ggatgggcgg tggcttttca
3181 caaaacattt agctcatctt attctctctt tgtcctctct cccctcctgc ccgcccgcac
3241 cctggaattg ccactcagtt cctctgggtg tgcacatatg tttggagaaa tagaggagag
```

3301 aaaagagggc cacgtaactg agagcttaca gtgccaatgc cgtttgtgtt ctggccagag
3361 tggagtgcgc agccctgact cccaggcgct gagattgttg cctggttacc caggaagctg
3421 ctgttccggc tgcccagcct ttctctgagc cagcggatgc acagtccgtg gccttcttca
3481 ggcttattga tgatgctttt tgcaaatgtt gaatcatggt tctgtttcta agttggatct
3541 tttttgtttt ctccttgcca ccctaatttg acatcaaaat tctctcttgt gcattgggcc
3601 ctgggtcatt caaacccagg tcacctcatt ccccttctct gttcacacct aatgtcttga
3661 agagtaggta gcagcagtgt gggctgaacc taggccagct tgcttagcgg gtcaccctgc
3721 tgtgaagtcc tggcaggtgt tggtaatgtg tggaaatgca gtcagcaagt ttgctgggga
3781 gtttgataaa agtataaaac aaaacaaaaa aagcctcggt ataattttgt tccacgactt
3841 cttctgtagc tttacaccag aaggaaggaa tgggctacag caggtagtgg aggaagaggg
3901 gggtgagcag gtgtattaaa atagcttacg ggtaaggcct aaaaggtcac ccctcggccc
3961 cctctccaaa agaagggcat gggcaccccc aggagaggat ggccccaaaa accttatttt
4021 tatacatgag agtaaataaa catatttttt ttacaaaaat aacttctgaa tttatcagtg
4081 ttttgccgtt aaaaatattc ctctatagta aattatttat tggaagatga ctttttttaaa
4141 gctgccgttt gccttggctt ggtttcatac actgatttat ttttctatgc caggcagtag
4201 agtctctctg cctctgagga gcaggctacc cgcatcccac tcagcccctc cctacccctc
4261 aagatttgat gaaaattcca accatgagga tgggtgcatc ggggaagggt gagaaggaga
4321 gcctgcctgc tcagggatcc aggctcgtag agtcactccc tgcccgtctc ccagagatgc
4381 ttcaccagca cctgcctctg agacctcgct ctctgttcca gcaaccctgg ttgggggtc
4441 agacttgata cactttcagg ttgggagtgg acccacccca gggcctgctg aggacagagc
4501 agccaggccg tcctggctca ctttgcagtt ggcactgggt tggggaggaa gagagctgat
4561 gagtgtggct tccctgagct ggggtttccc tgcttgtcca gttgtgagct gtcctcggtg
4621 ttaccgaggc tgtgcctaga gagtggagat ttttgatgaa aggtgtgctc gctctctgcg
4681 ttctatcttc tctctcctcc ttgttcctgc aaaccacaag ataaaggtag tggtgtgtct
4741 cgaccccatc agcctctcac ccactcccag acacacacaa gtcctcaaaa gtttcagctc
4801 cgtgtgtgag atgtgcaggt tttttctagg gggtaggggg agactaaaat cgaatataac
4861 ttaaaatgaa agtatacttt ttataatttt tctttttaaa acttggtgaa attatttcag
4921 atacatattt tagtgtcaag gcagattagt tatttagcca ccaaaaaaaa gtattgtgta
4981 caatttgggg cctcaaattt gactctgcct caaaaaaaag aaatatatcc tatgcagagt
5041 tacagtcaca aagttgtgta ttttatgtta caataaagcc ttcctctgaa gggaaaaaaa
5101 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
5161 aaaaaaaaaa a

FIG. 11F

Wild type amino acid sequence (homo sapiens) for STAT5B

```
   10         20         30         40         50         60
MAVWIQAQQL QGEALHQMQA LYGQHFPIEV RHYLSQWIES QAWDSVDLDN PQENIKATQL

