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(71) Applicant: **THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ARKANSAS** [US/US]; 2404 North University Avenue, Little Rock, AR 72207 (US).

(72) Inventors: **TIAN, Erming**; 2202 Maple Ridge Road, Little Rock, AR 72211 (US). **ZHANG, Qing**; 2404 North University Avenue, Little Rock, AR 72207 (US). **EPSTEIN, Joshua**; 132 Valley Club Circle, Little Rock, AR 72212 (US). **BARLOGIE, Barthel**; 3118 Edgerstoune Lane, Little Rock, AR 72205 (US).

(74) Agents: **COLLINS, Anne J.** et al.; Hamilton, Brook, Smith & Reynolds, P.C., 530 Virginia Rd, P.O. Box 9133, Concord, MA 01742-9133 (US).

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(54) Title: METHODS OF DETECTING 14Q32 TRANSLOCATIONS

(57) Abstract: The present invention provides methods to predict and detect 14q32 translocations in patients with B-cell malignancies, such as multiple myeloma, as well as methods of treating multiple myeloma.



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METHODS OF DETECTING 14Q32 TRANSLOCATIONS

RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 61/726,327, filed on November 14, 2012.

[0002] The entire teachings of the above application are incorporated herein by reference.

GOVERNMENT SUPPORT

[0003] This invention was made with government support under CA55819 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0004] Multiple myeloma (MM) is a heterogeneous plasma cell disorder characterized by genetic abnormalities, including chromosomal translocations, deletions, duplications and genetic mutations. Translocations involving the immunoglobulin heavy chain gene (IgH) on the long arm of chromosome 14 region 32 (t(14q32)) are detected in 50%-70% of multiple myeloma patients by fluorescence *in situ* hybridization (FISH), and increase in frequency with progression. The 14q32 translocations involve many chromosomal partners. Translocation of oncogenes into the 14q32 region may lead to their increased expression, contributing to disease initiation, disease progression and therapeutic resistance. Commercially available FISH probes for 14q32 translocations exist, but are significantly longer than a gene locus on a chromosome and are prone to false-positive errors. For example, t(4;14) is currently defined as any fusion between an IgH probe spanning approximately 1.5 Mb and containing sequences homologous to essentially the entire IgH locus, as well as sequence extending about 300 kb beyond the 3' end of the IgH locus, and a probe spanning approximately 900 kb.

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[0005] In addition to 14q32 translocations, hyperdiploidy is a common genetic abnormality in patients with multiple myeloma, as are deletions and amplifications. Thus, gene expression profiling (GEP), which provides information on genome-wide transcription levels, is another powerful tool, in addition to FISH, to query genetic abnormalities associated with myeloma plasma cells. GEP expression data from multiple myeloma patients can be used to stratify patients into those with expression levels of 14q32 translocation partners are similar to healthy donor plasma cells and those with GEP-based spikes (*i.e.* increased expression). However, current methods do not exist to differentiate GEP-based gene spikes representing 14q32 translocations from those reflecting changes in gene copy number.

[0006] Thus, there exists a need in the art for better tools and methods to simultaneously identify multiple 14q32 translocations, as well as methods to guide treatment decisions based on the presence or absence of 14q32 translocations.

SUMMARY OF THE INVENTION

[0007] In a first aspect, the invention provides methods to detect a 14q32 translocation in a subject with a B-cell malignancy. These methods entail performing fluorescence in-situ hybridization (FISH) on a biological sample from a subject with a B-cell malignancy using at least a first probe and a second probe, where the first probe comprises a nucleic acid sequence specific for IgHC and/or IgHV and the second probe comprises a nucleic acid sequence complementary to a chromosomal region encoding a genomic locus selected from the group consisting of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, and MAFB.

[0008] In another aspect, the invention provides methods to detect an IgHC translocation in a subject with a B-cell malignancy. These methods entail performing fluorescence in-situ hybridization (FISH) on a biological sample from the subject using at least a first probe and a second probe, where the first probe comprises a nucleic acid sequence specific for IgHC and the second probe comprises a nucleic acid sequence complementary to a chromosomal region encoding a genomic locus selected from the group consisting of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, and MAFB.

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[0009] A further aspect of the invention provides methods to establish a gene expression threshold value for a 14q32 translocation partner indicative of a 14q32 translocation in a subject with a B-cell malignancy. These methods entail (a) measuring the expression level for a 14q32 translocation partner in biological samples from subjects with 14q32 translocations and without 14q32 translocations, (b) determining the presence or absence of a 14q32 translocation by FISH using (at least) a pair of probes where the first probe comprises a nucleic acid sequence specific for IgHC or IgHV, and the second probe comprises a nucleic acid sequence complementary to a chromosomal region encoding the translocation partner whose expression was measured in (a), (c) comparing the expression level of the translocation partner with the presence or absence of a 14q32 translocation, and (d) determining a gene expression threshold value for the translocation partner above which indicates increased expression due to a 14q32 translocation and below which indicates increased expression not due to a 14q32 translocation.

[0010] In yet another aspect, the invention provides methods to predict a 14q32 translocation in a subject suspected to have multiple myeloma. These methods entail (a) measuring the expression level for one or more 14q32 translocation partners in a biological sample from the subject, and (b) comparing the expression level(s) to the gene expression threshold value(s) (determined by any suitable means), where an expression level for a 14q32 translocation partner in the biological sample that is greater than the threshold value, indicates that the subject has a 14q32 translocation.

[0011] The invention also provides methods to identify a multiple myeloma patient that would benefit from a treatment comprising bortezomib, or determine if a multiple myeloma patient would benefit from a treatment comprising bortezomib. These methods entail determining whether a patient has a 14q32 translocation according to any of the methods provided by the invention, wherein the presence or absence of a 14q32 translocation guides the treatment decision.

[0012] In another aspect, the invention provides methods to: identify a multiple myeloma patient that would benefit from a treatment comprising bortezomib or determine if a multiple myeloma patient would or would not benefit from a

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treatment comprising bortezomib. These methods entail determining whether a patient has a 14q32 translocation by measuring the gene expression level of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, MAFB, or a combination thereof, where elevated levels of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, or MAFB, relative to a suitable reference (*e.g.* predetermined reference values, positive and/or negative controls processed in parallel with the sample, *et cetera*), indicates the presence of a 14q32 translocation, and the presence or absence of a 14q32 translocation guides the treatment decision.

[0013] In a further aspect, the invention provides methods of treating multiple myeloma in a subject. These methods entail providing a suitable treatment comprising bortezomib or not comprising bortezomib to the subject on the basis of the the result of methods provided by the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0015] **FIG. 1** depicts a schematic representation of human chromosome 14. The Immunoglobulin Heavy (IgH) locus is positioned at region 3, band 2 of the long arm of chromosome 14 (14q32).

[0016] **FIGs. 2A-2B** depicts a schematic representation of human chromosome 14q32 (**FIG. 2A**) and 4p16 (**FIG. 2B**). In **FIG. 2A**, the green line represents a commercially available Locus Specific Identified (LSI) probe for the IgH locus, and the green arrows represent IgHC and IgHV specific probes of the invention. In **FIG. 2B**, the orange line represents a commercially available Locus Specific Identified (LSI) probe for the FGFR3/MMSET region of 4p16, and the red arrows represent FGFR3 and MMSET specific probes of the invention. Note, using the larger probes depicted in **FIG. 2A** and **FIG. 2B**, the definition of t(4;14) is if any fusion occurs between the two large probes (represented by lines). In contrast, using the probes of the invention (represented by arrows), specific gene fusions between IgHV/MMSET and FGFR3/IgHC can be detected, providing greater specificity.

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[0017] **FIG. 3A** depicts an illustration of human chromosome 14 and the binding location of the IgHC (green arrow) and IgHV (red arrow) FISH probes. **FIG. 3B** shows representative images of the probes binding normal chromosome 14.

[0018] **FIG. 4** shows normal 14q32 FISH using the IgHC (green) and IgHV (red) probes. The left panel shows normal G-banded chromosomes hybridized with IgHC and IgHV probes; the right panel shows DAPI-stained normal chromosomes hybridized with IgHC and IgHV probes.

[0019] **FIG. 5** presents images of normal plasma cell interphase 14q32 FISH using the IgHC (green) and IgHV (red) probes. Left panel: cytoplasmic immunoglobulin (cIg) *Kappa* light chain isotype stain; Right panel: *Lambda* cIg. The white bar indicates 2 micrometer (μm).

[0020] **FIG. 6A** depicts an illustration of human chromosome 4 and the binding location of the FGFR3 (green arrow) and MMSET (red arrow) FISH probes. **FIG. 6B** shows representative images of the probes binding normal chromosome 4.

[0021] **FIG. 7** shows normal 4q16 FISH using the FGFR3 (green) and MMSET (red) FISH probes. The right panel shows normal G-banded chromosomes hybridized with FGFR3 and MMSET probes; the left panel shows DAPI-stained normal chromosomes hybridized with FGFR3 and MMSET probes.

[0022] **FIG. 8** depicts schematic representation of reciprocal translocation between 4p16 and 14q32 ($t(4;14)(p16;q32)$). The images on the left of the arrow depict normal chromosome 4 (far left) and chromosome 14 (near left). Areas of interest are identified by the circles, representing the binding location of the FISH probes. The images on the right of the arrow depict the chromosomes after the translocation event. The circles again identify where the FISH probes bind, and illustrate that IgHV is now on chromosome 4 (near right) and FGFR3 is now on chromosome 14 (far right).

[0023] **FIG. 9** depicts a graph showing baseline GEP levels of MMSET expression in patients enrolled in TT4 and TT5 Protocols. MMSET expression levels quantified using the Affymetrix U133plus2.0 microarray probe set 209054_s_at are graphed along the y-axis. The gene expression threshold valued indicative of $t(4;14)$ and an IgHV/MMSET gene fusion was determined to be 2,405.

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[0024] **FIG. 10** depicts a graph showing baseline GEP levels of FGFR3 expression in patients enrolled in TT4 and TT5 Protocols. FGFR3 expression levels quantified using the Affymetrix U133plus2.0 microarray probe set 204379_s_at are graphed along the y-axis. The gene expression threshold valued indicative of t(4;14) and an FGFR3/IgHC gene fusion was determined to be 6,558.

[0025] **FIG. 11** depicts a graph showing baseline GEP levels of CCND1 expression in patients enrolled in TT4 and TT5 Protocols. CCND1 expression levels quantified using the Affymetrix U133plus2.0 microarray probe set 208711_s_at are graphed along the y-axis. The gene expression threshold valued indicative of t(11;14) and a CCND1/IgHC gene fusion was determined to be 5,525.

[0026] **FIG. 12** depicts a graph showing baseline GEP levels of CCND3 expression in patients enrolled in TT4 and TT5 Protocols. CCND3 expression levels quantified using the Affymetrix U133plus2.0 microarray probe set 201700_at are graphed along the y-axis. The gene expression threshold valued indicative of t(6;14) and a CCND3/IgHC gene fusion was determined to be 15,696.

[0027] **FIG. 13** depicts a graph showing baseline GEP levels of MAF expression in patients enrolled in TT4 and TT5 Protocols. MAF expression levels quantified using the Affymetrix U133plus2.0 microarray probe set 206363_at are graphed along the y-axis. The gene expression threshold valued indicative of t(14;16) and an MAF/IgHC gene fusion was determined to be 5,096.

[0028] **FIG. 14** depicts a graph showing baseline GEP levels of MAFB expression in patients enrolled in TT4 and TT5 Protocols. MAFB expression levels quantified using the Affymetrix U133plus2.0 microarray probe set 222670_s_at are graphed along the y-axis. The gene expression threshold valued indicative of t(14;20) and an MAFB/IgHC gene fusion was determined to be 19,850.

[0029] **FIGs. 15A-B** are two graphs showing overall survival (OS) of patients enrolled in TT2 (**FIG. 15A**) and TT3a/b (**FIG. 15B**) based on the presence or absence of t(4;14) predicted by the gene expression threshold value determined for MMSET. Blue lines indicate the survival probability (y-axis) over time (x-axis) for patients with MMSET expression levels less than the threshold value. Red lines indicate the survival probability (y-axis) over time (x-axis) for patients with

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MMSET expression levels equal to or greater than the threshold value. Patients in TT3 with t(4;14) predicted by the MMSET threshold value (**FIG. 15B**, red line) benefited from bortezomib therapy and had improved OS compared to patients not predicted to have t(4;14) (**FIG. 15B**, blue line), and which was not available to patients in TT2 (**FIG. 15A**). In these experiments, cRNA synthesis from cDNA was performed with an older GeneChip labeling kit (Affymetrix) no longer available.

[0030] **FIGs. 16A-B** are two graphs showing overall survival (OS) of patients enrolled in TT2 (**FIG. 16A**) and TT3a/b (**FIG. 16B**) based on the presence or absence of t(4;14) predicted by the gene expression threshold value determined for FGFR3. Blue lines indicate the survival probability (y-axis) over time (x-axis) for patients with FGFR3 expression levels less than the threshold value. Red lines indicate the survival probability (y-axis) over time (x-axis) for patients with FGFR3 expression levels equal to or greater than the threshold value. Patients in TT3 with t(4;14) predicted by the FGFR3 threshold value (**FIG. 16B**, red line) benefited from bortezomib therapy and had improved OS compared to patients not predicted to have t(4;14) (**FIG. 16B**, blue line), and which was not available to patients in TT2 (**FIG. 16A**). In these experiments, cRNA synthesis from cDNA was performed with an older GeneChip labeling kit (Affymetrix) no longer available.

[0031] **FIGs. 17A-B** are two graphs showing overall survival (OS) of patients enrolled in TT2 (**FIG. 17A**) and TT3a/b (**FIG. 17B**) based on the presence or absence of t(6;14) predicted by the gene expression threshold value determined for CCND3. Blue lines indicate the survival probability (y-axis) over time (x-axis) for patients with CCND3 expression levels less than the threshold value. Red lines indicate the survival probability (y-axis) over time (x-axis) for patients with MMSET expression levels equal to or greater than the threshold value. Patients in TT3 with t(6;14) predicted by the CCND3 threshold value (**FIG. 17B**, red line) benefited from bortezomib therapy and had improved OS compared to patients not predicted to have t(6;14) (**FIG. 17B**, blue line), and which was not available to patients in TT2 (**FIG. 17A**). In these experiments, cRNA synthesis from cDNA was performed with an older GeneChip labeling kit (Affymetrix) no longer available.

[0032] **FIGs. 18A-B** are two graphs showing overall survival (OS) of patients enrolled in TT2 (**FIG. 18A**) and TT3a/b (**FIG. 18B**) based on the presence or absence of t(11;14) predicted by the gene expression threshold value determined for CCND1. Blue lines indicate the survival probability (y-axis) over time (x-axis) for patients with CCND1 expression levels less than the threshold value. Red lines indicate the survival probability (y-axis) over time (x-axis) for patients with CCND1 expression levels equal to or greater than the threshold value. Patients in TT3 with t(11;14) predicted by the CCND1 threshold value (**FIG. 18B**, red line) benefited from bortezomib therapy and had improved OS compared to patients not predicted to have t(11;14) (**FIG. 18B**, blue line), and which was not available to patients in TT2 (**FIG. 18A**). In these experiments, cRNA synthesis from cDNA was performed with an older GeneChip labeling kit (Affymetrix) no longer available.

[0033] **FIGs. 19A-B** are two graphs showing overall survival (OS) of patients enrolled in TT2 (**FIG. 19A**) and TT3a/b (**FIG. 19B**) based on the presence or absence of t(14;20) predicted by the gene expression threshold value determined for MAFB. Blue lines indicate the survival probability (y-axis) over time (x-axis) for patients with MAFB expression levels less than the threshold value. Red lines indicate the survival probability (y-axis) over time (x-axis) for patients with MAFB expression levels equal to or greater than the threshold value. Patients in TT3 with t(14;30) predicted by the MAFB threshold value (**FIG. 19B**, red line) benefited from bortezomib therapy and had improved OS compared to patients not predicted to have t(14;20) (**FIG. 19B**, blue line), and which was not available to patients in TT2 (**FIG. 19A**). In these experiments, cRNA synthesis from cDNA was performed with an older GeneChip labeling kit (Affymetrix) no longer available.

[0034] **FIGs. 20A-B** are two graphs showing overall survival (OS) of patients enrolled in TT2 (**FIG. 20A**) and TT3a/b (**FIG. 20B**) based on the presence or absence of t(14;16) predicted by the gene expression threshold value determined for MAF. Blue lines indicate the survival probability (y-axis) over time (x-axis) for patients with MAF expression levels less than the threshold value. Red lines indicate the survival probability (y-axis) over time (x-axis) for patients with MAF expression levels equal to or greater than the threshold value. Patients in TT3 with

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t(14;16) predicted by the MAF threshold value (**FIG. 20B**, red line) benefited from bortezomib therapy and had worse OS compared to patients not predicted to have t(14;16) (**FIG. 20B**, blue line), and which was not available to patients in TT2 (**FIG. 20A**). In these experiments, cRNA synthesis from cDNA was performed with an older GeneChip labeling kit (Affymetrix) no longer available.

[0035] **FIG. 21** shows representative images of 14q32 translocations by FISH using a IgHC specific probe (green probe, top row) and a IgHV specific probe (green probe, bottom row) in multiple myeloma plasma cells. The second probe is either an FRFG3 specific probe (red probe, left panels), a MMSET specific probe (red probe, middle panels), or a MAF specific probe (red probe, right panels). The white bars indicate 2 micrometers.

[0036] **FIG. 22** shows IgHC more efficiently drives expression of MAFB (measured by GEP) compared to IgHV. Briefly, FISH with IgHC/MAFB or IgHV/MAFB probe combination was performed on individual spots of myeloma cells. GEP values of MAFB are shown in the far left column (e.g., 475 – 40,331.1). Open (white) circles indicate the number of FISH signal counts of IgHC or IgHV probe. Half-open circles indicate a t(14;20) occurred. Closed (black) circles indicate copy numbers of MAFB. Using GEP=19,850.9 as an example, FISH indicated myeloma cells had four copies of IgHC and four copies of IgHV, and a translocation was found involving a copy of IgHC and a copy of MAFB. In the case of GEP=853, two copies of IgHC and two copies of IgHV, and a translocation occurred between a copy of IgHV and a copy of MAFBs, but in these cells IgHV did not drive MAFB to spike (i.e., did not result in increased expression) as measured by GEP.

DETAILED DESCRIPTION OF THE INVENTION

[0037] The present invention provides, *inter alia*, new fluorescent *in situ* hybridization (FISH) probes and methods of using the same to identify translocations involving the immunoglobulin heavy chain (IgH) locus on the long arm of chromosome 14 region 32 (14q32 translocations). In addition to using the probes to more accurately identify 14q32 translocations, the probes may also be

used to establish for each translocation partner a gene expression threshold that is predictive of 14q32 translocations. The FISH probes and gene expression thresholds of the invention, and their methods of use are described below.

I. FISH probe

[0038] A probe provided by the invention encompasses, in certain embodiments, a nucleic acid suitable for use as a fluorescent *in situ* hybridization (FISH) probe. FISH is a cytogenetic technique used to visualize labeled DNA probes hybridized to a region of interest on a chromosome. FISH probes are designed to only bind those parts of the chromosome with which they show a high degree of sequence complementarity. Accordingly, as used to refer to probes herein, the term “complementary” includes “substantially complementary”, and refers to the ability of nucleic acids to hybridize by Watson-Crick base-pairing and form, at least partially, a double stranded structure. For example, a probe that hybridizes to a region of interest on a chromosome (*e.g.*, by Watson-Crick base-pairing) under the test conditions that are employed, and thus be useful for detecting and localizing the region, is said to be complementary or substantially complementary to the region of interest. Complementarity will be extensive enough so that the probes will form specific and/or stable hybrids with the target DNA under the hybridization conditions used. Persons of skill in the art will be able to determine suitable sequences through the general knowledge available in the art, and by routine experimentation, using the examples set forth herein below as guidelines.

[0039] In certain embodiments, complementary or substantially complementary means two sequences (*e.g.*, a probe and a target sequence) hybridize under highly stringent hybridization conditions. “Highly stringent hybridization” conditions, in some embodiments, refers to at least about 50% (v/v) Formamide with 2X SSC and 0.1% SDS at 42°C, with a first wash for 30 minutes at about 50°C with about 50% (v/v) Formamide in 2X SSC, and with a subsequent wash with 1XPBS and 0.01% IGEPAL CA-630 at room temperature. In other embodiments, “highly stringent hybridization” conditions refers to at least about 6X SSC and 1% SDS at 65°C, with a first wash for 10 minutes at about 42°C with about 20% (v/v) Formamide in 0.1X SSC, and with a subsequent wash with 0.2 X SSC and 0.1% SDS at 65°C. In certain

embodiments, complementarity or substantially complementary means a nucleic acid sequence is at least about: 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99%, or more (*e.g.* 100%) identical to the complement (*i.e.* reverse complement) of a region of interest.

[0040] Reference to a pair of probes, such as a pair of FISH probes, encompasses not only exactly two probes, such as a first probe and a second probe, but also, unless clearly indicated otherwise, additional probes, such as an additional 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more probes.

(a) Labeled DNA probes

[0041] In one aspect, a probe of the invention provides one or more labeled DNA probes. Labeled nucleic acid probes are synthetically and/or artificially labeled and are not naturally-occurring, but rather are products of human innovation. Probes may be indirectly labeled with haptens or directly labeled with a fluorochrome-conjugated nucleotide, *i.e.*, dUTP (2'-Deoxyuridine, 5'-Triphosphate) in place of dTTP. Non-limiting examples of suitable haptens include biotin and digoxigenin. Non-limiting examples of suitable fluorochromes include the Alexa fluor dye series, amino-methyl coumarin, cascade blue, cyanine 2^d, cyanine 3^d, cyanine 5^d, cyanine 7^d, DEAC, fluorescein, fluorescein isothiocyanate, rhodamine B and rhodamine derivatives (for example, 5(6)-carboxyrhodamine, 5-ROX, 5-TAMRA (5-carboxytetramethylrhodime), 6-TAMRA, *etc.*) and Texas Red. In some embodiments, the probe is directly labeled with a fluorochrome-conjugated nucleotide selected from the group consisting of DEAC-dUTP, 5-Fluorecein dUTP, 5(6)-Carboxyrhodamine 6G-dUTP, 5-TAMRA-dUTP, and 5-ROX-dUTP.