         70         80         90        100        110        120
LEGLVQELQK KAEHQVGEDG FLLKIKLGHY ATQLQNTYDR CPMELVRCIR HILYNEQRLV

        130        140        150        160        170        180
REANNGSSPA GSLADAMSQK HLQINQTFEE LRLVTQDTEN ELKKLQQTQE YFIIQYQESL

        190        200        210        220        230        240
RIQAQFGPLA QLSPQERLSR ETALQQKQVS LEAWLQREAQ TLQQYRVELA EKHQKTLQLL

        250        260        270        280        290        300
RKQQTIILDD ELIQWKRRQQ LAGNGGPPEG SLDVLQSWCE KLAEIIWQNR QQIRRAEHLC

        310        320        330        340        350        360
QQLPIPGPVE EMLAEVNATI TDIISALVTS TFIIEKQPPQ VLKTQTKFAA TVRLLVGGKL

        370        380        390        400        410        420
NVHMNPPQVK ATIISEQQAK SLLKNENTRN DYSGEILNNC CVMEYHQATG TLSAHFRNMS

        430        440        450        460        470        480
LKRIKRSDRR GAESVTEEKF TILFESQFSV GGNELVFQVK TLSLPVVVIV HGSQDNNATA

        490        500        510        520        530        540
TVLWDNAFAE PGRVPFAVPD KVLWPQLCEA LNMKFKAEVQ SNRGLTKENL VFLAQKLFNN

        550        560        570        580        590        600
SSSHLEDYSG LSVSWSQFNR ENLPGRNYTF WQWFDGVMEV LKKHLKPHWN DGAILGFVNK

        610        620        630        640        650        660
QQAHDLLINK PDGTFLLRFS DSEIGGITIA WKFDSQERMF WNLMPFTTRD FSIRSLADRL

        670        680        690        700        710        720
GDLNYLIYVF PDRPKDEVYS KYYTPVPCES ATAKAVDGYV KPQIKQVVPE FVNASADAGG

        730        740        750        760        770        780
GSATYMDQAP SPAVCPQAHY NMYPQNPDSV LDTDGDFDLE DTMDVARRVE ELLGRPMDSQ


WIPHAQS
```

FIG. 11G

Wild type nucleic acid sequence (homo sapiens) for IL2RG

```
   1 agaggaaacg tgtgggtggg gaggggtagt gggtgaggga cccaggttcc tgacacagac
  61 agactacacc cagggaatga agagcaagcg ccatgttgaa gccatcatta ccattcacat
 121 ccctcttatt cctgcagctg cccctgctgg gagtggggct gaacacgaca attctgacgc
 181 ccaatgggaa tgaagacacc acagctgatt tcttcctgac cactatgccc actgactccc
 241 tcagtgtttc cactctgccc ctcccagagg ttcagtgttt tgtgttcaat gtcgagtaca
 301 tgaattgcac ttggaacagc agctctgagc cccagcctac caacctcact ctgcattatt
 361 ggtacaagaa ctcggataat gataaagtcc agaagtgcag ccactatcta ttctctgaag
 421 aaatcacttc tggctgtcag ttgcaaaaaa aggagatcca cctctaccaa acatttgttg
 481 ttcagctcca ggacccacgg gaacccagga gacaggccac acagatgcta aaactgcaga
 541 atctggtgat cccctgggct ccagagaacc taacacttca caaactgagt gaatcccagc
 601 tagaactgaa ctggaacaac agattcttga accactgttt ggagcacttg gtgcagtacc
 661 ggactgactg ggaccacagc tggactgaac aatcagtgga ttatagacat aagttctcct
 721 tgcctagtgt ggatgggcag aaacgctaca cgtttcgtgt tcggagccgc tttaacccac
 781 tctgtggaag tgctcagcat tggagtgaat ggagccaccc aatccactgg gggagcaata
 841 cttcaaaaga gaatcctttc ctgtttgcat tggaagccgt ggttatctct gttggctcca
 901 tgggattgat tatcagcctt ctctgtgtgt atttctggct ggaacggacg atgccccgaa
 961 ttcccaccct gaagaaccta gaggatcttg ttactgaata ccacgggaac ttttcggcct
1021 ggagtggtgt gtctaaggga ctggctgaga gtctgcagcc agactacagt gaacgactct
1081 gcctcgtcag tgagattccc ccaaaaggag gggcccttgg ggaggggcct ggggcctccc
1141 catgcaacca gcatagcccc tactgggccc ccccatgtta caccctaaag cctgaaacct
1201 gaaccccaat cctctgacag aagaacccca gggtcctgta gccctaagtg gtactaactt
1261 tccttcattc aacccacctg cgtctcatac tcacctcacc ccactgtggc tgatttggaa
1321 ttttgtgccc ccatgtaagc acccccttcat ttggcattcc ccacttgaga attacccttt
1381 tgccccgaac atgtttttct tctccctcag tctggccctt ccttttcgca ggattcttcc
1441 tccctccctc tttccctccc ttcctctttc catctaccct ccgattgttc ctgaaccgat
1501 gagaaataaa gtttctgttg ataatcatca aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
```

FIG. 11H

Wild type amino acid sequence (homo sapiens) for IL2RG