[0042] Labeling the probe can be accomplished by any suitable method known in the art. Briefly, depending on the source of the nucleic acid, probes are either labeled by nick translation and PCR, or labeled by PCR using region specific primers, primers to vector/plasmid sequences, universal primers or degenerative oligonucleotide primers. For greater detail, see Bayani J and Squire JA 2004 "Fluorescent In Situ Hybridization (FISH)" *Current Protocols in Cell Biology*, incorporated by reference herein.

(b) Hybridize to a region of interest on a chromosome

[0043] In another aspect, a probe provided by the invention hybridizes (i.e., is complementary or substantially complementary) to a region of interest on a chromosome. A probe can be designed to hybridize to any region on a chromosome. Generally, a probe of the invention is to be used to detect translocations involving the immunoglobulin heavy locus (IgH) on the long arm of chromosome 14 region 32 (**FIG. 1**). As used herein, translocations involving IgH on the long arm of chromosome 14 region 32 may be abbreviated as “14q32 translocations” or “t14q32”. The IgH locus includes variable (IgHV), diversity (IgHD), joining (IgHJ), and constant (IgHC) segments (**FIG. 1 and FIG. 2A**). 14q32 translocations involve many chromosomal partners (translocation partners). For example, 14q32 translocation partners in multiple myeloma may include, but are not limited to, Fibroblast Growth Factor Receptor 3 (FGFR3, human GeneID No. 2261), Wolf-Hirschhorn syndrome candidate 1 (WHSC1 or MMSET or WHSC1/MMSET, human GeneID No. 7468), Cyclin D3 (CCND3, human GeneID No. 896), Cyclin D1 (CCND1, human GeneID No. 595), V-maf musculoaponeurotic fibrosarcoma oncogene homolog (MAF, human GeneID No. 4094), V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB, human GeneID No. 9935) and c-MYC (human GeneID No. 4609).

[0044] Several factors determine the length of a FISH probe. For example, probes designed to identify chromosomal translocations would be sufficiently long to hybridize to a region of interest on a chromosome (e.g., some or all of a chromosomal region encoding the IgH genomic locus or a genomic locus of a 14q32 translocation partner) but short enough to exclude other potential translocation partners. As used herein, genomic locus refers to the specific location of a DNA sequence on a chromosome that encodes a polypeptide or RNA chain that has a function in the organism, and includes all associated regulatory regions, transcribed regions, termination sequences, and other functional sequence regions. Preferably, a probe of the invention is specific for a single genomic locus. As used herein, “specific” means a probe comprises a nucleic acid sequence that is complementary to a particular sequence of interest, e.g., a genomic locus or target transcript thereof.

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A probe specific for a (*e.g.*, single) genomic locus is not complementary or substantially complementary to, for example, a second genomic locus or a target transcript thereof. For some genomic loci, it is more advantageous to design a probe specific for only a portion of, or a segment of, a single locus. For example, the IgH locus is approximately 1,250 kb in length and comprises the four segments described above (*i.e.*, IgHC, IGHI, IGHD, IGHV), which encode an estimated 170-176 different genes. It has been discovered that (i) smaller probes to the IgH locus reduce the false positive rate of 14q32 translocations identified by current commercially available probes, and (ii) when a 14q32 translocation event occurs, IgHC drives expression of its translocation partner more efficiently than IGHV drives expression of its translocation partner, and increased expression driven by IgHC can be used prognostically to identify patients who will be responsive bortezomib therapy. This is described in more detail in **Example 4**.

[0045] In some embodiments, a probe comprises a DNA sequence complementary to a chromosomal region comprising an entire genomic locus. In other embodiments, a probe comprises a DNA sequence complementary to a chromosomal region comprising a portion of a genomic locus. In still other embodiments, a probe comprises a DNA sequence complementary to a chromosomal region comprising some or all of a genomic locus, as well as DNA sequences upstream and/or downstream of the genomic locus. In exemplary embodiments, a probe comprises a DNA sequence specific for a single genomic locus. In other exemplary embodiments, a probe comprises a DNA sequence specific for segment of a genomic locus. In some preferred embodiments, a probe comprises a DNA sequence complementary to a chromosomal region encoding a genomic locus comprising IgHC, IGHV, FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, MAFB, c-MYC, or a combination thereof. In other preferred embodiments, a probe comprises a DNA sequence specific to a genomic locus comprising IgHC, IGHV, FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, MAFB, c-MYC, or a combination thereof.

[0046] A skilled artisan will appreciate, therefore, that the length of the probe can and will vary. Factors including, but not limited to, the genomic locus and the

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proximity of adjacent loci on the chromosome may influence the maximal length of the probe. Generally, a probe of the invention is about 1 to about 500 kilobases (kb) in length or about 50 to about 500 kb in length. In some embodiments, a probe is about 1 to about 100 kb in length. For example, a probe is about 1 to about 50 kb, or about 50 to about 100 kb in length. In other embodiments, a probe is about 100 to about 200 kb in length. For example, a probe is about 100 to about 150 kb, or about 150 to about 200 kb in length. In still other embodiments, a probe is about 200 to about 300 kb in length. For example, a probe is about 200 to about 250 kb, or about 250 to about 300 kb in length. In still other embodiments, a probe is about 300 to about 400 kb in length. For example, a probe is about 300 to about 350 kb, or about 350 to about 400 kb in length. In yet other embodiments, a probe is about 400 to about 500 kb in length. For example, a probe is about 400 to about 450 kb, or about 450 to about 500 kb in length. In some exemplary embodiments, a probe is about 100-300 kb in length. In other exemplary embodiments, a probe is about 200-400 kb in length. In still other exemplary embodiments, a probe is about 300-500 kb in length.

[0047] Any suitable source (*i.e.*, template) of nucleic acid may be used to produce a probe of the invention. Generally, the source is a vector comprising the DNA sequence complementary to a region of interest on a chromosome. As used herein, "vector" refers to an autonomously replicating nucleic acid unit. The present invention can be practiced with any known type of vector, including viral, cosmid, phasmid, artificial chromosomes, and plasmid vectors. Non-limiting examples of artificial chromosomes include BACs (bacterial artificial chromosomes), PACs (P1 bacteriophage-derived artificial chromosome), and YACs (yeast artificial chromosomes). Alternative sources of DNA for FISH include, but are not limited to, genomic DNA from flow sorted chromosomes and genomic DNA from microdissected DNA. In exemplary embodiments, the source of DNA for a probe is a BAC. In other exemplary embodiments, a source of DNA for a probe is a PAC. In still other exemplary embodiments, a source of DNA for a probe is a YAC.

[0048] Vectors containing DNA complementary to a region of interest on a chromosome may be purchased through commercial vendors, or may be generated

by the methods known in the art (Osoegawa K., *et al.*, 2001 Genome Res 11(3):483-96). In some embodiments, a source of DNA for a probe is selected from the group of BAC clones listed in **Table 1**.

II. Use of the probe

[0049] A FISH probe of the invention may be used for cytogenetic and genomic analyses using methods well known in the art. Generally, locus specific probes are used to detect gene fusions, translocations, deletions, and amplifications. According to the invention, methods of the invention encompass means to detect 14q32 translocations and to establish for each translocation partner a gene expression threshold that is predictive of 14q32 translocations.

(a) Method to detect 14q32 translocations

[0050] In one aspect, a method of the invention provides a means to detect 14q32 translocations in a sample by metaphase FISH or interphase FISH using (at least) a pair of FISH probes. Chromosomal translocations can be detected as gene fusions. A gene fusion may be created when the translocation joins two otherwise separated genes. Non-limiting examples of gene fusions associated with 14q32 translocations include IgHV/MMSET, FGFR3/IgHC, CCND3/IgHC, CCND1/IgHC, MAF/IgHC, and MAFB/IgHC. To detect a translocation as a gene fusion, the two probes are labeled with different fluorochromes, each fluorochrome emitting at different ultraviolet-wavelengths. When a translocation occurs, the two probes are brought into close proximity. The gene fusion may appear as two colors near each other, or the two colors may appear as a third color (for example, a red probe and green probe may produce a yellow signal). In some embodiments, a first probe comprises a DNA sequence complementary to a chromosomal region encoding a genomic locus or a segment of a genomic locus selected from the group consisting of IgH, IgHC, and IgHV, and a second probe comprises a nucleic acid sequence complementary to a chromosomal region encoding a genomic locus selected from the group consisting of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, and MAFB. In other embodiments, a first probe comprises a nucleic acid sequence specific for a genomic locus or a segment of a genomic locus selected from the

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group consisting of IgH, IgHC, and IgHV, and a second probe comprises a DNA specific for a genomic locus selected from the group consisting of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, and MAFB. In still other embodiments, a first probe comprises a DNA sequence specific for a genomic locus or a segment of a genomic locus selected from the group consisting of IgH, IgHC, or IgHV, and a second probe comprises a DNA sequence complementary to a chromosomal region encoding a genomic locus selected from the group consisting of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, and MAFB. In yet other embodiments, a first probe comprises a DNA sequence complementary to a chromosomal region encoding a genomic locus or a segment of a genomic locus selected from the group consisting of IgH, IgHC, or IgHV, and a second probe comprises a DNA sequence specific for a genomic locus selected from the group consisting of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, and MAFB. Substantially similar probes to any of the forgoing probes (the reference probes) can also be used consonant with the invention. Substantially similar probes are those that hybridize to the reference probes, or their complements, under highly stringent hybridization conditions or, in other embodiments, are at least about: 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99%, or more identical to the reference probe. In a preferred embodiment, a first probe comprises a DNA sequence specific for IgHC, and a second probe comprises a DNA sequence complementary to a chromosomal region encoding a genomic locus selected from the group consisting of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, or MAFB. Suitable fluorochromes and probes are further described in **Section I**. In particular embodiments, probes provided by the invention and/or useful in the methods provided by the invention, as described above, are those described in **Table 1**, or probes substantially similar to those in **Table 1**.

[0051] The methods of the invention may be performed on any sample comprising a nucleic acid, for example, containing any cell type with a nucleus. A cell may or may not have a 14q32 translocation. In some embodiments, a cell has a 14q23 translocation. In other embodiments, a cell does not have a 14q32 translocation. In some embodiments, a sample is comprised of cells grown *in vitro*.

In other embodiments, a sample is a biological sample. As used herein, “biological sample” refers to a sample derived (*i.e.*, isolated) from a subject. In some embodiments, a biological sample is modified, *e.g.*, through staining or detectable labeling, such that the biological sample is a new res, that is not naturally occurring and is instead a product of human ingenuity. Suitable subjects may include a human, a livestock animal, a companion animal, a laboratory animal, or a zoological animal. In one embodiment, a subject may be a rodent, *e.g.*, a mouse, a rat, a guinea pig, *etc.* In another embodiment, a subject may be a livestock animal. Non-limiting examples of suitable livestock animals may include pigs, cows, horses, goats, sheep, llamas and alpacas. In yet another embodiment, a subject may be a companion animal. Non-limiting examples of companion animals may include pets such as dogs, cats, rabbits, and birds. In yet another embodiment, a subject may be a zoological animal. As used herein, a “zoological animal” refers to an animal that may be found in a zoo. Such animals may include non-human primates, large cats, wolves, and bears. In yet another embodiment, a subject may be a laboratory animal. Non-limiting examples of a laboratory animal may include rodents, canines, felines, and non-human primates. In a preferred embodiment, a subject is human.

[0052] The subject may or may not have been diagnosed with a disease or condition associated with a 14q32 translocation. IgH translocations are common in B-cell malignancies. Non-limiting examples of B-cell malignancies include precursor B-cell acute lymphoblastic leukemia (B-ALL), B-cell chronic lymphoblastic leukemia (B-CLL), B-cell non-Hodgkin’s lymphoma (including, but not limited to, follicular, mantle cell, Burkitt, diffuse large B-cell, marginal cell, mucosa-associated lymphoid tissue (MALT) lymphoma, and lymphoplasmacytic lymphoma) and myeloma. In some embodiments, the subject has no clinical signs or symptoms of disease. In other embodiments, the subject has clinical signs of disease. In yet other embodiments, the subject may be at risk for disease. In still other embodiments, the subject has been diagnosed with disease. In some exemplary embodiments, a subject has a tumor. In other exemplary embodiments, a subject has lymphoma. In still other exemplary embodiments, a subject has leukemia. In yet other exemplary embodiments, a subject has a B-cell malignancy.

In a preferred embodiment, a subject has multiple myeloma. “Multiple myeloma” includes symptomatic myeloma, asymptomatic myeloma, and monoclonal gammopathy of undetermined significance (MGUS), as defined in Kyle and Rajkumar *Leukemia* 23:3-9 (2009, PubMedID 18971951, incorporated by reference in its entirety), as well as the other stratifications and stages described in Kyle and Rajkumar 2009. In particular embodiments, multiple myeloma is symptomatic myeloma.

[0053] The method of the invention may be used with any of the numerous types of biological samples known in the art. Non-limiting examples may include tissue samples or bodily fluids. In some embodiments, the biological sample is a tissue sample such as a tissue biopsy—*e.g.* a sample containing plasma cells, such as a bone marrow sample. The tissue biopsy may be a biopsy of a tumor. The biopsied tissue may be fixed, embedded in paraffin or plastic, and sectioned, or the biopsied tissue may be frozen and cryosectioned. Alternatively, the biopsied tissue may be processed into individual cells or an explant, or processed into a homogenate, a cell extract, or a membranous fraction. The sample may also be primary and/or transformed cell cultures derived from tissue from the subject. In other embodiments, the sample may be a bodily fluid. Non-limiting examples of bodily fluids include cerebrospinal fluid, interstitial fluid, blood, serum, plasma, saliva, sputum, semen, tears, lymph and urine. The fluid may be used “as is”, the cellular components may be isolated from the fluid, or a protein fraction may be isolated from the fluid using standard techniques. In some preferred embodiments, the sample may be selected from the group consisting of blood, plasma, serum or lymph. In other preferred embodiments, the sample is a biopsy of a tumor.

[0054] As will be appreciated by a skilled artisan, the method of collecting a biological sample can and will vary depending upon the nature of the biological sample and the type of analysis to be performed. Any of a variety of methods generally known in the art may be utilized to collect a biological sample. Generally speaking, the method preferably maintains the integrity of the sample such that it can be accurately measured according to the method of the invention. For example, metaphase FISH requires the availability of viable cells from which to produce

chromosome preparations for analysis, whereas interphase FISH is able to make use of preserved cellular material and may thus be extended to include the use of paraffin embedded biopsy sections.

[0055] Methods of sample preparation for interphase FISH and metaphase FISH are well known in the art. Briefly, a sample is fixed, the fixed sample is hybridized in a buffer containing the labeled probe(s) at a temperature which favors the specific binding of the probe(s) to target, the sample is washed, and then the hybridized sample is analyzed by fluorescence microscopy or by flow cytometry. For greater detail, see the **Examples** and Bayani J and Squire JA 2004 “Fluorescent In Situ Hybridization (FISH)” *Current Protocols in Cell Biology*.

(b) Method to detect IgHC translocations

[0056] In another aspect, a method of the invention provides a means to detect IgHC translocations in a sample by interphase FISH or metaphase FISH using at least a pair of FISH probes. In some embodiments, a first probe comprises a DNA sequence specific for IgHC, and a second probe comprises a DNA sequence complementary to a chromosomal region encoding a genomic locus selected from the group consisting of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, and MAFB. In other embodiments, a first probe comprises a DNA sequence specific for IgHC, and a second probe comprises a DNA sequence specific for a genomic locus selected from the group consisting of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, and MAFB. In exemplary embodiments, a first probe comprises a clone, such as a BAC clone (*e.g.*, as described in **Table 1**), and a second probe comprises a clone selected from the group consisting of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, or MAFB (*e.g.*, as described in **Table 1**). Suitable fluorochromes and probes are further described in **Section I**. Suitable samples and methods of interphase and metaphase FISH are described in **Section II(a)**.

(c) Method to establish a gene expression threshold value

[0057] In another aspect, a method of the invention provides a means to establish a gene expression threshold value for a 14q32 translocation partner, above which a gene expression level indicates a 14q32 translocation in a subject with a B-

cell malignancy. Current methods do not exist to differentiate gene expression profile (GEP)-based spikes in expression representing 14q32 translocations from those reflecting changes in other genetic abnormalities in a subject with a B-cell malignancy that may result in increased expression levels, for example hyperdiploidy or gene amplification. It has been discovered that IgHC drives expression of its translocation partner more efficiently than IgHV drives expression of its translocation partner. Further, a unique GEP threshold value can be defined for each gene fusion that results from a 14q32 translocation (*e.g.*, IgHV-translocation partner gene fusion or translocation partner-IgHC gene fusion), such that the gene expression threshold value can be used to determine whether increased expression is either a result of a 14q32 translocation with the translocation partner or gene copy number driven increases in expression.

[0058] Accordingly, in some embodiments, a method of the invention comprises (a) obtaining biological samples from subjects with 14q32 translocations and without 14q32 translocations, (b) measuring the expression level of a 14q32 translocation partner in the samples, (c) determining the presence or absence of a 14q32 translocation by FISH using at least a pair of probes, wherein the first probe comprises a DNA sequence specific for IgHC or IgHV, and the second probe comprises a DNA sequence complementary to a chromosomal region encoding the translocation partner whose expression was measured in (b), (d) comparing the expression level of the translocation partner with the presence or absence of a 14q32 translocation, and (e) determining a gene expression threshold value for the translocation partner above which indicates increased expression due to a 14q32 translocation and below which indicates increased expression not due to a 14q32 translocation. Measuring the expression level of two or more 14q32 translocation partners in a single sample is also contemplated, and within the scope of the invention. Accordingly, the presence or absence of a 14q32 translocation will then need to be determined by FISH for each translocation partner (*e.g.*, a different second probe will need to be for each FISH analysis), and a unique gene expression threshold will need to be determined for each translocation partner. Suitable biological samples and FISH probes are described in above in **Section I**. Suitable

samples, sample preparation and methods of interphase and metaphase FISH are described in **Section II(a)**.

[0059] Suitable methods to measure gene specific expression levels are well known in the art, and further detailed in the **Examples**. In more particular embodiments, the gene expression levels are tested by quantitative polymerase chain reaction (qPCR), quantitative real-time polymerase chain reaction (qRT-PCR), digital droplet PCR, (ddPCR), sequencing (including next generation sequencing, including various sequencing by synthesis or sequencing by ligation methodologies), microarray hybridization (to any microarray platform with suitable probes for detecting 14q32 translocation partners), northern blotting, or Southern blotting.

[0060] In some embodiments, expression levels of 14q32 translocation partners are measured by microarray analysis. For example, gene expression levels of one or more of FGFR3, WHSC1/MMSET, CCND3, CCND1, MAF, or MAFB may be measured with, for example, the Affymetrix U133 Plus 2.0 Array GeneChip, using probes 204379_s_at, 209054_s_at, 201700_at, 208711_s_at, 206363_at, and 222670_s_at (*see, e.g., Table 2*), respectively, or substantially similar probes. For clarity, the method of the invention is not limited to the use of these specific probes. Any suitable probe and/or gene chip may be used to establish a threshold value specific for that probe/chip. In other embodiments, expression levels of 14q32 translocation partners are measured by quantitative RT-PCR.

[0061] In some embodiments, the (at least) pair of probes is selected from a group consisting of (i) a first probe comprising a DNA sequence specific for IgHV and a second probe comprising a DNA sequence specific for MMSET, (ii) a first probe comprising a DNA sequence specific for IgHC and a second probe comprising a DNA sequence specific for FGFR3, (iii) a first probe comprising a DNA sequence specific for IgHC and a second probe comprising a DNA sequence specific for CCND1, (iv) a first probe comprising a DNA sequence specific for IgHC and a second probe comprising a DNA sequence specific for CCND3, (v) a first probe comprising a DNA sequence specific for IgHC and a second probe comprising a DNA sequence specific for MAF, and (vi) a first probe comprising a DNA sequence

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specific for IgHC and a second probe comprising a DNA sequence specific for MAFB.

[0062] In some exemplary embodiments, the translocation is t(4;14), the gene fusion is MMSET/IgHV, the expression of MMSET is measured by microarray analysis using the Affymetrix U133 Plus 2.0 Array GeneChip 209054_s_at, and the threshold value predictive of a translocation in a subject with multiple myeloma is 2,405. In other exemplary embodiments, the translocation is t(4;14), the gene fusion is FGFR3/IgHC, the expression of FGFR3 is measured by microarray analysis using the Affymetrix U133 Plus 2.0 Array GeneChip 204379_s_at, and the threshold value predictive of a translocation in a subject with multiple myeloma is 6,558. In still other exemplary embodiments, the translocation is t(6;14), the gene fusion is CCND3/IgHC, the expression of CCND3 is measured by microarray analysis using the Affymetrix U133 Plus 2.0 Array GeneChip 201700_at, and the threshold value predictive of a translocation in a subject with multiple myeloma is 15,696. In yet other exemplary embodiments, the translocation is t(11;14), the gene fusion is CCND1/IgHC, the expression of CCND1 is measured by microarray analysis using the Affymetrix U133 Plus 2.0 Array GeneChip 208711_s_at, and the threshold value predictive of a translocation in a subject with multiple myeloma is 5,525. In different exemplary embodiments, the translocation is t(14;16), the gene fusion is MAF/IgHC, the expression of MAF is measured by microarray analysis using the Affymetrix U133 Plus 2.0 Array GeneChip 206363_at, and the threshold value predictive of a translocation in a subject with multiple myeloma is 5,096. In alternative exemplary embodiments, the translocation is t(14;20), the gene fusion is MAFB/IgHC, the expression of MAFB is measured by microarray analysis using the Affymetrix U133 Plus 2.0 Array GeneChip 222670_s_at, and the threshold value predictive of a translocation in a subject with multiple myeloma is 19,850.

(d) Method to predict a 14q32 translocation

[0063] In another aspect, a method of the invention provides a means to predict/detect one or more 14q32 translocations in a subject suspected to have a B-cell malignancy, such as multiple myeloma, the method comprising (a) obtaining a biological sample from the subject, (b) measuring the expression level for one or

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more 14q32 translocation partners in the biological sample, and (c) comparing the expression level to a gene expression threshold value indicative of a 14q32 translocation for each translocation partner, wherein if the expression level for a 14q32 translocation partner in the biological sample is greater than the threshold value, then the subject is determined to have a 14q32 translocation.

Advantageously, when expression levels are measured by GEP, the method of the invention allows a skilled artisan to simultaneously identify multiple 14q32 translocations in a single analysis, such as 2, 3, 4, or 5 translocations simultaneously. In contrast, identification of multiple 14q32 translocations by FISH would require multiple samples to be processed in parallel. In some embodiments, the expression level for one or more 14q32 translocation partners is measured in the biological sample. In other embodiments, the expression level for two or more 14q32 translocation partners is measured in the biological sample. In still other embodiments, the expression level for three or more 14q32 translocation partners is measured in the biological sample. In yet other embodiments, the expression level for four or more 14q32 translocation partners is measured in the biological sample. In some other embodiments, the expression level for five or more 14q32 translocation partners is measured in the biological sample. In different embodiments, the expression level for six or more 14q32 translocation partners is measured in the biological sample.

[0064] Suitable biological samples are described above in **Section I**, as are methods to obtain samples from a subject. Suitable methods to measure gene specific expression levels and determine a gene expression threshold value indicative of a 14q32 translocation are described above in **Section II(c)**, and further detailed in the **Examples**.

(e) Method to predict response to therapy

[0065] In another aspect, a method of the invention provides means to predict response to therapy. For example, gene expression data and/or metaphase or interphase FISH may be used to stratify subjects with B-cell malignancies, such as multiple myeloma, into those with predicted or confirmed 14q32 translocations, and predict outcome (e.g., response to therapy) with the presence or absence of a

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response to a therapy. As used herein, patients who respond to therapy are said to have benefited from therapy. Typical responses to therapy measured in clinical practice include, but are not limited to, overall survival, event free survival, time to progression, time to death, partial response, and complete response. These terms are well known in the art and are intended to refer to specific parameters measured during clinical trials and in clinical practice by a skilled artisan. As detailed in **Example 4**, it has been discovered that myeloma patients with t(14,16) in their tumor cells did not benefit from bortezomib (VELCADE®, *see, e.g.*, ChemID 387447) therapy. In fact, the median survival was reduced from 8 years (TT2, without bortezomib) to 3 years for TT3a/3b subgroup of patients (treated with bortezomib) compared to other groups of 14q32 translocations. In contrast, patients with other types of 14q32 translocations benefited from bortezomib therapy. One skilled in the art would appreciate that the method of the invention may be applied to any therapy suitable for the treatment of B-cell malignancies, not just bortezomib. For example, B-cell malignancies may be treated with chemotherapy, radiotherapy, immunotherapy, and bone marrow transplant. Non-limiting examples of chemotherapy include proteasome inhibitors, alkylating agents (*e.g.*, melphalan, cyclophosphamide, cisplatin, carboplatin, oxaliplatin), anti-metabolites, taxanes (paclitaxel, docetaxel), vinca alkaloids, such as vincristine, vinblastine, vinorelbine, vindesine), topoisomerase inhibitors (etoposide, irinotecan, topotecan), cytotoxic antibiotics (doxorubicin, daunorubicin, epirubicin, bleomycin, mitomycin), histone deacetylase inhibitors, dexamethasone, thalidomide, and inhibitors of vascular endothelial growth factors. In some embodiments, the treatment is chemotherapy. In other embodiments, the treatment is radiotherapy. In still other embodiments, the treatment is immunotherapy. In yet other embodiments, the treatment is bone marrow transplant. In exemplary embodiments, the therapy is a proteasome inhibitor. In preferred embodiments, the therapy is bortezomib.

[0066] In some embodiments, a 14q32 translocation indicates a patient should receive a therapy or treatment. For example, a t(4;14), t(6;14), t(11;14), or t(14;20) translocation in a tumor cell from a subject with myeloma indicates a subject should receive treatment comprising bortezomib. In other embodiments, a 14q32

translocation indicates a patient should not receive a therapy or treatment comprising bortezomib. For example, a t(14;16) translocation in a tumor cell from a subject with myeloma indicates a subject should not receive a treatment comprising bortezomib.

[0067] Accordingly, in related aspects, the invention provides methods of treating B cell malignancies, such as multiple myeloma. These methods entail providing a treatment comprising bortezomib or not comprising bortezomib to a subject (such as a human subject with multiple myeloma), as appropriate, on the basis of the presence of a 14q32 translocation, where the presence of a 14q32 translocation is determined by a method provided by the invention—*e.g.*, either by FISH or GEP. For example, a subject with a translocation selected from t(4;14), t(6;14), t(11;14), or t(14;20) is administered a treatment comprising bortezomib. A subject with a t(14;16) translocation is administered a treatment that does not comprise bortezomib.

[0068] In certain embodiments, the invention also provides non-transient computer readable media comprising instructions that, when executed by a processor, cause the processor to perform steps consonant with the invention (*e.g.*, the methods of detecting translocations, predicting responses to therapy, as well as calculating thresholds for any of the foregoing, *et cetera*). For example, the instructions may entail accepting data representing expression levels of 14q32 translocation partners, comparing the values to suitable references (*e.g.* controls) and providing an evaluation of the data—*e.g.* whether, and which, translocation is be present in a sample, and any implication (*e.g.* whether a treatment comprising bortezomib should or should not be administered). The invention also provides systems comprising these computer readable media and a processor adapted to execute the instructions.

[0069] Method provided by the invention, in some embodiments, entail comparison to reference values (*e.g.* controls), including, for example predetermined reference values (*e.g.*, for known outcomes or determinations), positive and/or negative controls processed in parallel with the sample, *et cetera*.

EXAMPLES

[0070] The following examples illustrate various aspects of the invention.

Example 1: Novel panel of fluorescent *in situ* hybridization probes to identify 14q32 translocations

[0071] In order to identify 14q32 translocations (t14q32) more accurately, a panel of fluorescent *in situ* hybridization (FISH) probes that are specific for a single gene and do not cross-hybridize to other chromosomal loci were developed. Briefly, bacterial artificial chromosome (BAC) and P1 artificial chromosome (PAC) libraries were screened to identify individual clones encoding Fibroblast Growth Factor Receptor 3 (FGFR3), Wolf-Hirschhorn syndrome candidate I (MMSET), Cyclin D3 (CCND3), Cyclin D1 (CCND1); V-maf musculoaponeurotic fibrosarcoma oncogene homolog (MAF), V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB), IgH constant region (IgHC), and IgH variable region (IgHV).

[0072] Initially, a small radioactive (^{32}P) DNA probe was designed for screening BAC libraries (filters) or for performing PCR to amplify target PAC clone in an archive. After pinning down the clone locations (address; **Table 1**), BAC or PAC clones containing the chromosome segments of interest were purchased from the vendors. The bacterial colonies were re-examined for the DNA sequences, then, the plasmid was propagated from a single colony in large scale cultures. A high quality preparation of the plasmid was prepared using a commercially available kit and the probes were labeled in Nick Translation reactions to incorporate fluorochrome (green or red) conjugated dUTP into BAC/PAC DNA templates replacing dTTP. The reaction was optimized to fragment the template into 300-600 base pairs. Human placental DNA and herring sperm DNA were used to block repetitive sequences. All probes were validated by FISH on **normal** metaphase chromosomes. Representative images of the probes designed for IgHV, IgHC, FGFR3 and MMSET are presented in **FIGs. 3-6**.

Table 1: DNA probes used in FISH analyses

BAC/PAC Probes	Gene Cytogenetic Bands	Chromosomal Locations (mega-base-pairs from p arm telomere)	Length of the Inserts (kilo-base-pairs)
<i>IGHC</i>	14q32	106.08Mb – 106.32Mb	240
<i>IGHV</i>	14q32	106.69Mb – 106.84Mb	150
<i>MMSET</i>	4p16	1.86Mb – 2.06Mb	190
<i>FGFR3</i>	4p16	1.59Mb – 1.79Mb	190
<i>CCND3</i>	6p21	41.80Mb – 41.97Mb	170
<i>CCND1</i>	11q13	69.15Mb – 69.37Mb	220
<i>MAF</i>	16q23	80.63Mb – 80.80Mb	170
<i>MAFB</i>	20q12	39.19Mb – 39.34Mb	155

Example 2: Fluorescent *in situ* hybridization on healthy donor and myeloma patient cells using the new probes

[0073] To validate that the probes could identify 14q32 translocations in myeloma cells, FISH was applied to healthy donor and myeloma patient cells at interphase. One green and one red fluorescently labeled probe was used for each reaction—one for the selected region on chromosome 14q32, the other for its partner gene. Interphase FISH requires additional immunocytochemistry steps to recognize plasma cells in whole cell populations in bone marrow or blood using an immunoglobulin light chain specific antibody. All probes were documented for the correct locations in the human genome; and the 14q32 probe panel was optimized and suitable for all FISH applications.

[0074] 14q32 chromosomal translocations were identified by the merging of the two colors; green and red labeled probes yielding yellow fluorescence indicates

chromosomal translocation of two genes. Using these probes, it was found that t(4;14) were reciprocal fusions of IgHC/FGFR3 and IGHV/MMSET respectively (FIGs. 7 and 21).

Example 3: Development of a GEP-based expression threshold is indicative of 14q32 translocation event

[0075] To determine whether GEP-based expression spikes of t14q32 partner genes represent chromosomal translocations or changes in gene copy numbers, GEP-based expression data from 268 multiple myeloma (MM) patients was correlated with cytogenetic data developed using the new FISH probes. MM patients were enrolled Total Therapy (TT) 4 and 5 clinical protocols at the Myeloma Institute for Research and Therapy of University of Arkansas for Medical Sciences. The TT4 and TT5 protocols, initiated in late 2008, were the first myeloma treatment protocols at any facility to use risk-adapted therapy based on a robust predictive model built from GEP data. In the experiments described below, expression data on t14q32 partner genes was obtained using an Affymetrix U133plus2.0 chip (Table 2) and FISH was performed using the combinations of either the IgHC or IgHV probe with each probe of the partner genes.

[0076] First, GEP-based expression spikes confirmed to be associated with t(4;14) were analyzed. GEP-based expression spikes (*i.e.*, levels) of IgHC/FGFR3 were 5 fold higher than IgHV/MMSET spikes, suggesting IgHC is a highly efficient enhancer of expression. Other 14q32 translocations were also examined with the two IgH probes combined with each of the partners: t(6;14), t(11;14), t(14; 16), and t(14;20). It was found that IgHC was potent to facilitate the spikes in expression of t14q32 partner genes, while IgHV weakly promotes gene expression to the levels equivalent to gene copy increase (see FIG. 22).

[0077] Next, expression levels of the Affymetrix probe sets were correlated with FISH data and a signal threshold indicative of 14q32 translocation was defined (Table 3 and FIG. 9-14). Above this threshold, the expression level was clearly indicative of translocations between IgHC and the partners: t(4;14)(p16;q32); t(6;14)(p21;q32), t(11;14)(q13;q32); t(14;16)(q32;q23), or t(14;20)(q32;q11). Below

these thresholds, increased signals reflected gene copy numbers or the translocations between IgHV and the partners.

[0078] Overall, of the patients enrolled in the TT 4 and 5 clinical protocols, 42.2% (113/268) were confirmed to have 14q32 translocations: 14.9% had t(4;14), 3.4% t(6;14), 19.4% t(11;14), 3.4% t(14;16), and 1.1% t(14;20). In cases where the GEP analysis indicated simultaneous spikes of more than one 14q32 translocation partners in the same patient, we discovered that 14q32 translocation were mutually exclusive - one 14q32 translocation per clone. The spikes of the other 14q32 partner gene(s) shown by GEP reflected copy number driven increases, or in a few cases, the co-existence of distinct clones, each with a unique 14q32 translocation in the same patient.

Table 2: Affymetrix U133plus GeneChip probe sets

Gene	Probe Set
MMSET	209054_s_at
FGFR3	204379_s_at
CCND3	201700_at
CCND1	208711_s_at
MAF	206363_at
MAFB	222670_s_at

Table 3: GEP thresholds indicating 14q32 translocation

Probe	Translocation	GEP threshold
209054_s_at	t(4;14) MMSET / IGHV	2,405
204379_s_at	t(4;14) FGFR3 / IGHC	6,558
201700_at	t(6;14) CCND3 / IGHC	15,696
208711_s_at	t(11;14) CCND1 / IGHC	5,525
206363_at	t(14;16) MAF/ IGHC	5,096
222670_s_at	t(14;20) MAFB/ IGHC	19,850

Example 4: Validation of the GEP-based expression threshold and correlation with treatment outcome

[0079] The threshold expression levels identified with the TT4 and TT5 training set were used to predict 14q32 translocations in an earlier MM data set, patients enrolled in the TT2 and TT3 protocols. TT4 and TT5 were designed based on the learnings from TT2 and TT3, both of which incorporated new agents (thalidomide in TT2 and bortezomib in TT3) and adjusted timing of therapy to support stem cell transplants. Using threshold expression levels equivalent to those developed in the TT4 and TT5 training set, it was predicted that 42.3% (335/792) of patients treated on TT2 and TT3 protocols had 14q32 translocations at baseline, as follows; 14.9% t(4;14), 1.4% t(6;14), 19.8% t(11;14), 4.4% (14;16), and 1.8% t(14;20) (Table 4).

Table 4: FISH confirmed t14q32 in TT4/TT5 and GEP predicted t14q32 in TT2/TT3

14q32 translocations	TT4/TT5 (n=268) GEP spikes/FISH	TT2/TT3 (n=792) GEP predicted
t(4;14)(p16;q32)	14.9% (n=40)	14.9% (n=118)
t(4;14)(p16;q32) FGFR3/IGHC	- (n=27)	- (n=77)
t(6;14)(p21;q32)CCND3/I GHC	3.4% (n=9)	1.4% (n=11)
t(11;14)(q13;32)CCND1/I GHC	19.4% (n=52)	19.8% (n=157)
t(14;16)(q32;q23) MAF/IGHC	3.4% (n=9)	4.4% (n=35)
t(14;20)(q32;q11)MAFB/I GHC	1.1% (n=3)	1.8% (n=14)
Total	42.2%	42.3%

[0080] Next, the effects of 14q32 translocations were correlated with overall survival (OS) of patients treated in TT2 and TT3. As noted above, the chemotherapy regimen used in TT2 included thalidomide, while TT3 included thalidomide and also introduced bortezomib. Thus, it was possible to determine if the addition of bortezomib extended OS by comparing data from TT2 and TT3, and,

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by layering on top of the OS data information regarding 14q32 translocations, it was possible to discover prognostic indicators associated with 14q32 translocation.

[0081] Patients whose GEP spikes predicted t(4;14) and t(6;14) significantly benefited from the inclusion of bortezomib in TT3 (FIGs. 15-17). Patients with t(11;14) and t(14;20) also benefited from bortezomib, showing an improved median survival in 8 years follow-ups of TT2 and TT3 (FIGs. 18 and 19, respectively). In contrast, patients with t(14;16) as predicted by high MAF spikes clearly did not benefit from the inclusion of the proteasome inhibitor in TT3 (FIG. 20).

[0082] In conclusion, by combining interphase FISH and GEP, an expression-level threshold that is predictive of 14q32 translocations in multiple myeloma was established. Furthermore, 14q32 translocation thresholds have been correlated with response to bortezomib therapy. Hence, either FISH or GEP-based thresholds can be used to define molecular subgroups of patients and guide treatment decisions. This single -gene approach to define molecular subgroups was expedient and provided important information of prognostic significance in myeloma and other cancer treatments.

Example 5: Novel FISH probes are more accurate than commercially available probes.

[0083] An important difference between the new FISH probes and commercially available probes is that the new probes are made of gene locus-based BAC or PAC DNA templates while commercial probes are usually significantly longer than a gene locus on a chromosome. For example, using FISH probes offered for sale by Abbott, t(4;14) is currently defined as any fusion between an IgH probe spanning approximately 1.5 Mb and containing sequences homologous to essentially the entire IGH locus (IgHC, IgHJ, IgHD and IgHV), as well as sequence extending about 300 kb beyond the 3' end of the IGH locus, and an FGFR3 probe spanning approximately 900 kb and containing sequences homologous to both FGFR3 and MMSET. In our probe panel, two probes are assigned to 4p16 (one each for FGFR3 and MMSET) and two probes are assigned to 14q32 (one each for IgHC and IgHV).

[0084] As described above, FISH and GEP-based analyses were applied to two sets of baseline myeloma samples collected from 1998 to 2007 (TT2/TT3) and 2007

to present (TT4/TT5). The overall incidences of t14q32 and predicted t14q32 subgroups were found to be the same in two patient populations (see **Table 4**). The lower incidence rates of t14q32 found by these studies, whose analysis is based on the new FISH probes, compared to a 75% event rate cited in the early publications (Avet-Loiseau H., Facon T., *et al.*, 2002 *Blood* 99(6):2185-91; Avet-Loiseau H., Brigaudeau C., *et al.*, 1999 *Genes Chromosomes Cancer* 24(1):9–15) suggests non-specific fusions of large- size probes from the commercial sources could state false positive 10%-30% more than 14q32 translocations detected using the new FISH probes and/or the GEP thresholds established with the new FISH probes.

[0085] It should be understood that for all numerical bounds describing some parameter in this application, such as “about,” “at least,” “less than,” and “more than,” the description also necessarily encompasses any range bounded by the recited values. Accordingly, for example, the description at least 1, 2, 3, 4, or 5 also describes, *inter alia*, the ranges 1-2, 1-3, 1-4, 1-5, 2-3, 2-4, 2-5, 3-4, 3-5, and 4-5, *et cetera*.

[0086] For all patents, applications, and other references cited herein, such as non-patent literature and reference sequence or chemical information, it should be understood that it is incorporated by reference in its entirety for all purposes as well as for the proposition that is recited. Where any conflict exists between a document incorporated by reference and the present application, this application will control.

[0087] Headings used in this application are for convenience only and do not affect the interpretation of this application.

[0088] The described computer-readable implementations may be implemented in software, hardware, or a combination of hardware and software. Examples of hardware include computing or processing systems, such as personal computers, servers, laptops, mainframes, and micro-processors. Any of the computer-readable implementations provided by the invention may, optionally, comprise a step of providing a visual output to a user, such as a visual representation on a screen or a physical printout.

[0089] Preferred features of each of the aspects provided by the invention are applicable to all of the other aspects of the invention *mutatis mutandis* and, without

limitation, are exemplified by the dependent claims and also encompass combinations and permutations of individual features (*e.g.*, elements, including numerical ranges and exemplary embodiments) of particular embodiments and aspects of the invention including the working examples. For example, particular experimental parameters exemplified in the working examples can be adapted for use in the claimed invention piecemeal without departing from the invention. For example, for material that are disclosed, while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein, as are methods of making and using such compounds. Thus, if a class of elements A, B, and C are disclosed as well as a class of elements D, E, and F and an example of a combination of elements, A-D is disclosed, then even if each is not individually recited, each is individually and collectively contemplated. Thus, in this example, each of the combinations A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. Likewise, any subset or combination of these is also specifically contemplated and disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. This concept applies to all aspects of this application including, elements of a composition of matter, and steps of method of making or using the compositions.

[0090] The forgoing aspects of the invention, as recognized by the person having ordinary skill in the art following the teachings of the specification, can be claimed in any combination or permutation to the extent that they are novel and non-obvious over the prior art. Thus, to the extent an element is described in one or more references known to the person having ordinary skill in the art, they may be excluded from the claimed invention by, *inter alia*, a negative proviso or disclaimer of the feature or combination of features.

[0091] While this invention has been particularly shown and described with references to example embodiments thereof, it will be understood by those skilled in

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the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

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CLAIMS

What is claimed is:

1. A method to identify a multiple myeloma patient that would benefit from a treatment comprising bortezomib, or determine if a multiple myeloma patient would or would not benefit from a treatment comprising bortezomib, the method comprising determining whether a patient has a 14q32 translocation by measuring the gene expression level of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, MAFB, or a combination thereof, wherein elevated levels of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, or MAFB, relative to a suitable reference, indicates the presence of a 14q32 translocation, and the presence or absence of a 14q32 translocation guides the treatment decision.
2. A method to identify a multiple myeloma patient that would benefit from a treatment comprising bortezomib, or determine if a multiple myeloma patient would benefit from a treatment comprising bortezomib, the method comprising determining whether a patient has a 14q32 translocation according to the any of the methods of claims 11-22 and 29, wherein the presence or absence of a 14q32 translocation guides the treatment decision.
3. The method of claim 1 or 2, wherein the presence of a t(4;14), t(6;14), t(11;14), or t(14;20) translocation indicates that the subject would benefit from a treatment comprising bortezomib.
4. The method of claim 1 or 2, wherein the presence of a t(14;16) translocation indicates that the subject would not benefit from a treatment comprising bortezomib.
5. The method of any one of claims 1 to 4, wherein the benefit is increased overall survival.

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6. A method of treating multiple myeloma in a subject in need thereof, comprising providing a treatment comprising bortezomib or not comprising bortezomib to the subject on the basis of the outcome of the method of any one of Claims 1-5.
7. The method of Claim 6, wherein a treatment comprising bortezomib is administered to the subject on the basis of detecting the presence of a t(4;14), t(6;14), t(11;14), or t(14;20) translocation in the subject.
8. The method of Claim 7, wherein the subject was not a candidate for bortezomib therapy before detecting the translocation.
9. The method of Claim 6, wherein a treatment not comprising bortezomib is administered on the basis of detecting the presence of a t(14;16) translocation in the subject.
10. The method of Claim 9, wherein the subject was a candidate for therapy comprising bortezomib before detecting the translocation.
11. A method to detect a 14q32 translocation in a subject with a B-cell malignancy, the method comprising performing fluorescence in-situ hybridization (FISH) on a biological sample from a subject with a B-cell malignancy using at least a first probe and a second probe, wherein the first probe comprises a nucleic acid sequence specific for IgHC or IgHV and the second probe comprises a nucleic acid sequence complementary to a chromosomal region encoding a genomic locus selected from the group consisting of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, and MAFB.
12. The method of claim 11, wherein the second probe comprises a nucleic acid sequence specific for FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, and MAFB.