```
  10         20         30         40         50         60
MLKPSLPFTS LLFLQLPLLG VGLNTTILTP NGNEDTTADF FLTTMPTDSL SVSTLPLPEV

         70         80         90        100        110        120
QCFVFNVEYM NCTWNSSSEP QPTNLTLHYW YKNSDNDKVQ KCSHYLFSEE ITSGCQLQKK

       130        140        150        160        170        180
EIHLYQTFVV QLQDPREPRR QATQMLKLQN LVIPWAPENL TLHKLSESQL ELNWNNRFLN

       190        200        210        220        230        240
HCLEHLVQYR TDWDHSWTEQ SVDYRHKFSL PSVDGQKRYT FRVRSRFNPL CGSAQHWSEW

       250        260        270        280        290        300
SHPIHWGSNT SKENPFLFAL EAVVISVGSM GLIISLLCVY FWLERTMPRI PTLKNLEDLV

       310        320        330        340        350        360
TEYHGNFSAW SGVSKGLAES LQPDYSERLC LVSEIPPKGG ALGEGPGASP CNQHSPYWAP


PCYTLKPET
```

FIG. 12

Selected recurrently mutated genes in T-PLL by whole genome and whole exome sequencing.

Number	Gene	Chr	Position	Ref	Alt	Consequence	Amino Acid Residue	Number of Cases	Confirmed Somatic	Total Number Confirmed Somatic	Novel
1	*IL2RG*	chrX	70,328,495	CATGGAGGC		Frameshift	G268_M270del	1	Somatic	1	Novel
2			70,327,753	T	C	Missense	K315E	1	Somatic	1	Novel
3	*JAK1*	chr1	65,313,222	TATCCC		Deletion	R629_D630del	1	Somatic	1	Novel
4			65,312,347	C	A	Missense	V658F	2	Somatic	2	COSMIC
5			65,311,203	C	A	Missense	S703I	1	Somatic	1	COSMIC
6			65,305,426	G	C	Missense	T901R	1	Somatic	1	Novel
7	*JAK3*	chr19	17,948,006	G	A	Missense	A573V	2	Somatic	2	Previous
8			17,948,745	TGCAGTTCT		Deletion	K563_C565del	1	Somatic	1	Novel
9			17,945,970	G	A	Missense	R657W	1	N/A		Novel
10			17,949,121	T	G	Missense	Q507P	1	Somatic	1	COSMIC
11			17,949,108	C	T	Missense	M511I	5	Somatic	5	Previous
12			17,949,108	C	G	Missense	M511I	2	Somatic	2	Previous
13			17,949,108	C	A	Missense	M511I	3	Somatic	3	COSMIC
14	*STAT5B*	chr17	40,362,212	G	C	Missense	T628S	3	Somatic	3	Novel
15			40,359,729	T	G	Missense	N642H	11	Somatic	11	Previous
16			40,359,678	G	T	Missense	R659C	1	Somatic	1	Novel
17			40,359,659	T	A	Missense	Y665F	2	Somatic	2	Previous
18			40,354,787	T	A	Missense	Q706L	1	Somatic	1	Novel
19	*ATM*	chr11	108,099,934	AG		Frameshift	E73fs	1	Somatic	1	
20			108,115,522	A	G	Missense	K224E	1	Somatic	1	
21			108,117,745	T	G	Missense	L319R	1	Somatic	1	
22			108,122,676	AATC		Frameshift	S575fs	1	Somatic	1	
23			108,124,604	A		Frameshift	T655fs	1	Somatic	1	
24			108,124,723	T	G	Missense	L694R	1	Somatic	1	
25			108,178,665	C	T	Nonsense	Q1906*	1	Somatic	1	
26			108,186,590	A	G	Missense	D2016G	1	Somatic	1	
27			108,199,938	T	G	Missense	L2427R	1	N/A		

28			108,199,938	T	C	Missense	L2427P	1	N/A		
29			108,200,975	G	A	Missense	D2448N	1	Somatic	1	
30			108,200,991	G	C	Missense	R2453P	1	Somatic	1	COSMIC
31			108,206,576	TGA		Deletion	D2720del	1	Somatic	1	