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13. The method of claim 11 or claim 12, wherein the source of nucleic acid for the first and second probe is independently selected from the group consisting of a BAC, a PAC, and a YAC.
14. The method of any one of claims 11-13, wherein the B-cell malignancy is selected from the group consisting of precursor B-cell acute lymphoblastic leukemia (B-ALL), B-cell chronic lymphoblastic leukemia (B-CLL), B-cell non-Hodgkin's lymphoma, and multiple myeloma.
15. The method of any one of claims 11-14, wherein the B-cell malignancy is multiple myeloma, the first probe comprises a nucleic acid sequence specific for IgHC or IgHV, and the second probe comprises a nucleic acid sequence specific for FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, MAFB, or a combination thereof.
16. The method of claim 15, wherein the second probe comprises a nucleic acid sequence specific for a gene selected from the group consisting of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, MAFB, or a combination thereof.
17. A method to detect an IgHC translocation in a subject with a B-cell malignancy, the method comprising performing fluorescence in-situ hybridization (FISH) on a biological sample from the subject using at least a first probe and a second probe, wherein the first probe comprises a nucleic acid sequence specific for IgHC and the second probe comprises a nucleic acid sequence complementary to a chromosomal region encoding a genomic locus selected from the group consisting of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, and MAFB.
18. The method of claim 17, wherein the second probe comprises a nucleic acid sequence specific for FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, MAFB, or a combination thereof.

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19. The method of claim 17 or 18, wherein the source of nucleic acid for the first and second probe is independently selected from the group consisting of a BAC, a PAC, and a YAC.
20. The method of any one of claims 17-19, wherein the B-cell malignancy is selected from the group consisting of precursor B-cell acute lymphoblastic leukemia (B-ALL), B-cell chronic lymphoblastic leukemia (B-CLL), B-cell non-Hodgkin's lymphoma, and multiple myeloma.
21. The method of any one of claims 17-20, wherein the B-cell malignancy is multiple myeloma, the first probe is specific for IgHC, and the second probe comprises a nucleic acid sequence specific for FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, MAFB, or a combination thereof.
22. The method of claim 21, wherein the second probe comprises a nucleic acid sequence specific to a gene selected from the group consisting of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, and MAFB.
23. A method to establish a gene expression threshold value for a 14q32 translocation partner indicative of a 14q32 translocation in a subject with a B-cell malignancy, the method comprising (a) measuring the expression level for a 14q32 translocation partner in biological samples from subjects with 14q32 translocations and without 14q32 translocations, (b) determining the presence or absence of a 14q32 translocation by FISH using at least a first probe and a second probe, wherein the first probe comprises a nucleic acid sequence specific for IgHC or IgHV, and the second probe comprises a nucleic acid sequence complementary to a chromosomal region encoding the translocation partner whose expression was measured in (a), (c) comparing the expression level of the translocation partner with the presence or absence of a 14q32 translocation, and (d) determining a gene expression threshold value for the translocation partner

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above which indicates increased expression due to a 14q32 translocation and below which indicates increased expression not due to a 14q32 translocation.

24. The method of claim 23, wherein the expression level for a 14q32 translocation partner is measured by quantitative RT-PCR or microarray analysis.
25. The method of claim 23 or 24, wherein the at least first probe and second probe are selected from a group consisting of (i) a first probe comprising a nucleic acid sequence specific for IgHV and a second probe comprising a nucleic acid sequence specific for MMSET, (ii) a first probe comprising a nucleic acid sequence specific for IgHC and a second probe comprising a nucleic acid sequence specific for FGFR3, (iii) a first probe comprising a nucleic acid sequence specific for IgHC and a second probe comprising a nucleic acid sequence specific for CCND1, (iv) a first probe comprising a nucleic acid sequence specific for IgHC and a second probe comprising a nucleic acid sequence specific for CCND3, (v) a first probe comprising a nucleic acid sequence specific for IgHC and a second probe comprising a nucleic acid sequence specific for MAF, and (vi) a first probe comprising a nucleic acid sequence specific for IgHC and a second probe comprising a nucleic acid sequence specific for MAFB.
26. The method of any one of claims 23-25, wherein the B-cell malignancy is selected from the group consisting of precursor B-cell acute lymphoblastic leukemia (B-ALL), B-cell chronic lymphoblastic leukemia (B-CLL), B-cell non-Hodgkin's lymphoma, and myeloma.
27. The method of any one of claims 23-26, wherein:
- (i) the translocation is t(4;14), the gene fusion is MMSET/IgHV, the expression of MMSET is measured by microarray analysis using the Affymetrix U133 Plus 2.0 Array GeneChip 209054_s_at, and the

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threshold value predictive of the translocation in a subject with multiple myeloma is about 2,405,

- (ii) the translocation is t(4;14), the gene fusion is FGFR3/IgHC, the expression of FGFR3 is measured by microarray analysis using the Affymetrix U133 Plus 2.0 Array GeneChip 204379_s_at, and the threshold value predictive of the translocation in a subject with multiple myeloma is about 6,558,
- (iii) the translocation is t(6;14), the gene fusion is CCND3/IgHC, the expression of CCND3 is measured by microarray analysis using the Affymetrix U133 Plus 2.0 Array GeneChip 201700_at, and the threshold value predictive of the translocation in a subject with multiple myeloma is about 15,696,
- (iv) the translocation is t(11;14), the gene fusion is CCND1/IgHC, the expression of CCND1 is measured by microarray analysis using the Affymetrix U133 Plus 2.0 Array GeneChip 208711_s_at, and the threshold value predictive of the translocation in a subject with multiple myeloma is about 5,525,
- (v) the translocation is t(14;16), the gene fusion is MAF/IgHC, the expression of MAF is measured by microarray analysis using the Affymetrix U133 Plus 2.0 Array GeneChip 206363_at, and the threshold value predictive of the translocation in a subject with multiple myeloma is about 5,096, or
- (vi) the translocation is t(14;20), the gene fusion is MAFB/IgHC, the expression of MAFB is measured by microarray analysis using the Affymetrix U133 Plus 2.0 Array GeneChip 222670_s_at, and the threshold value predictive of the translocation in a subject with multiple myeloma is about 19,850.

28. The method of claim 23, wherein the first probe comprises a nucleic acid sequence specific for a gene selected from the group consisting of IgHC and IgHV, and the second probe comprises a nucleic acid sequence specific for a

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gene selected from the group consisting of FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF, and MAFB.

29. A method to predict a 14q32 translocation in a subject suspected to have multiple myeloma, the method comprising (a) measuring the expression level for one or more 14q32 translocation partners in a biological sample from the subject, and (b) comparing the expression level(s) to the gene expression threshold value(s) of any one of claims 23-28, wherein an expression level for a 14q32 translocation partner in the biological sample that is greater than the threshold value, indicates that the subject has a 14q32 translocation.
30. The method of claim 29, wherein the expression level for two or more 14q32 translocation partners are measured.

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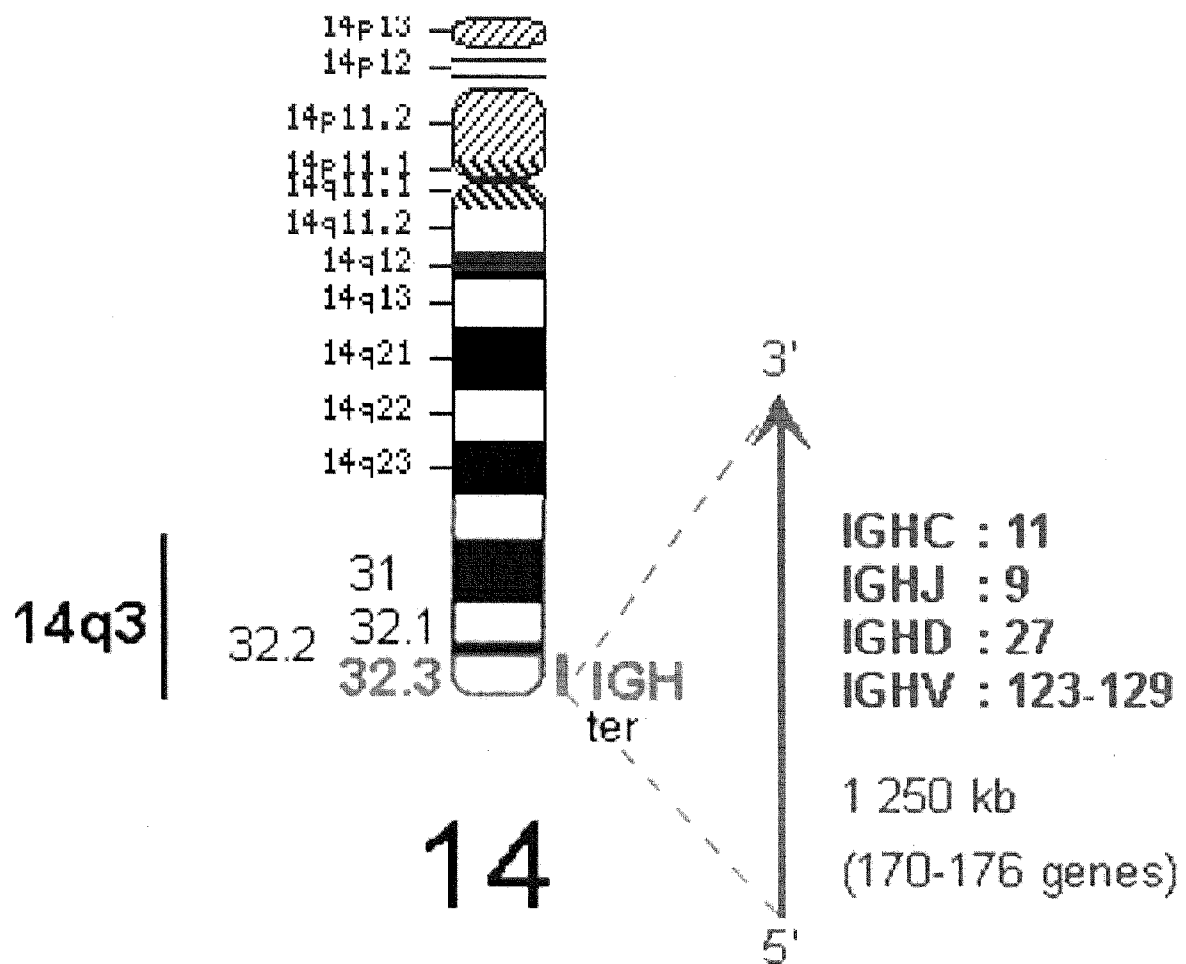


FIG. 1

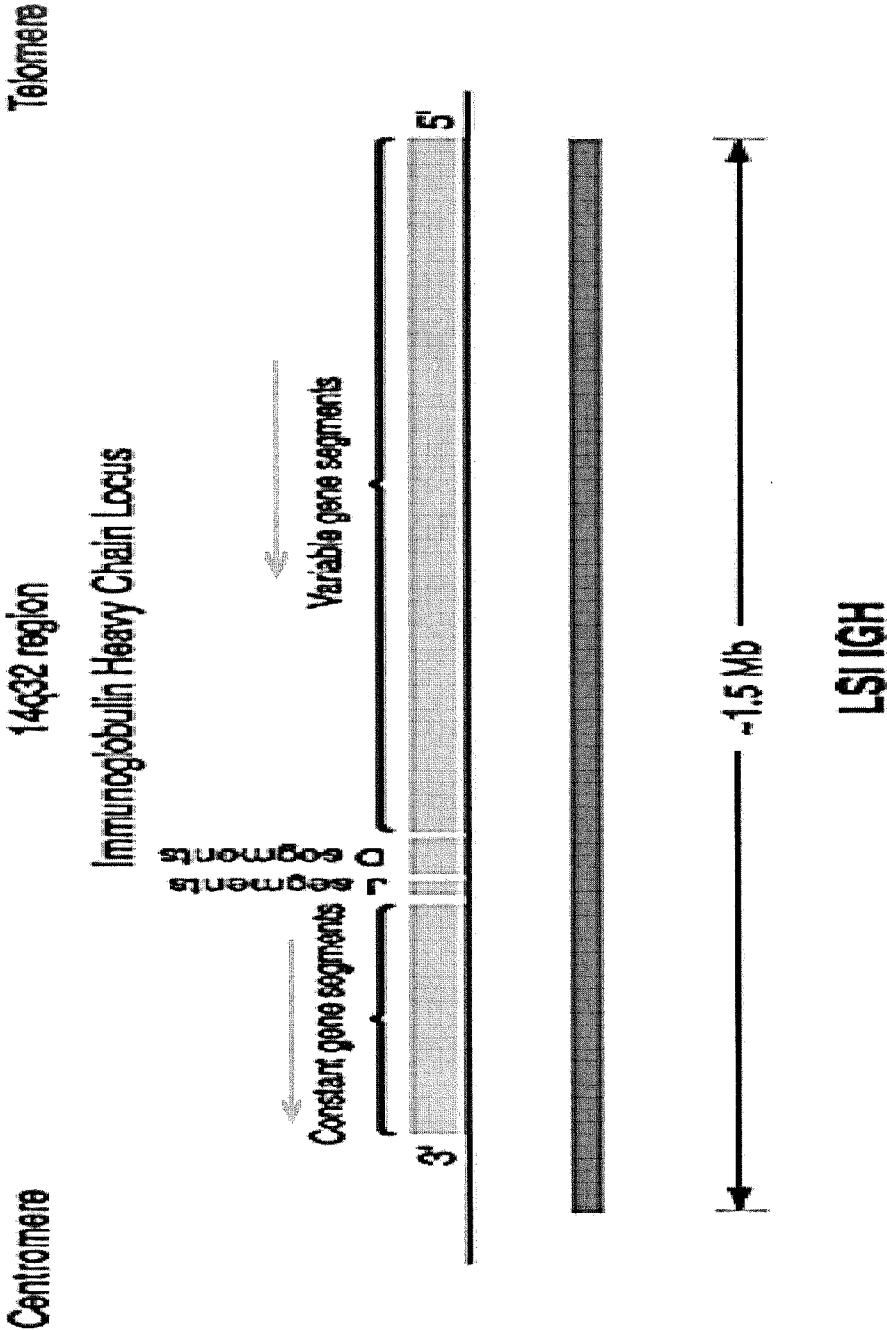


FIG. 2A

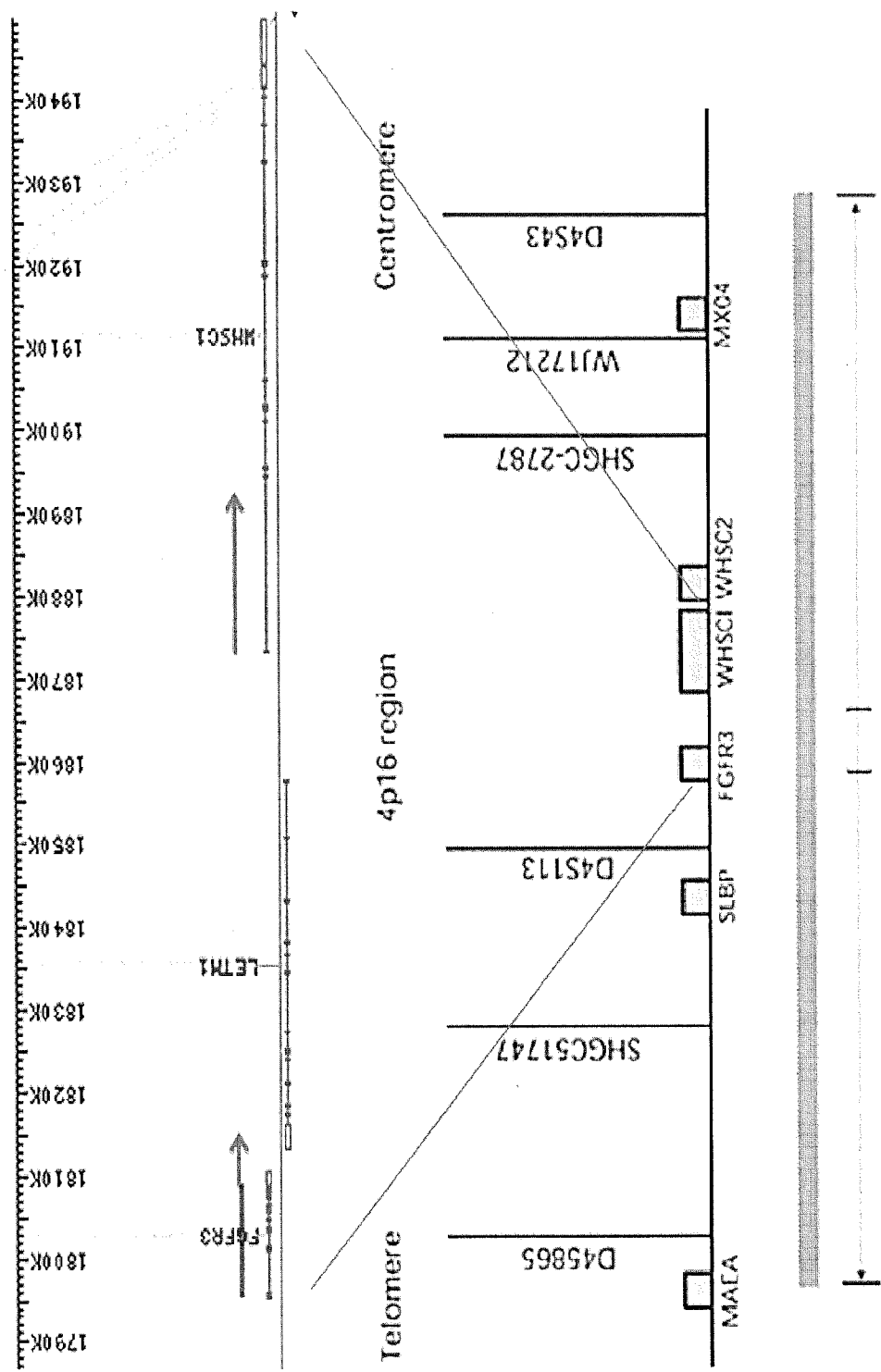


FIG. 2B

Total Probe Size = 900kb
LSI - FGFR3 -SO

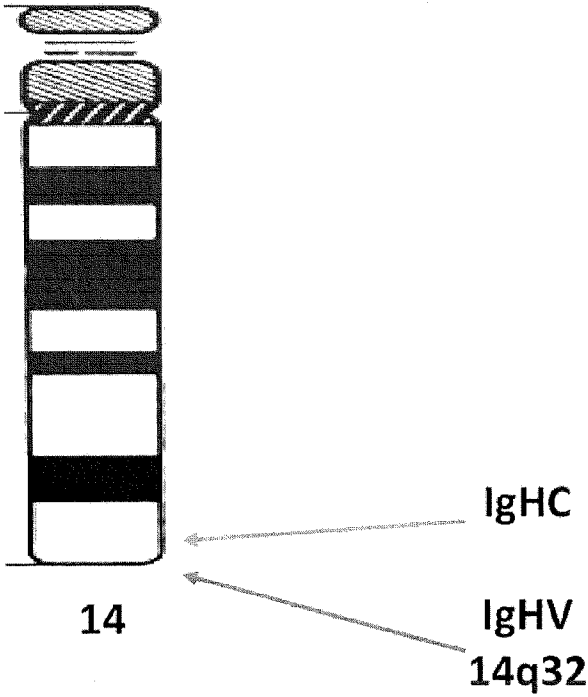


FIG. 3A

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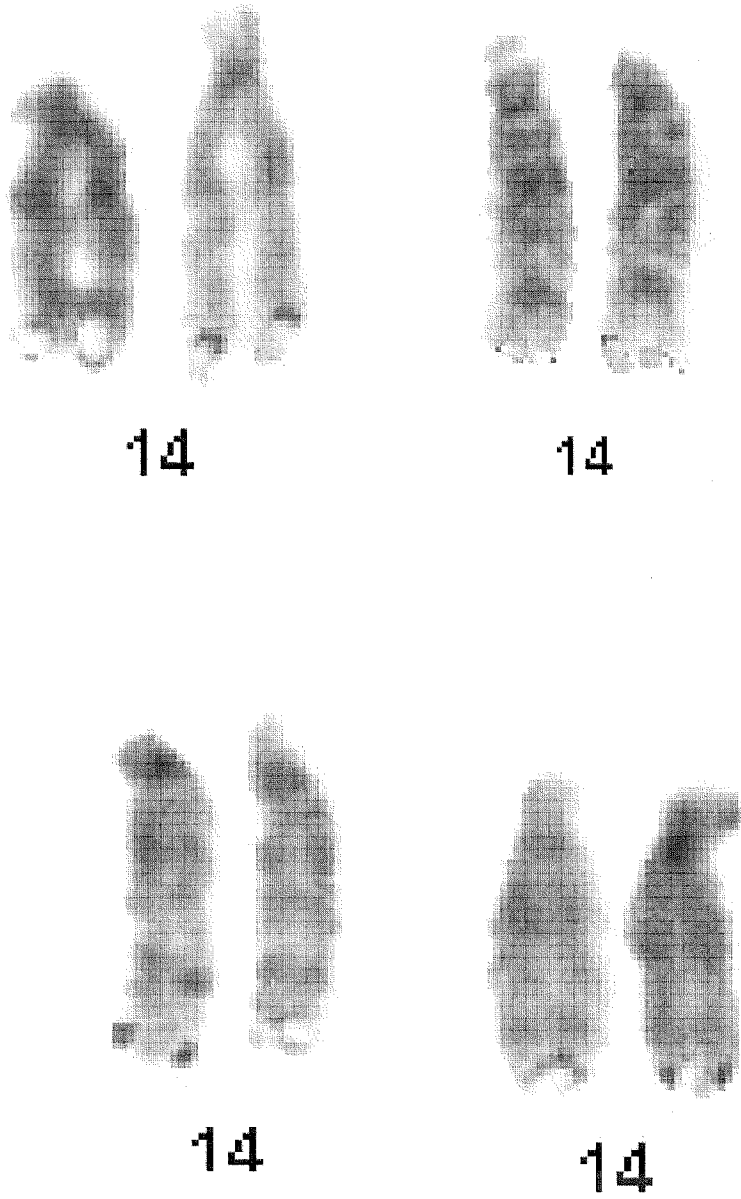


FIG. 3B

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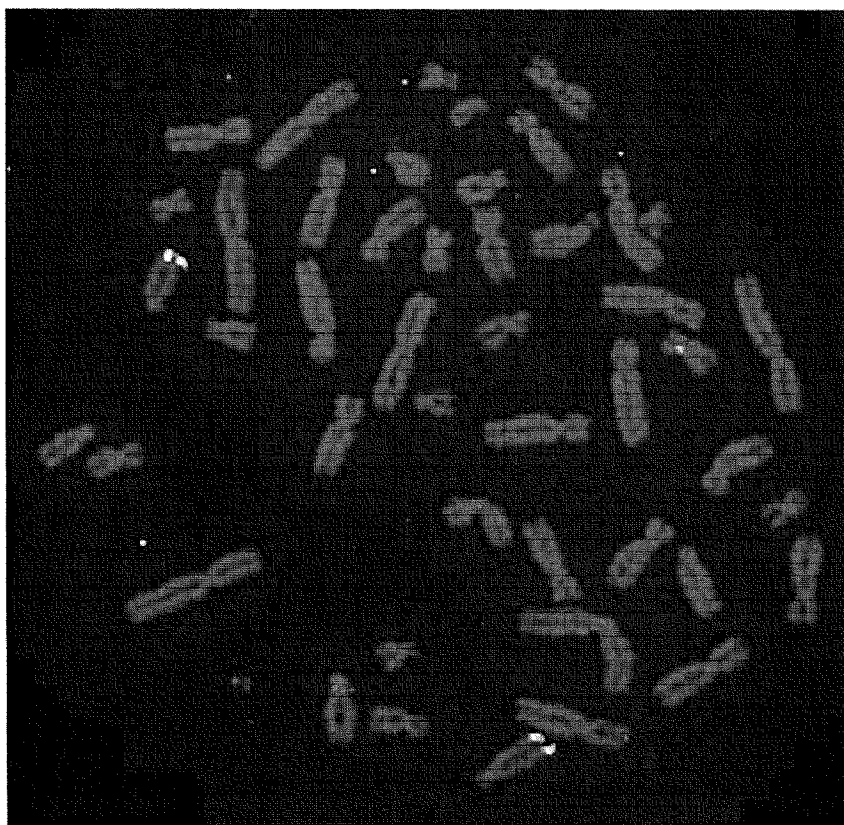
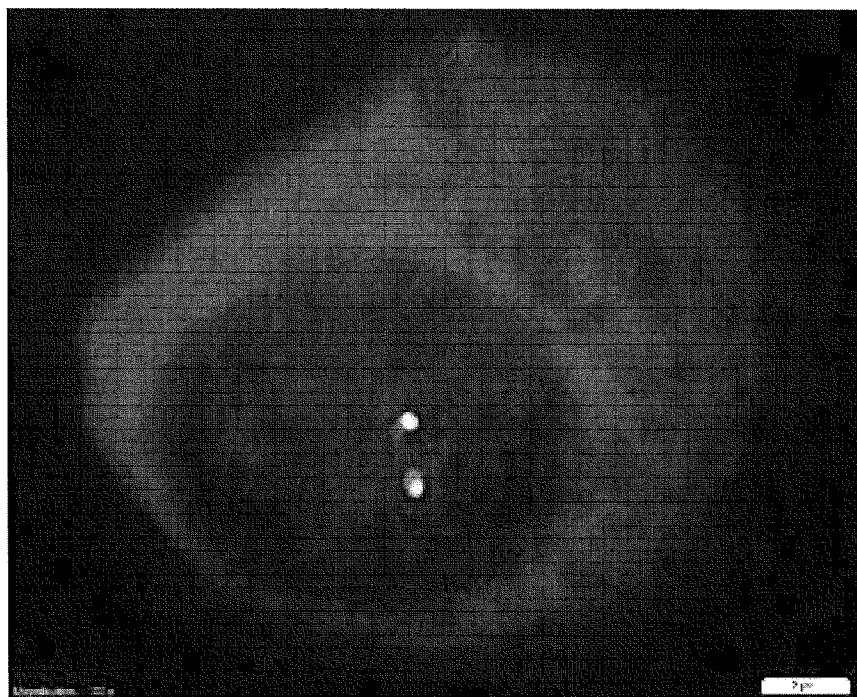


FIG. 4

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A



B

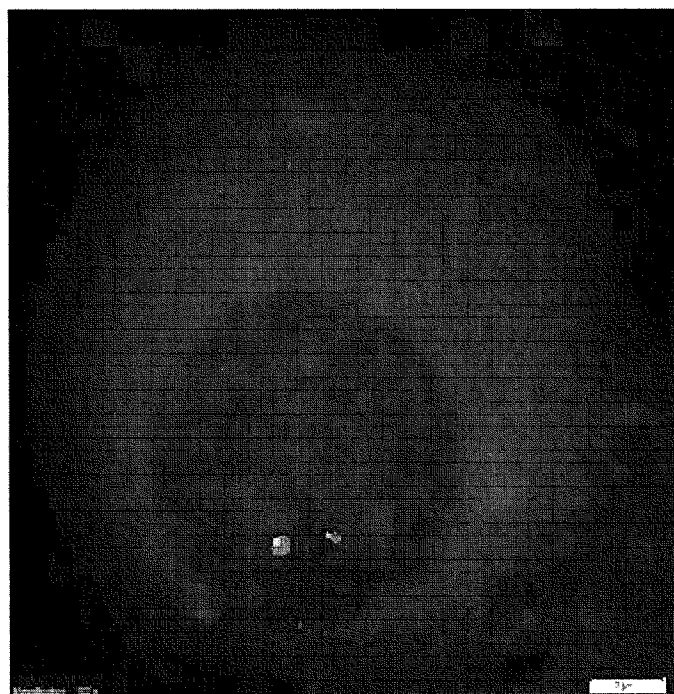


FIG. 5

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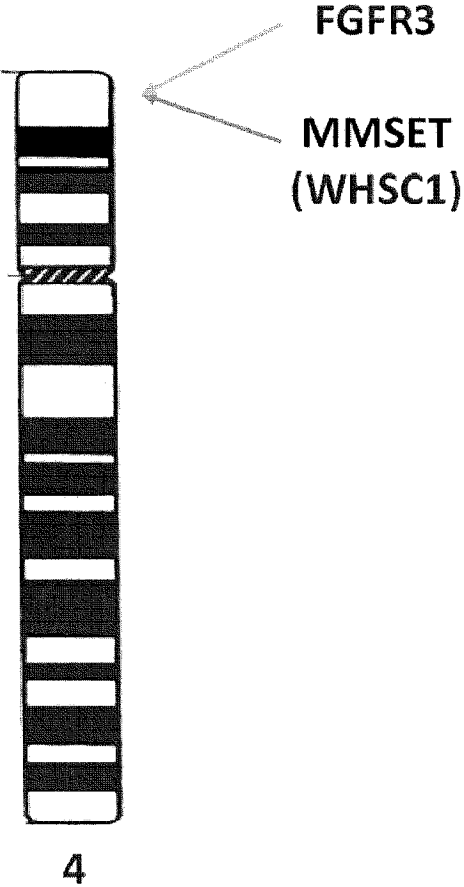


FIG. 6A

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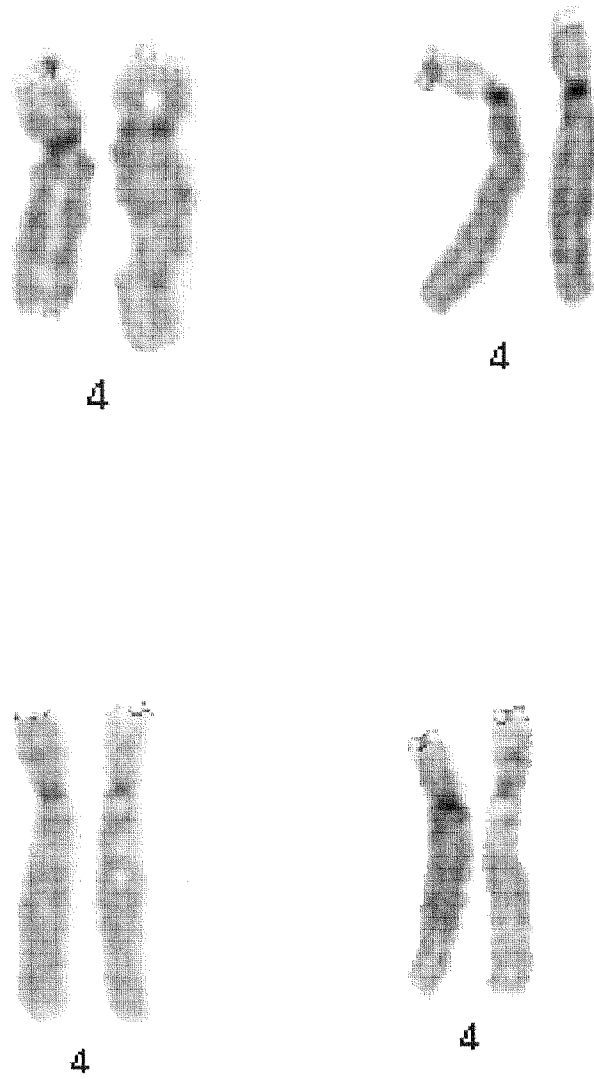


FIG. 6B

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FIG. 7

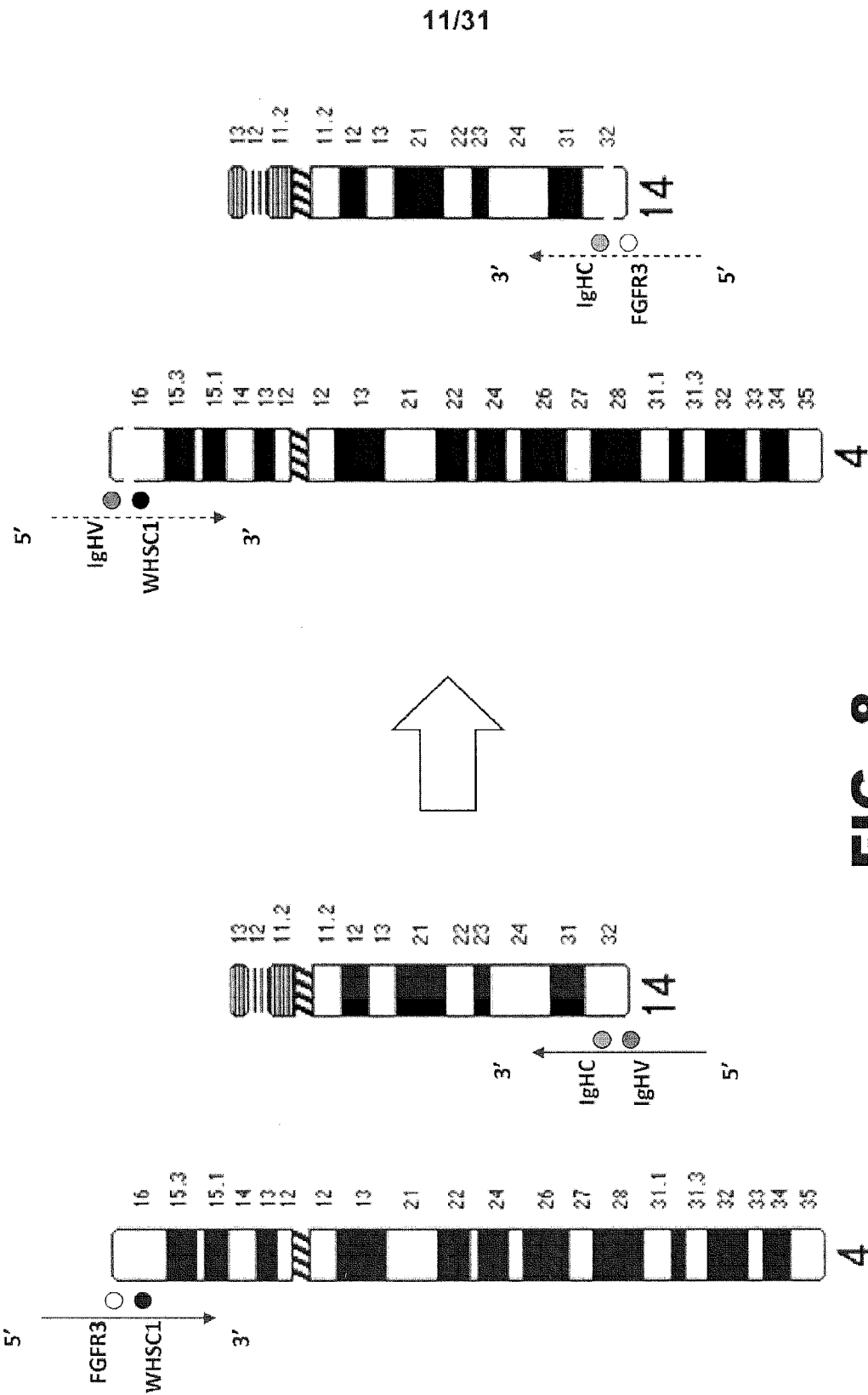


FIG. 8

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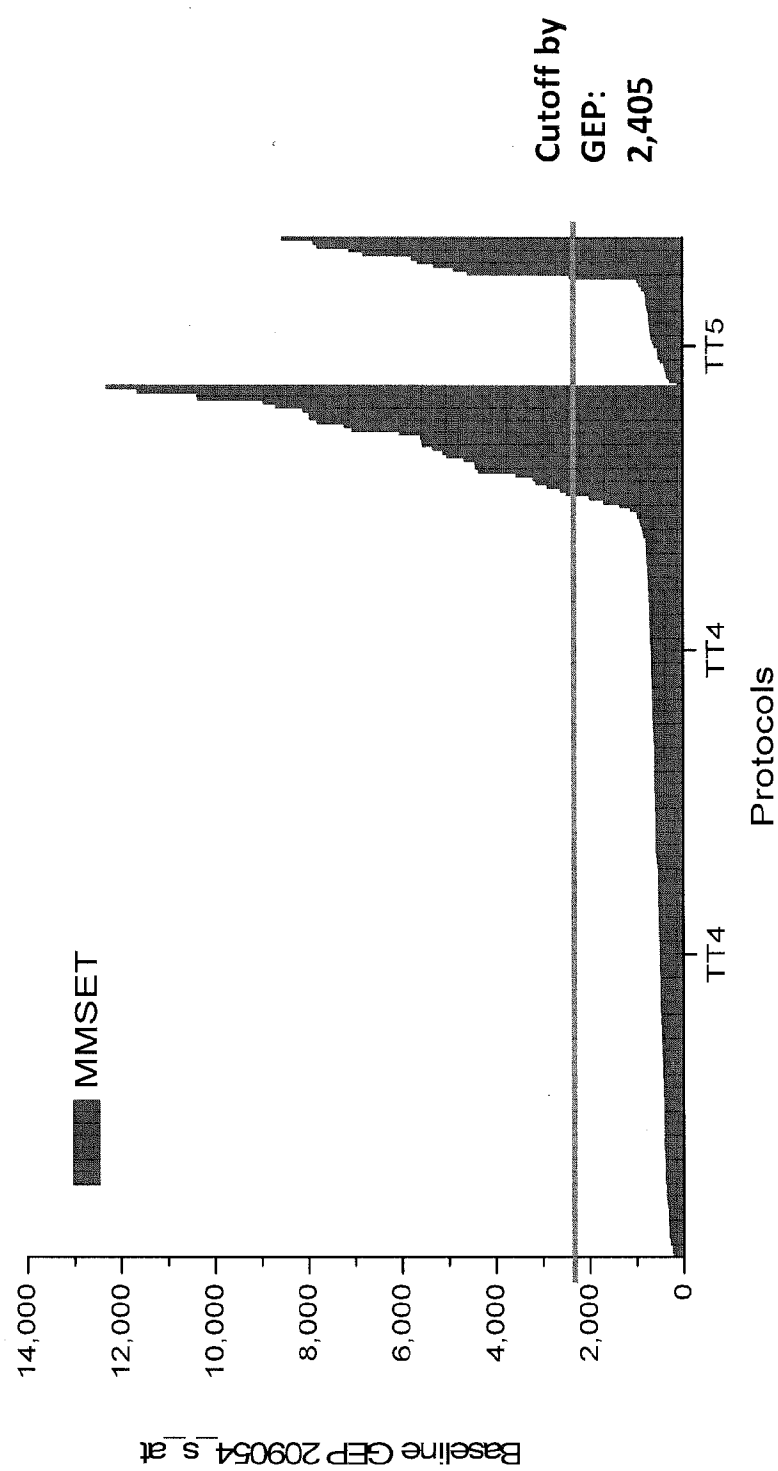


FIG. 9

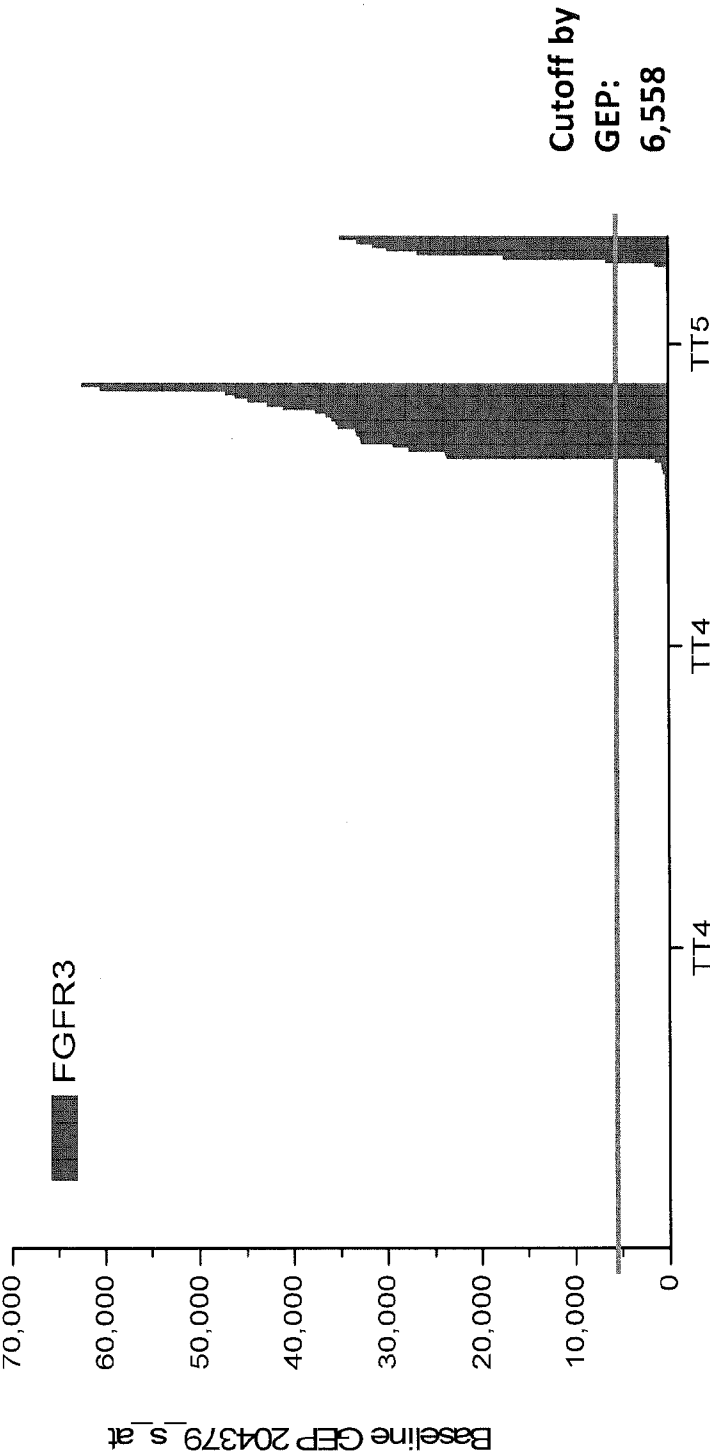


FIG. 10

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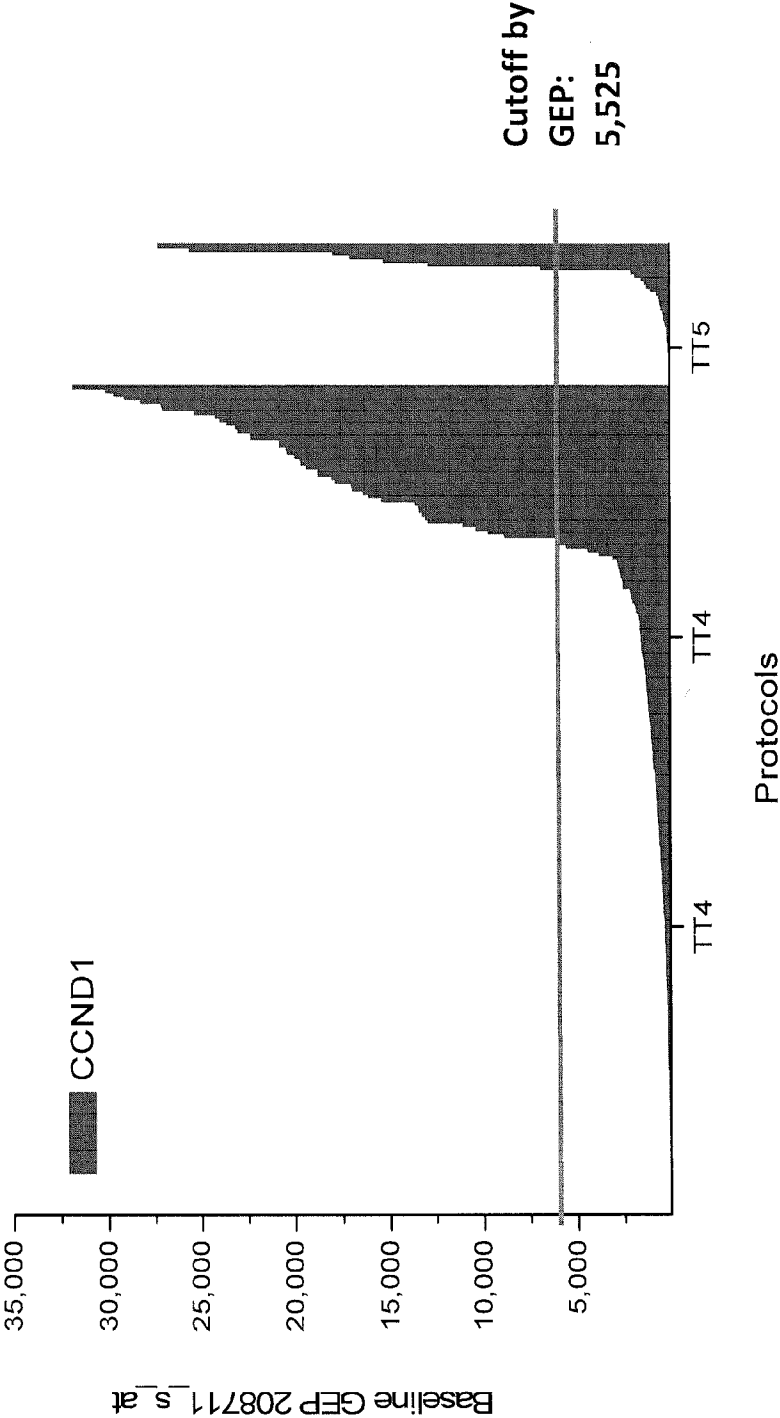