32			108,206,581	G	A	Missense	D2721N	1	Somatic	1	COSMIC
33			108,206,594	A	T	Missense	D2725V	1	Somatic	1	
34			108,206,595	T	A	Missense	D2725E	2	Somatic	2	
35			108,206,600	T	G	Missense	V2727G	1	N/A		
36			108,216,627	C	T	Missense	S2859F	1	Somatic	1	
37			108,218,052	G	C	Missense	L2877F	1	Somatic	1	
38			108,224,492	G	A	Missense	G2890D	1	Somatic	1	
39			108,218,092	G	C	Missense	G2891R	1	Somatic	1	COSMIC
40			108,224,493	G	A	Missense	G2891D	1	N/A		
41			108,224,518	C	G	Missense	I2899M	1	N/A		
42			108,235,809	G	A	Missense	V2951I	1	Somatic	1	
43			108,236,080	G	A	Missense	A3006T	1	Somatic	1	
44			108,236,086	C	T	Missense	R3008C	1	Somatic	1	
45	*CHEK2*	chr22	29,091,856	G		Frameshift	T146fs	1	N/A		Novel
46			29,083,962	G	C	Missense	R298G	1	Somatic	1	COSMIC
47	*EZH2*	chr7	148,526,858	A	C	Missense	L110R	1	N/A		Novel
48			148,516,705	G	A	Nonsense	Q284*	1	Somatic	1	Novel
49			148,506,474	C	T	Missense	V624M	1	N/A		Novel
50			148,506,468	C	T	Missense	A626T	1	Somatic	1	Novel
51			148,506,245	AA		Frameshift	K703fs	1	N/A		Novel
52	*FBXW10*	chr17	18,653,403	G	A	Missense	D318N	1	Somatic	1	Novel
53			18,661,631	C	T	Nonsense	R416*	1	N/A		Novel
54			18,682,350	AA		Frameshift	K966fs	1	Somatic	1	Novel
								77		66	

FIG. 13

JAK-STAT mutational status associated with patient demographic and clinical diagnostic and prognostic information.

PLL_ID	Age at Dx	Sex	*ATM*	*ATM*	inv(14)/TCL1+	iso(8)	*IL2RG*	*JAK1*	*JAK3*	*STAT5B*	*JAK-STAT* Mutation Status	Previous treatment status	Time to relapse (days)	Time to death (days)	Survival
PLL_index1	47	M		T655fs	X,14	yes		S703I			Confirmed somatic	untreated	335	622	Dead
PLL_index2	89	M	DELETION		inv(14)	yes		V658F		Q706L	Confirmed somatic	untreated		160	Dead
PLL_index3	53	F		H2037R	inv(14)		none-genome	none-genome	none-genome	none-genome	No mutation	unknown	1008	1077	Dead
PLL_index4		M	unknown		inv(14)	yes	none-genome	none-genome	none-genome	none-genome	No mutation	untreated		548	Dead
PLL_1	71	M	DELETION	E73fs						T628S	Confirmed somatic	untreated		1288	Dead
PLL_2	69	F					none-exome	none-exome	none-exome	none-exome	No mutation	treated		2674	Dead
PLL_3	81	F	DELETION	G2891R*	add(14)				M511I		Confirmed somatic	untreated		103	Dead
PLL_4	68	M			TCL1+			T901R			Confirmed somatic	untreated			Alive
PLL_5	58	M		L2427R	TCL1+					N642H	Confirmed somatic	treated	791	1084	Dead
PLL_6	61	F	unknown	unknown		yes	none-Sanger	none-Sanger	none-Sanger	none-Sanger	No mutation	untreated		192	Dead
PLL_7	71	M		D2725V	inv(14)			V658F			Confirmed somatic	untreated			Alive
PLL_8	68	M	DELETION	V2727G	TCL1+					Y665F	Confirmed somatic	untreated		130	Dead
PLL_9	68	M			del(14)				M511I		Confirmed somatic	treated		361	Dead
PLL_10	60	M	DELETION		inv(14)				M511I		Confirmed somatic	treated	924		Alive