FIG. 11

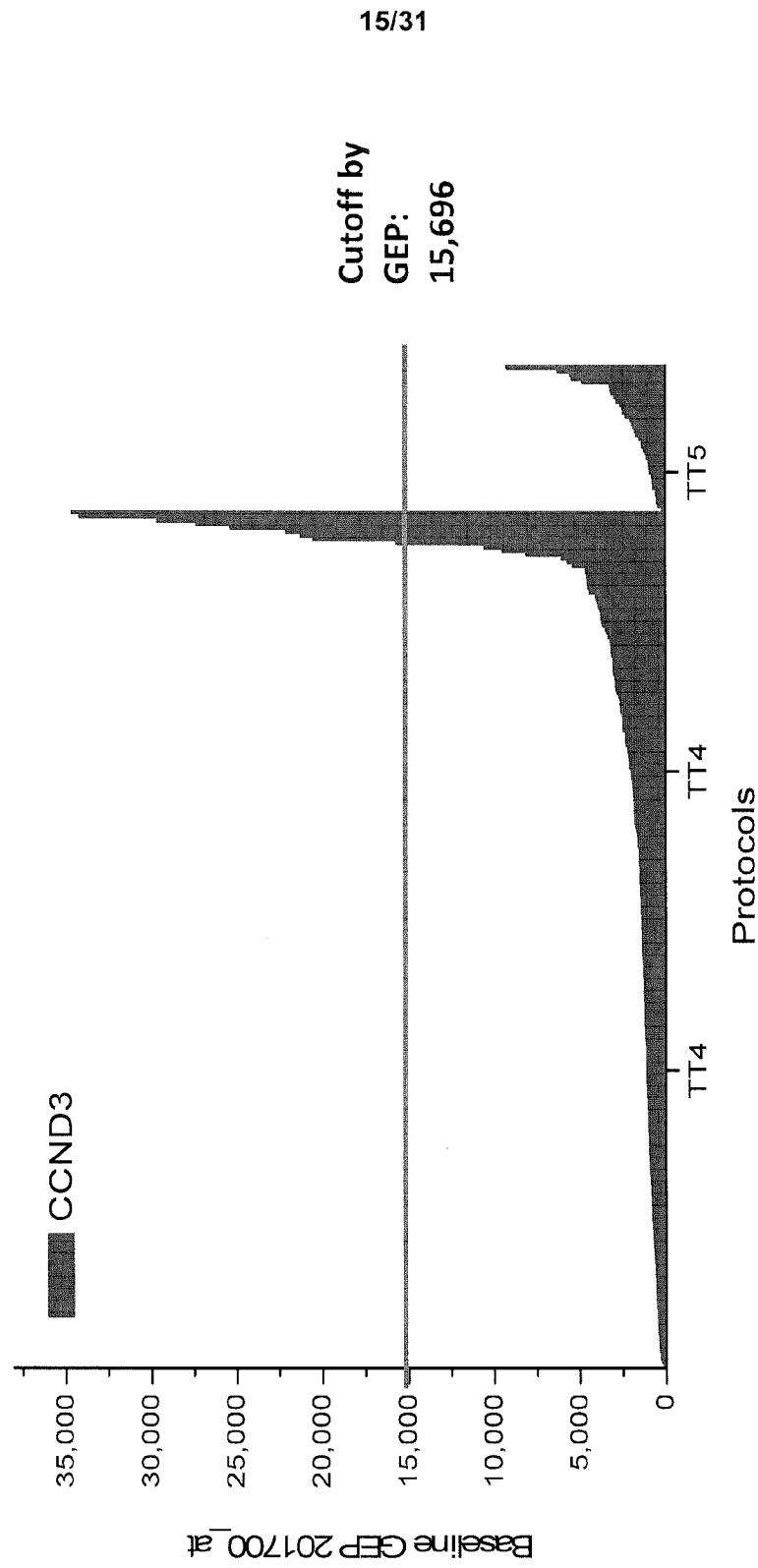


FIG. 12

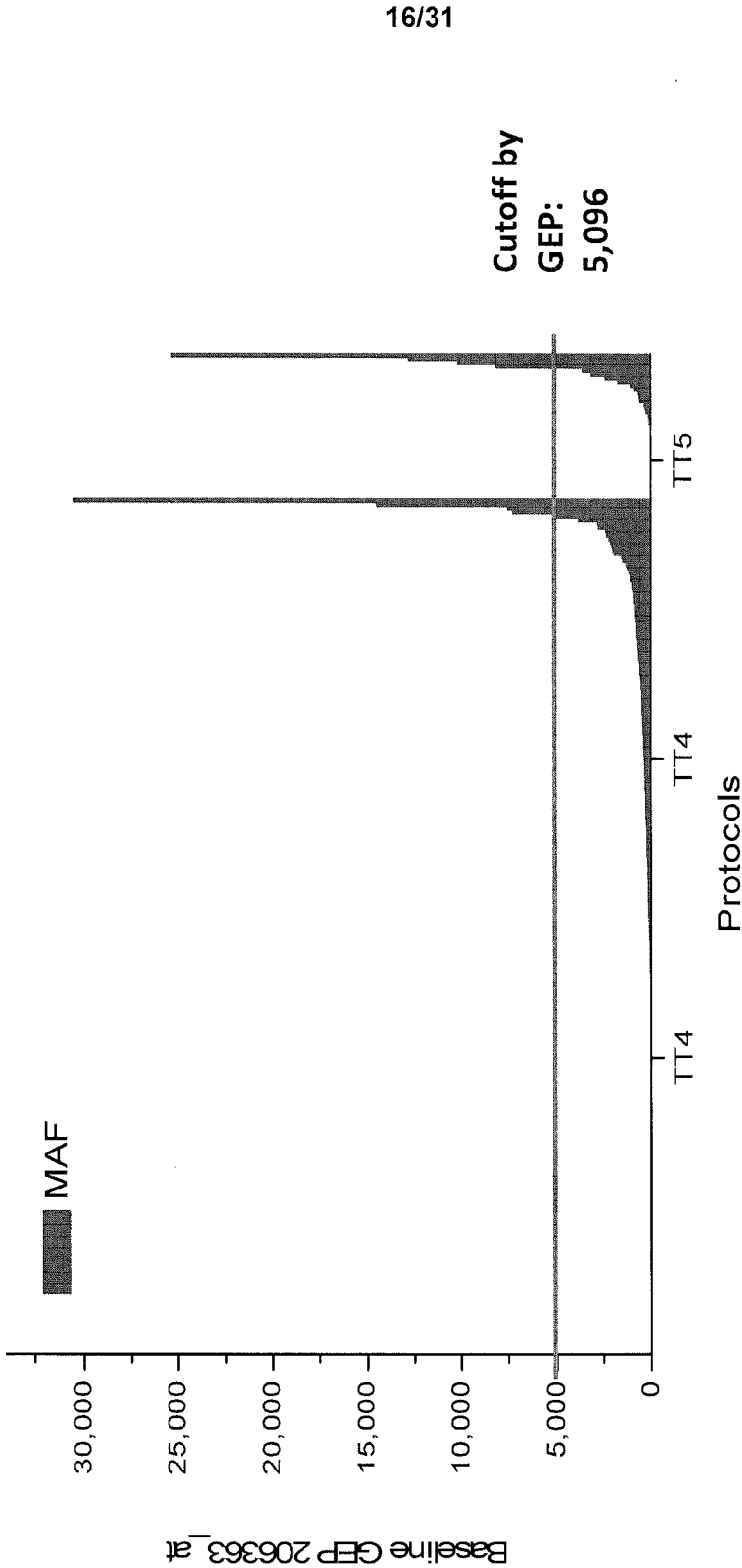


FIG. 13

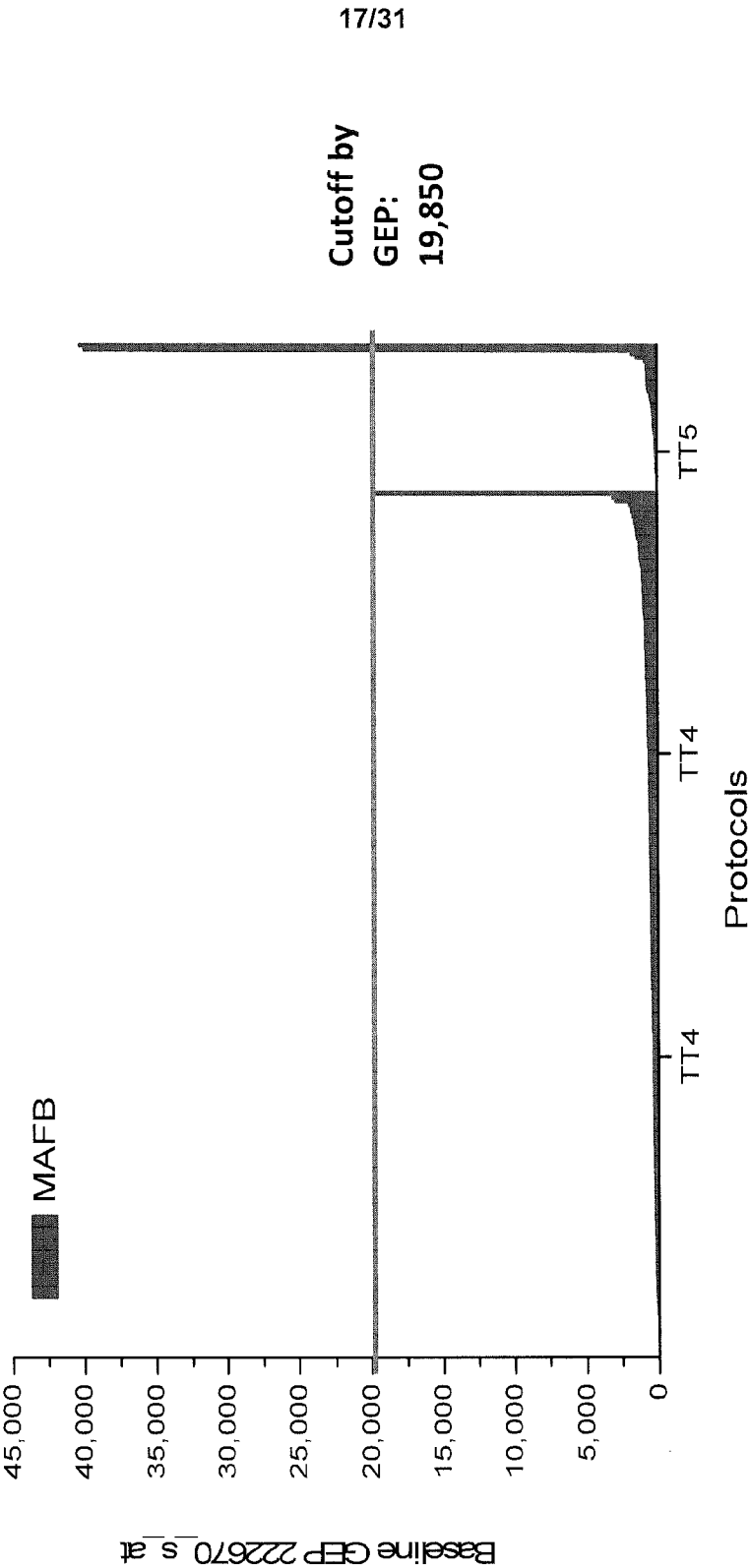
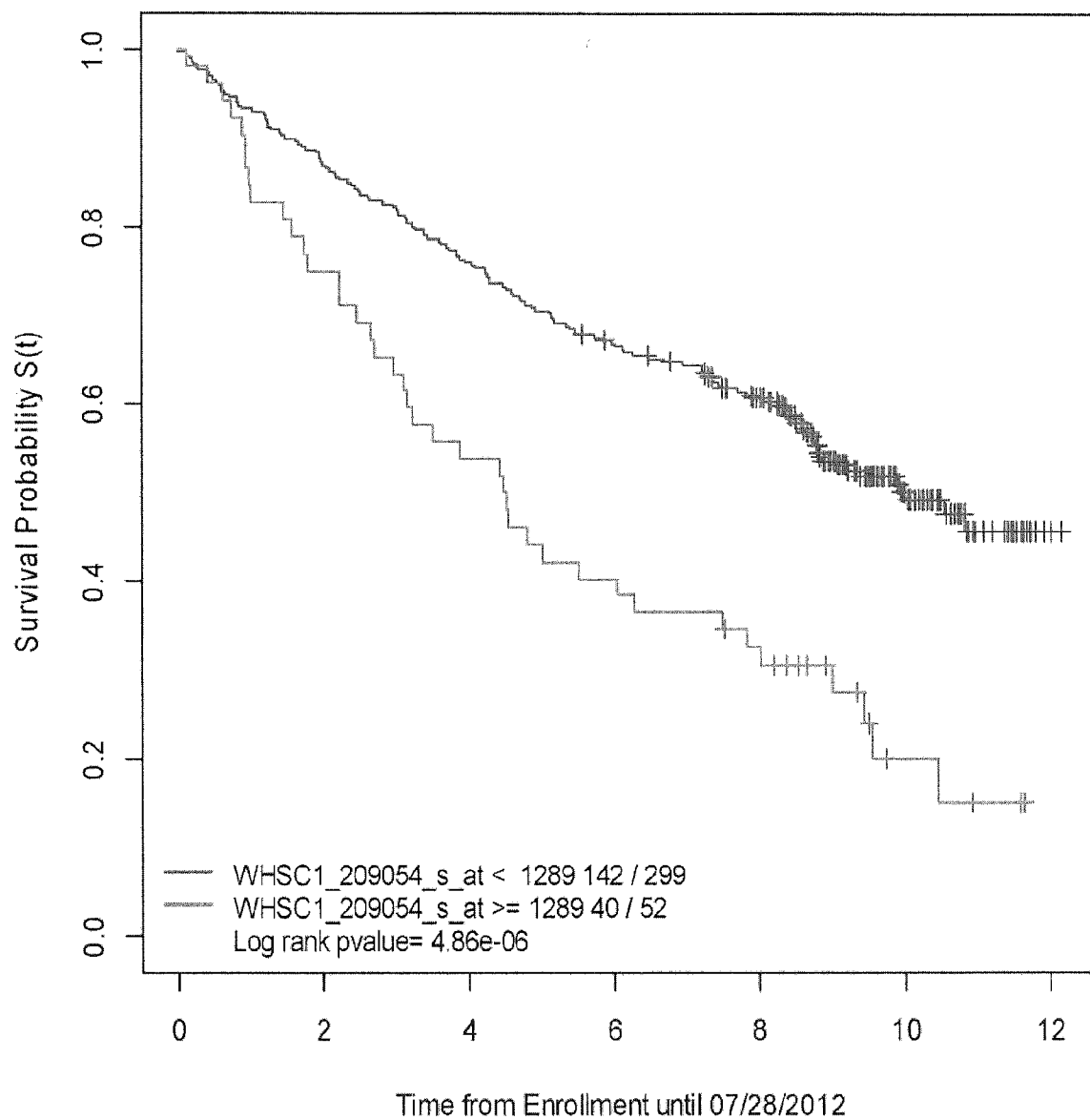
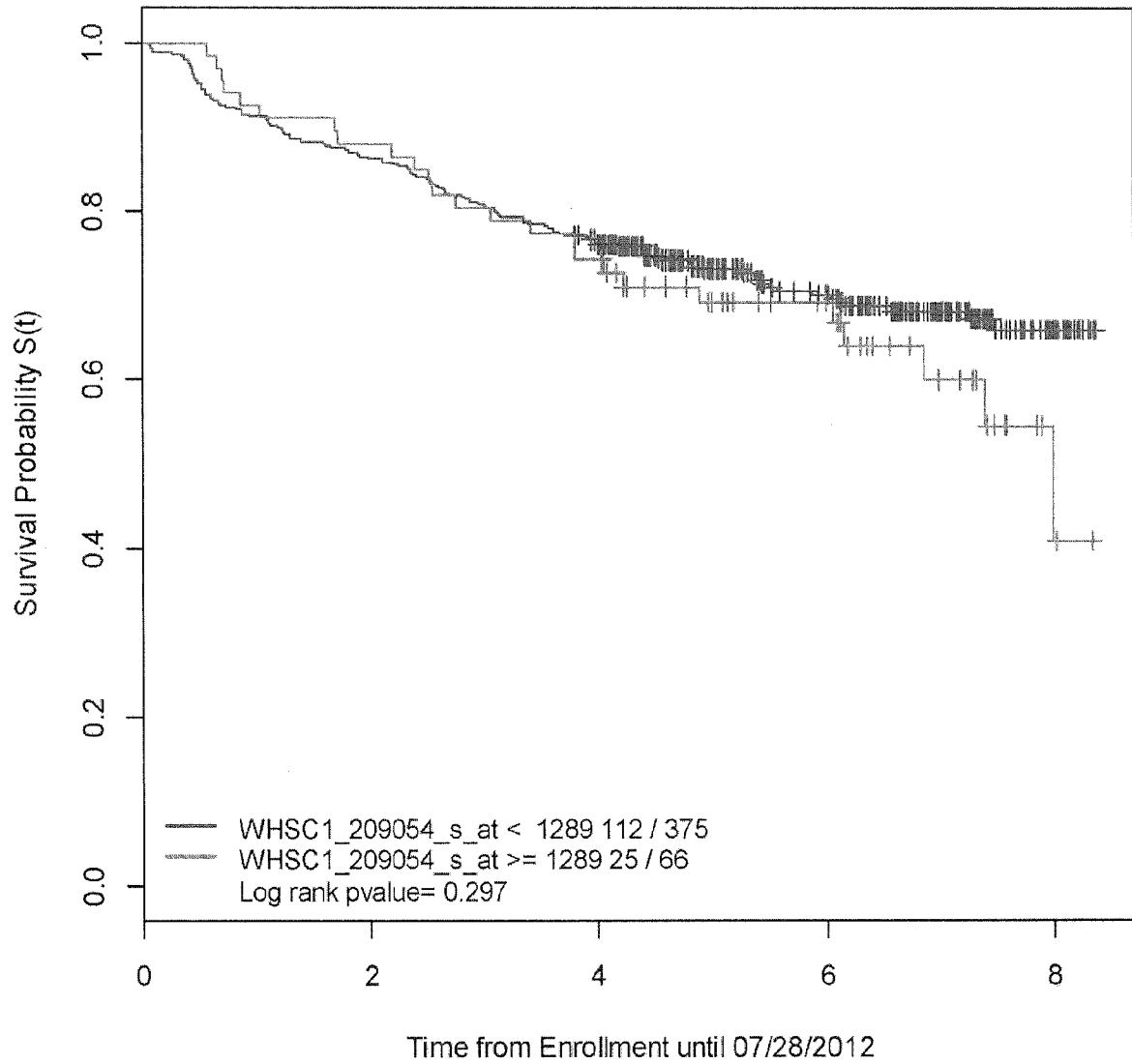


FIG. 14

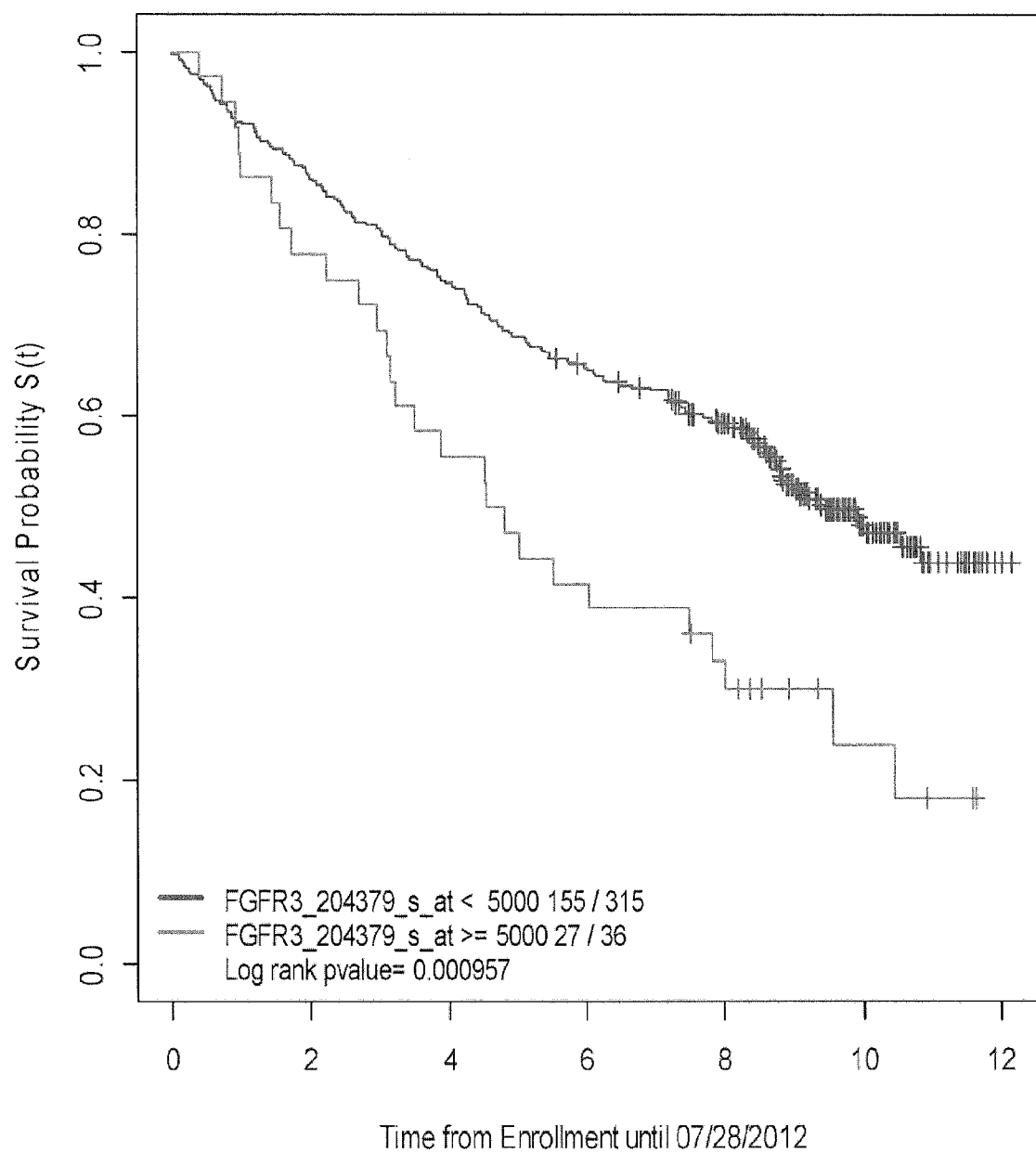
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**FIG. 15A**

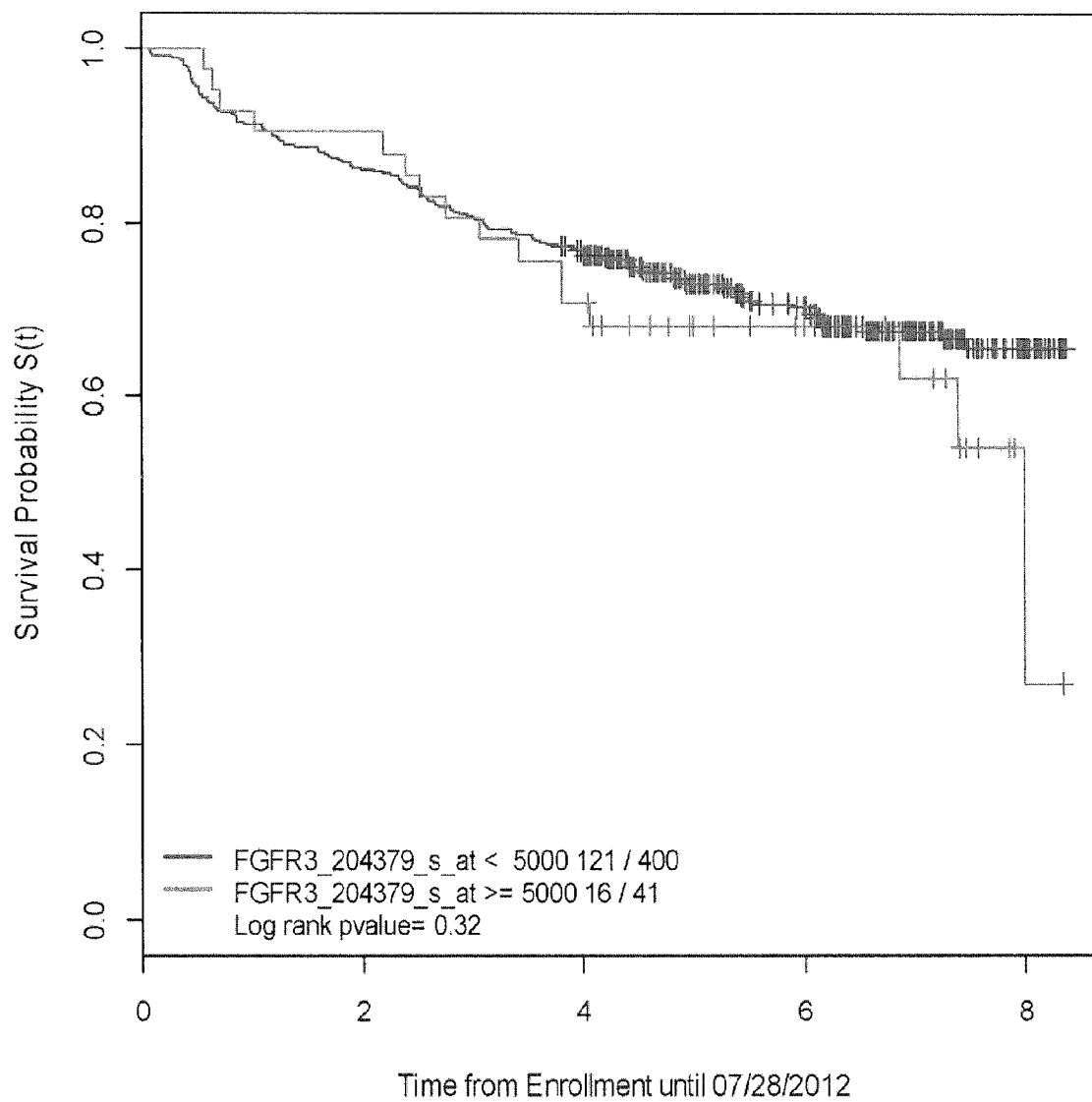
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**FIG. 15B**

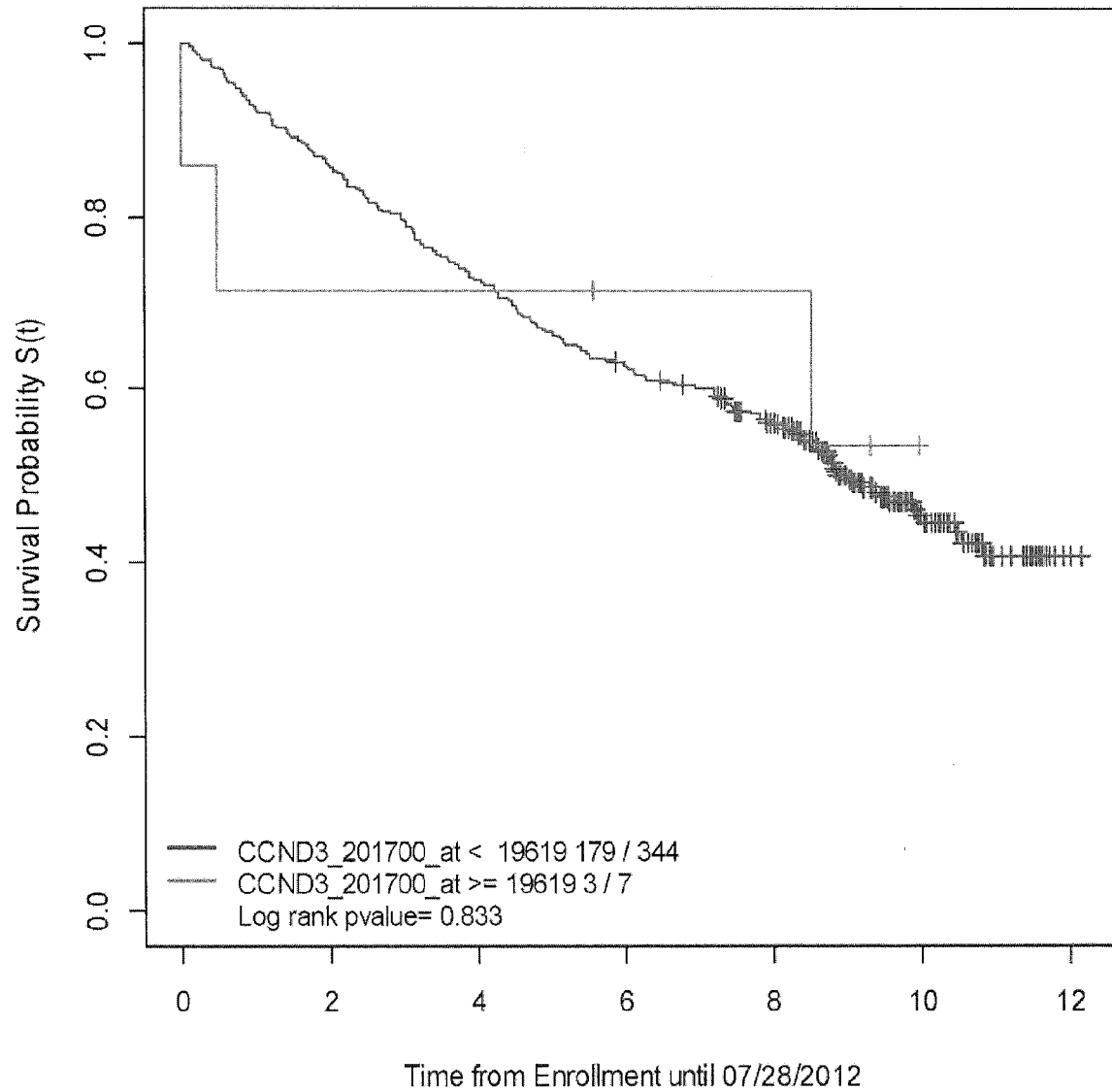
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**FIG. 16A**

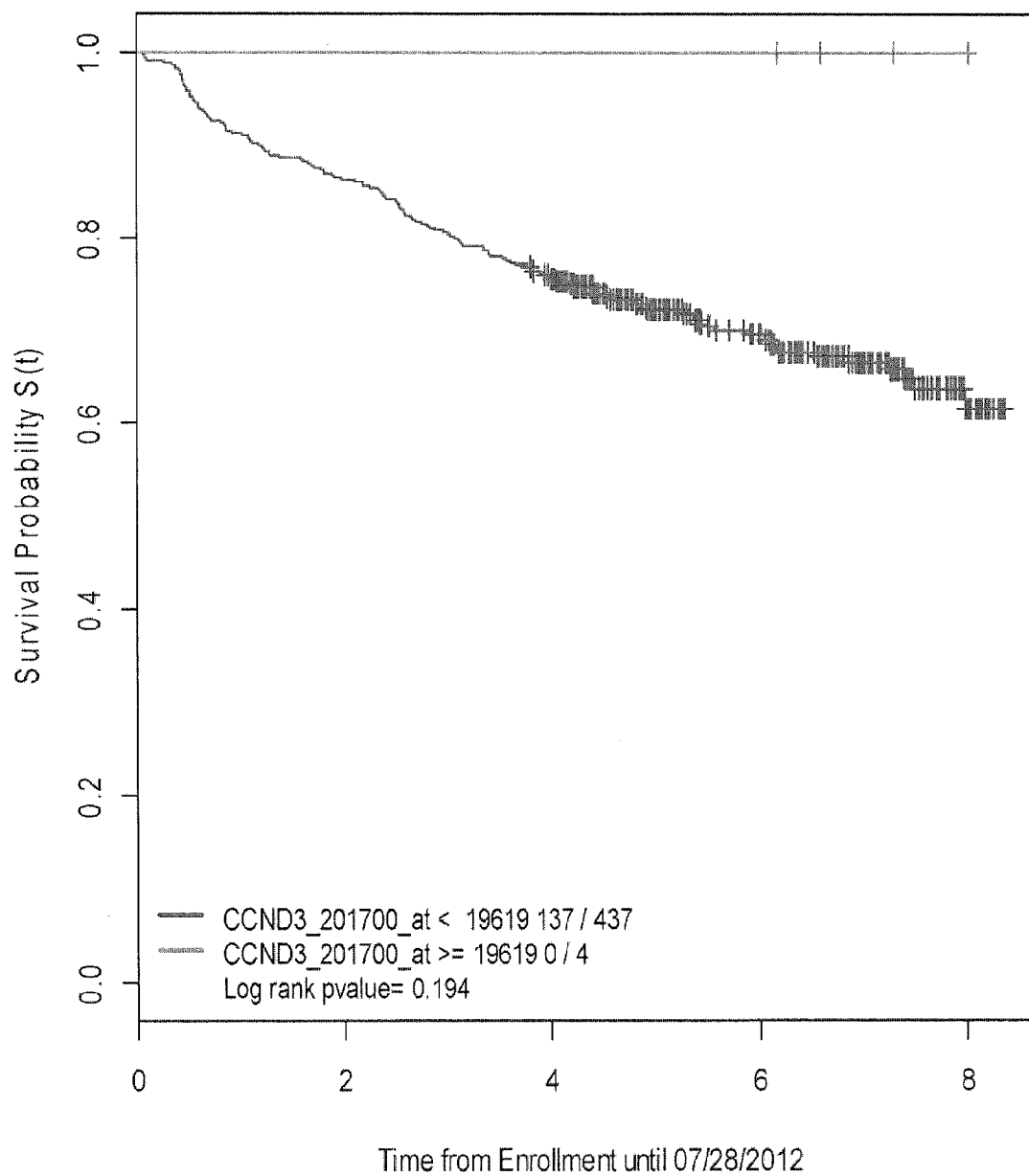
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**FIG. 16B**

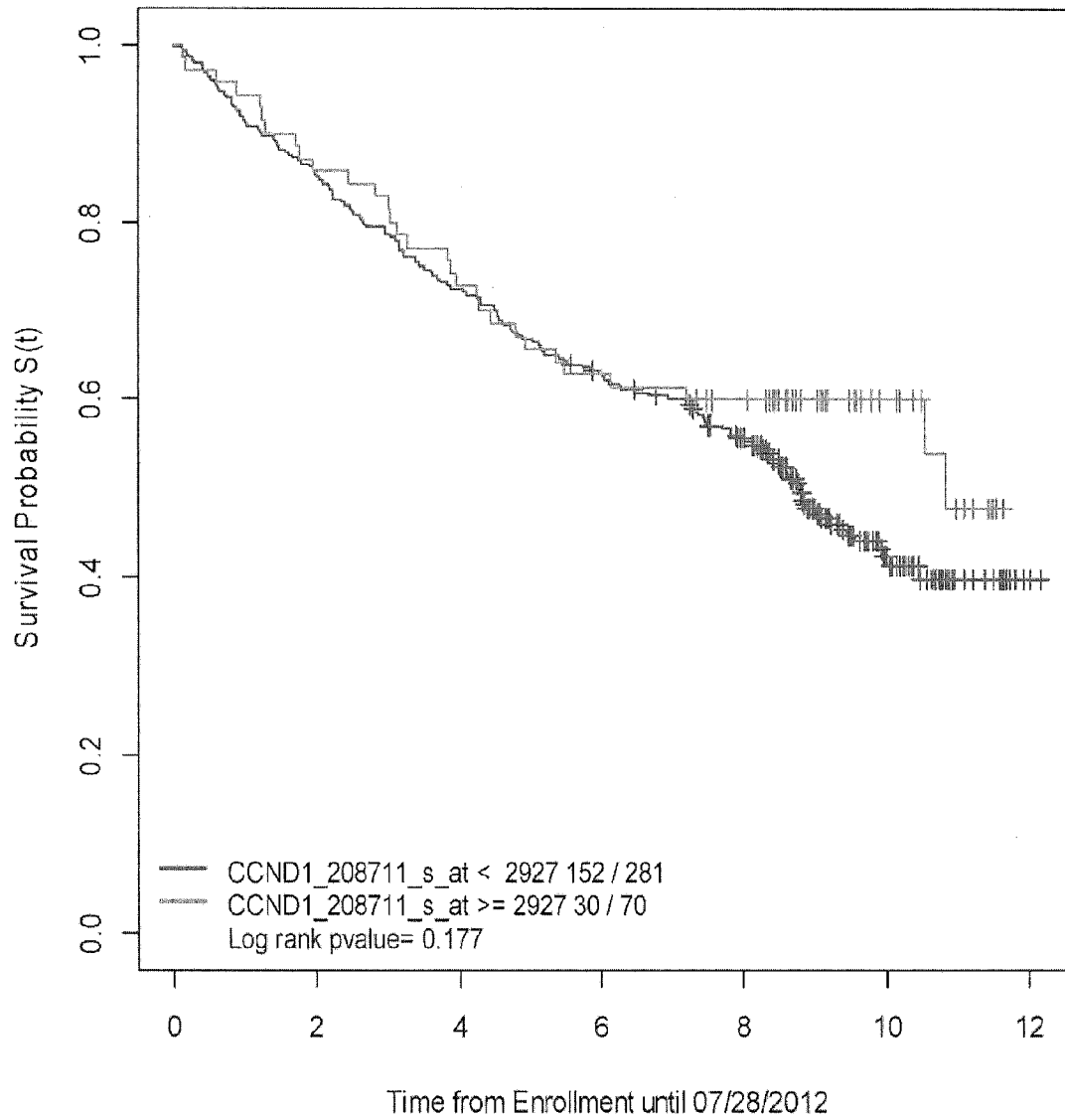
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**FIG. 17A**

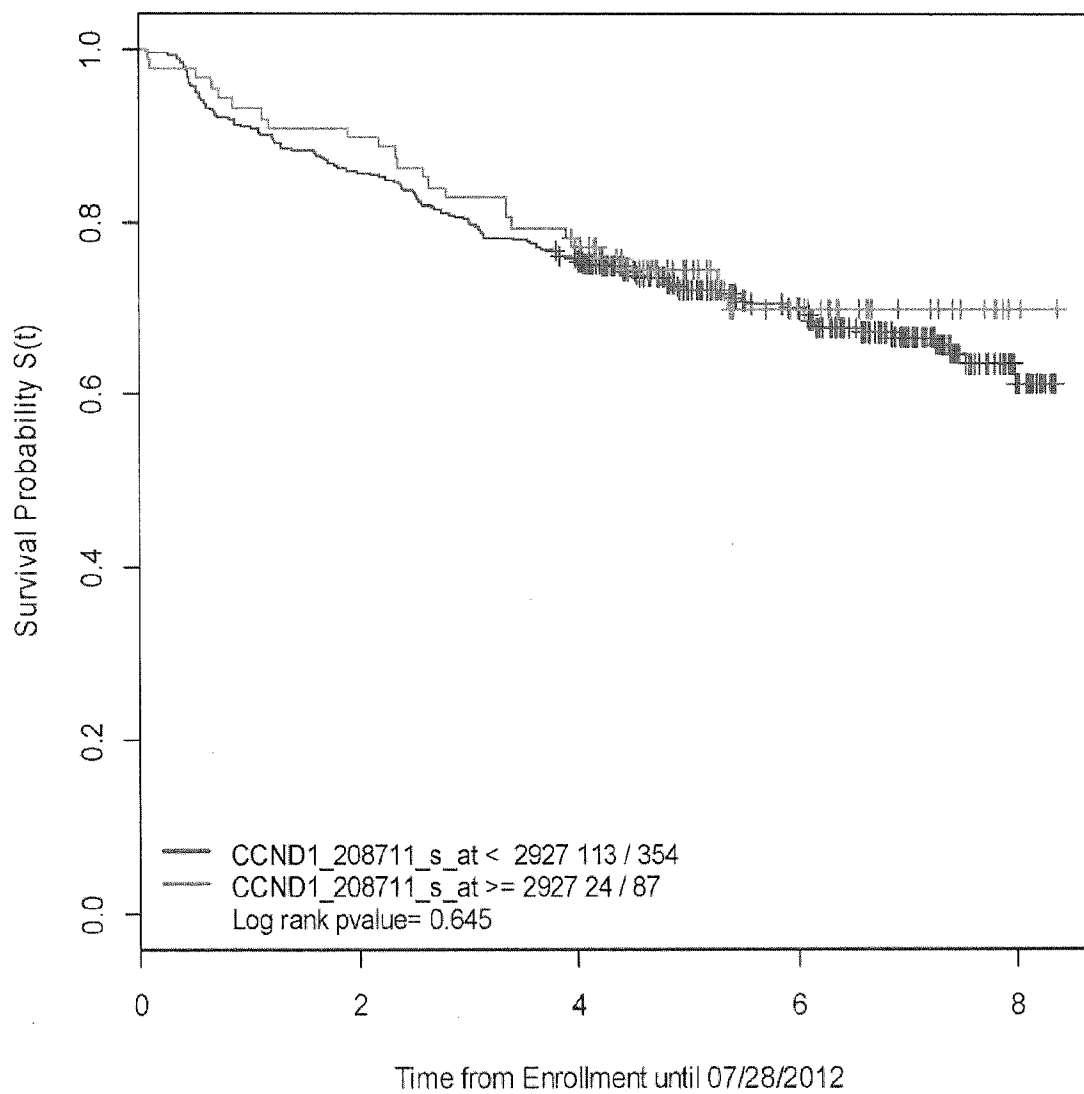
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**FIG. 17B**