PLL_11	79	M	DELETION	D2719del	inv(14)		none-exome	none-exome	none-exome	none-exome	No mutation	untreated			Alive
PLL_12	80	M			TCL1+	yes			M511I		Confirmed somatic	untreated	189	190	Dead
PLL_13	62	M			inv(14)				M511I		Confirmed somatic	treated	335	754	Dead
PLL_14	77	F	DELETION	I2899M	inv(14)				M511I		Confirmed somatic	treated	2522	2871	Dead
PLL_15	27	M								N642H	Confirmed somatic	treated	2680		Alive
PLL_16	36	F	DELETION	D2725E	inv(14)				M511I		Confirmed somatic	untreated		561	Death
PLL_17	43	M			TCL1+					T628S	Confirmed somatic	untreated			Alive
PLL_18	71	M	DELETION	D2721N*	10,14	yes			K563_C565del		Confirmed somatic	untreated			Alive
PLL_19	75	F	DELETION	S575fs					A573V		Confirmed somati	treated	973	1122	Death

FIG. 13 (continued)

PLL_20			DELETION	G2891D						N642H	Confirmed somatic	unknown			
PLL_21	73	M	DELETION	A3006T		+8				N642H	Confirmed somatic	treated	212	824	Dead
PLL_22	74	F	unknown	unknown	inv(14)	+8	none-Sanger	none-Sanger	none-Sanger	none-Sanger	No mutation	untreated			Alive
PLL_23	61	F	DELETION	L2427P	inv(14)	iso(8)				Y665F	Confirmed somatic	untreated	486		Alive
PLL_24	60	M	DELETION	H2038D	TCL1+	unknown				T628S	Confirmed somatic	untreated			Alive
PLL_25	78	F	DELETION	L694R	inv(14)		G628_S630del and K315E				Confirmed somatic	untreated			Alive
PLL_26	72	M			TCL1+	unknown	none-exome	none-exome	none-exome	none-exome	No mutation	untreated	109	132	Dead
PLL_27	45	M	DELETION	D2448N	TCL1+	unknown				N642H	Confirmed somatic	untreated	111	342	Dead
PLL_28	62	F	unknown	unknown	TCL1+	+MYC	none-Sanger	none-Sanger	none-Sanger	none-Sanger	No mutation	untreated		120	Dead
PLL_29	63	M	DELETION	D2016G	t(14q11)	+8q24			M511I		Confirmed somatic	untreated		238	Dead
PLL_30	63	M	unknown	unknown			none-Sanger	none-Sanger	none-Sanger	none-Sanger	No mutation	untreated			Alive
PLL_31	50	F		V2951I	TCL1+	unknown			Q507P		Confirmed somatic	untreated	443	560	Dead
PLL_32	56	M	DELETION	R2453P	TCL1+	+MYC			R657W		Mutation but normal tissue not available	treated	823	941	Dead
PLL_33	52	M	DELETION	L319R	TCL1+	unknown				N642H	Confirmed somatic	untreated	1643	2179	Dead
PLL_34	58	M			TCL1+					N642H	Confirmed somatic	untreated		609	Dead
PLL_35	48	M	DELETION	D2725E and K224E	inv(14)	+MYC				N642H	Confirmed somatic	untreated	276		Alive
PLL_36	72	M		L2877F	TCL1+	unknown				N642H	Confirmed somatic	untreated		129	Dead
PLL_37	72	M			TCL1+	+MYC	none-exome	none-exome	none-exome	none-exome	No mutation	untreated		612	Dead
PLL_38	68	M		R3008C	inv(14)				A573V		Confirmed somatic	untreated			Alive
PLL_39	64	M	DELETION	S2859F	TCL1+	unknown		R629_D630del			Confirmed somatic	untreated			Alive
PLL_40	71	M		Q1906*	TCL1+	unknown				N642H	Confirmed somatic	untreated			Alive
PLL_41	67	F	unknown	unknown	inv(14)		none-Sanger	none-Sanger	none-Sanger	none-Sanger	No mutation	untreated		722	Dead
PLL_42	67	M	unknown	unknown	unknown	unknown				N642H	Confirmed somatic	untreated		92	Dead
PLL_43	54	M			inv(14)	yes			M511I		Confirmed somatic	unknown	1461	1990	Dead
PLL_44	73	M							M511I		Confirmed somatic	untreated		18	Dead
PLL_45	58	M	DELETION		inv(14)	yes	none-Sanger	none-Sanger	none-Sanger	none-Sang	No mutati	untreated		135	Dead

FIG. 13 (continued)

					er			
PLL_46	57	M			R659C	on Confirmed somatic	untreated	Alive

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/051099

A. CLASSIFICATION OF SUBJECT MATTER

C12Q 1/68(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q 1/68

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: mature T-cell leukemia, JAK/STAT, variant, T-PLL(T-cell prolymphocytic leukemia), Sezary syndrome

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KOSKELA et al., `Somatic STAT3 mutations in large granular lymphocytic leukemia` The New England Journal of Medicine, Vol.366, No.20, pp.1905-1913 (2012) See abstract; pages 1905-1907, 1909-1912; and figures 1-4.	27-29,41-44
A		30-40,45-55
A	VAINCHENKER et al., `JAK/STAT signaling in hematological malignancies` Oncogene, Vol.32, No.21, pp.2601-2613 (06 August 2012) See abstract and pages 2601-2606.	27-55
A	DEARDEN, `How I treat prolymphocytic leukemia` Blood, Vol.120, No.3, pp.538-551 (2012) See abstract and pages 538-545.	27-55
A	RAVANDI et al., `Mature T-cell leukemias` Cancer, Vol.104, No.9, pp.1808-1818 (2005) See the whole document.	27-55
A	WO 2009-106372 A1 (ISTITUTO SUPERIORE DI SANATA) 03 September 2009 See abstract and claims 1-29.	27-55

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 November 2014 (12.11.2014)	**13 November 2014 (13.11.2014)**

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/051099

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	KIEL et al., `Integrated genomic sequencing reveals mutational landscape of T-cell prolymphocytic leukemia` Blood, Vol.124, No.9, pp.1460-1472 (Epub. 13 May 2014) See the whole document, especially figures 1-5 and tables 1-2.	27-55

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/051099

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-26
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1-26 pertain to methods for treatment of the human body by therapy, as well as diagnostic methods, and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.

2. ☒ Claims Nos.: 22
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Although claim 22 is a dependent claim referring to other claim, the number of the referred claim is missed (PCT Article 6).

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2014/051099

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009-106372 A1	03/09/2009	None	