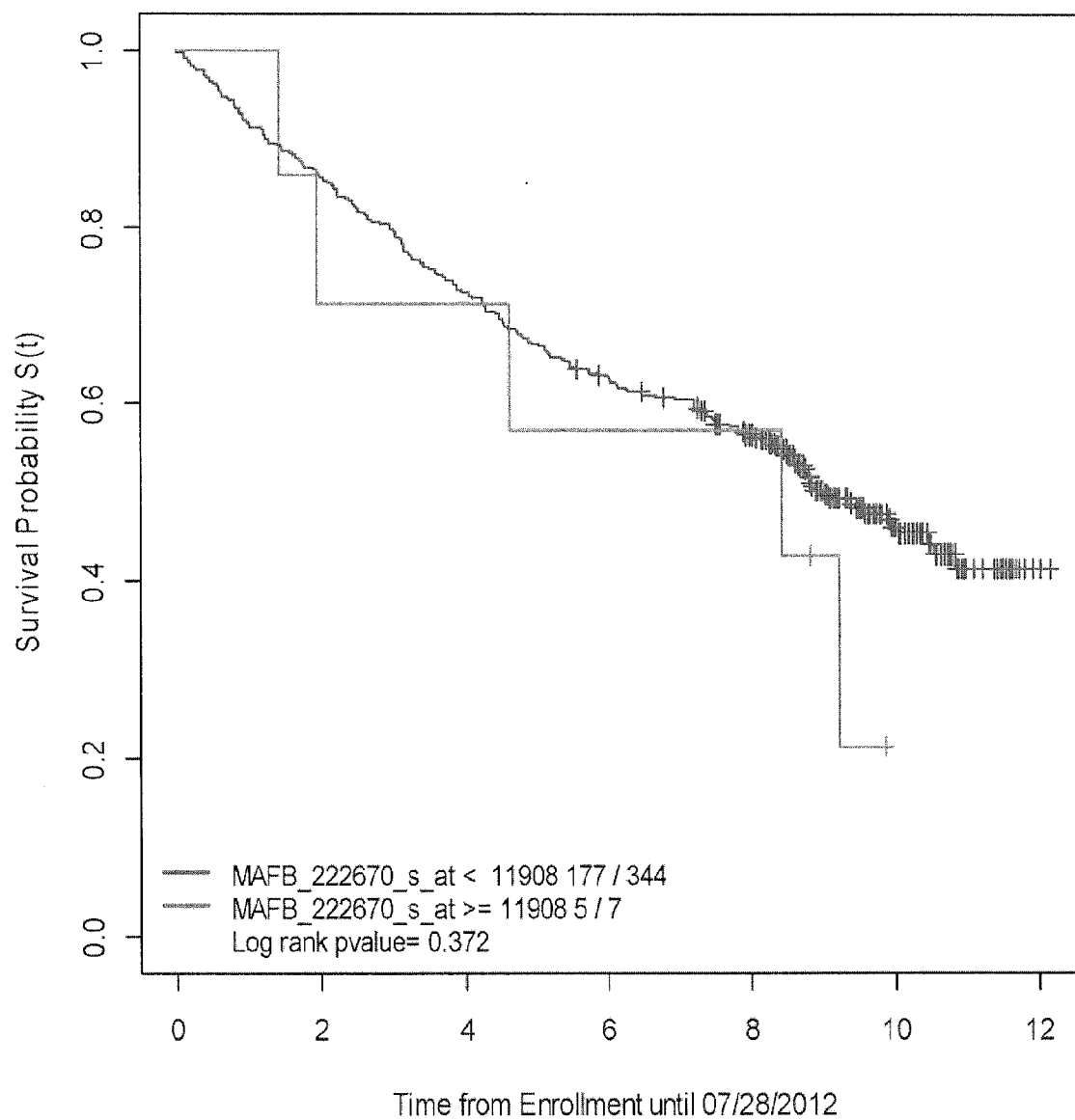
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**FIG. 18A**

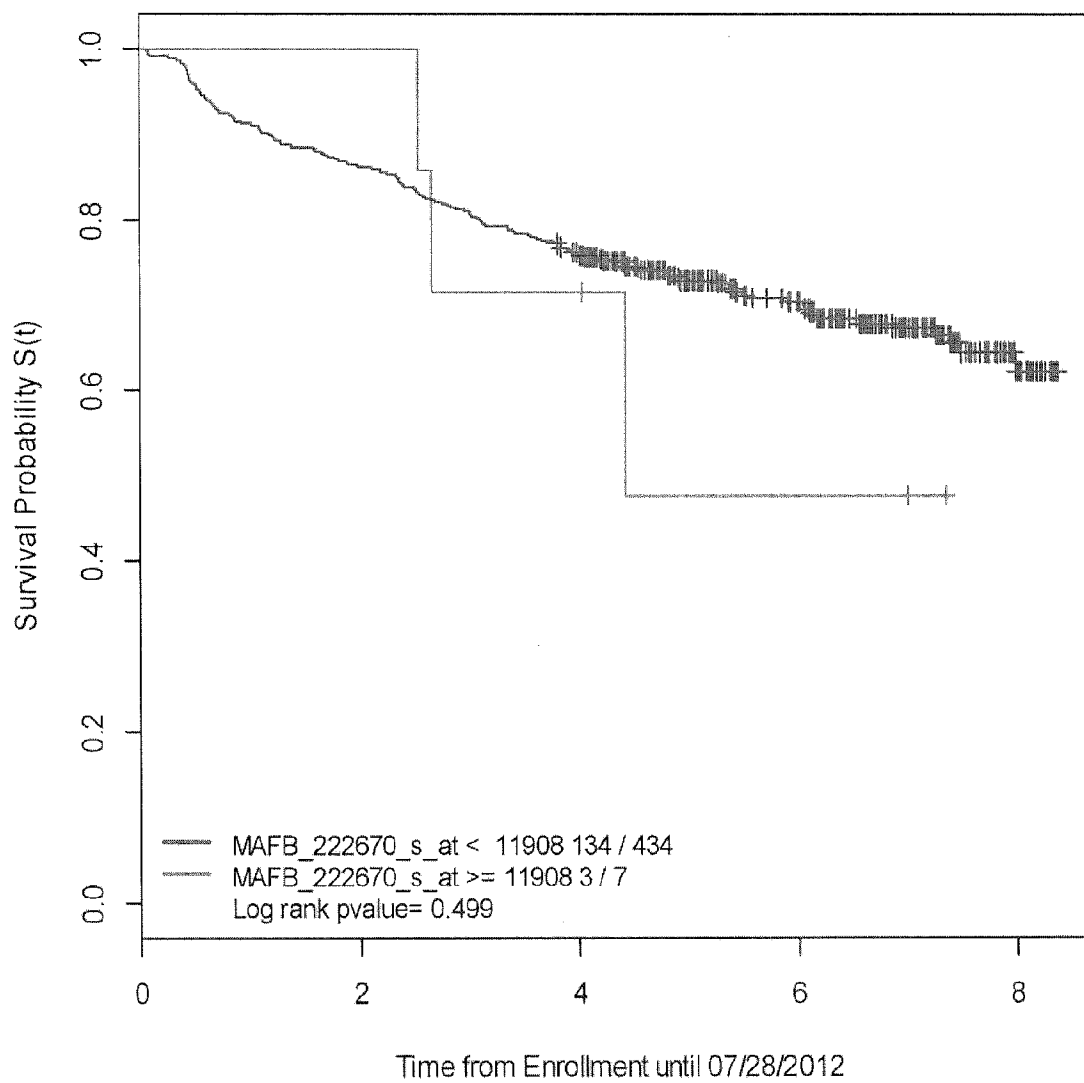
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**FIG. 18B**

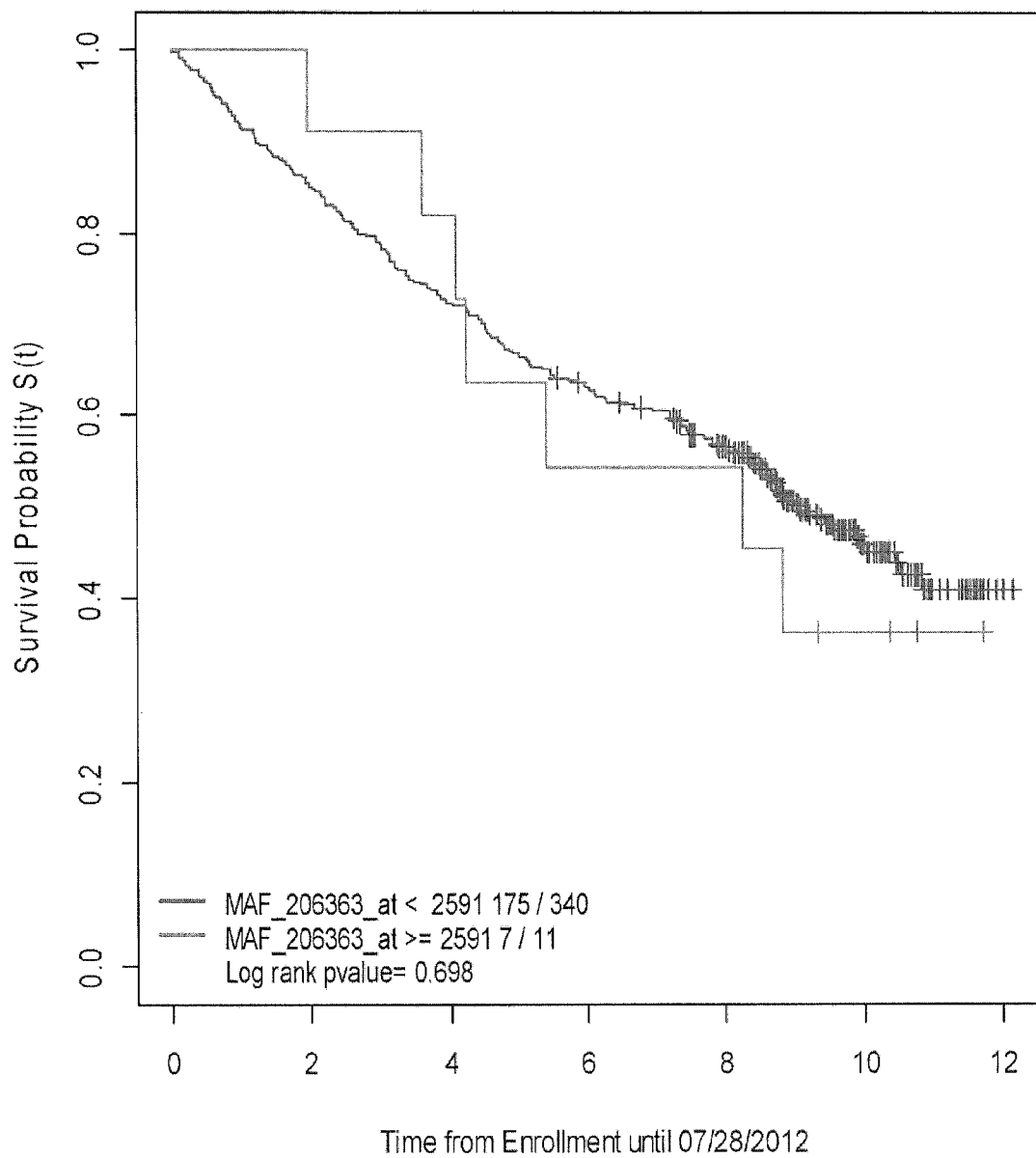
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**FIG. 19A**

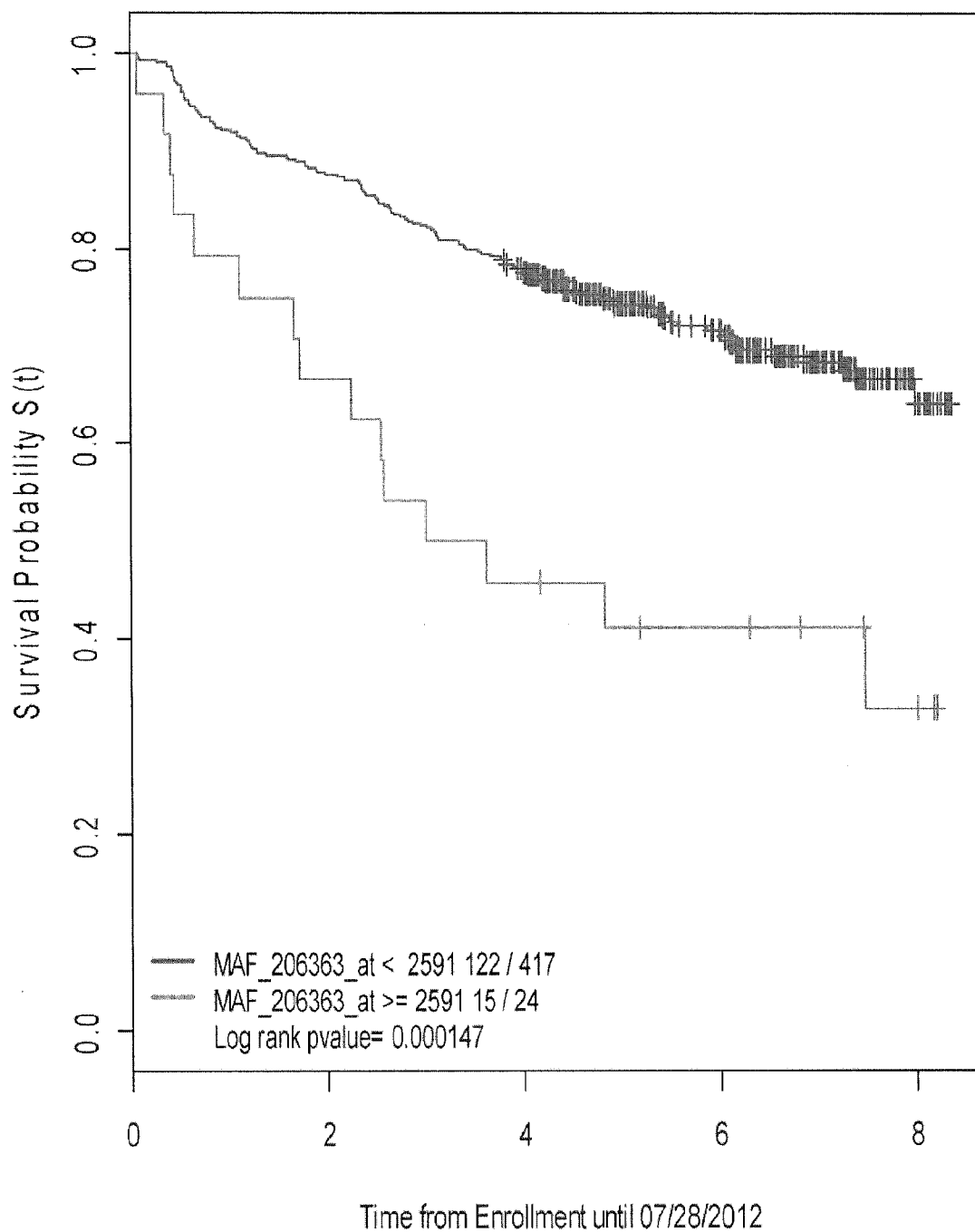
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**FIG. 19B**

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**FIG. 20A**

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**FIG. 20B**

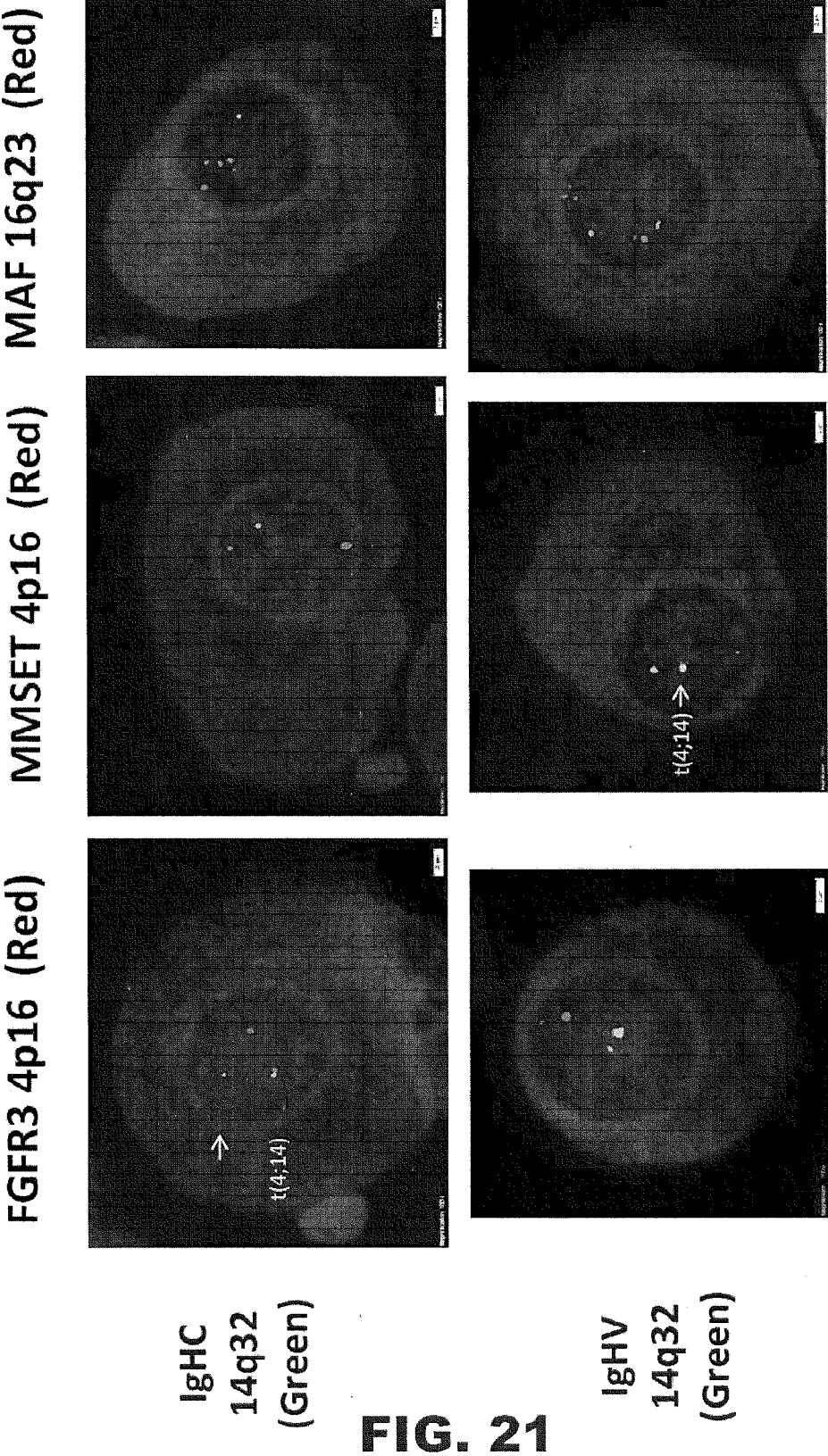


FIG. 21

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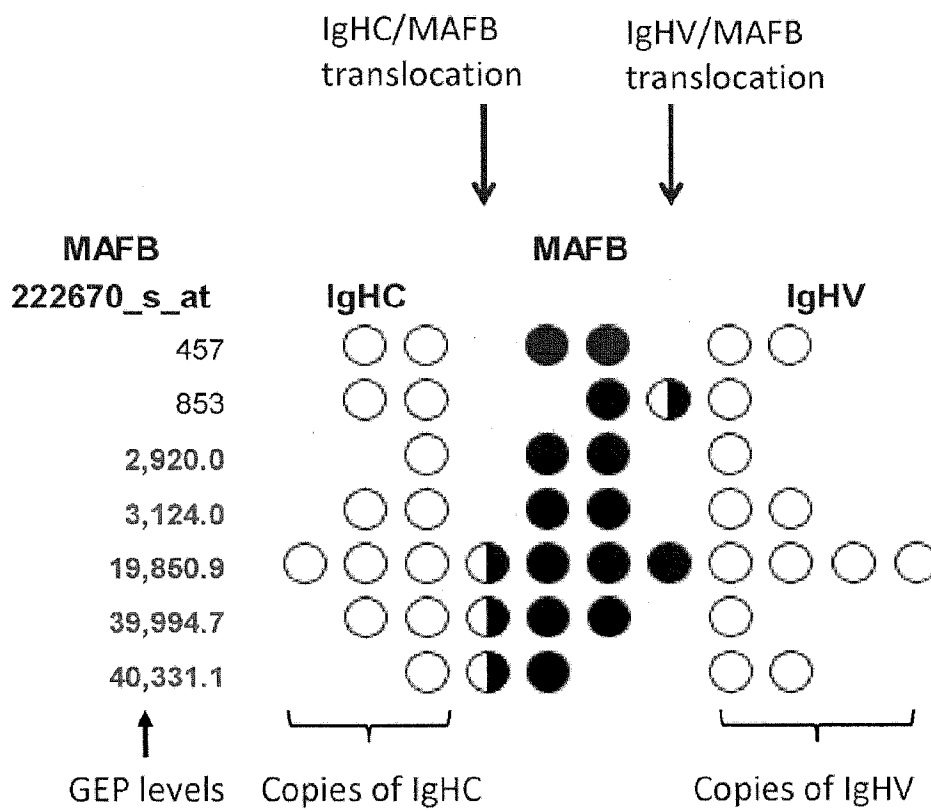


FIG. 22

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/070149

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PINEDA-ROMAN MAURICIO ET AL: "Sustained complete remissions in multiple myeloma linked to bortezomib in total therapy 3: comparison with total therapy 2", BRITISH JOURNAL OF HAEMATOLOGY, vol. 140, no. 6, 1 March 2008 (2008-03-01), pages 625-634, XP055093698, ISSN: 0007-1048, DOI: 10.1111/j.1365-2141.2007.06921.x abstract page 628, column 1, paragraph 1 page 631, column 1 - column 2; figures 5D-F page 632, column 2, paragraph 3 - paragraph 4 ----- -/--	1,3,5-8



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

3 March 2014

Date of mailing of the international search report

07/03/2014

Name and mailing address of the ISA/

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

Authorized officer

Werner, Andreas

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/070149

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.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
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:
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:

see additional sheet

1. ☐ As all required additional 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 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.:

1, 3, 5-8(all partially)

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

International application No

PCT/US2013/070149

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KALFF A ET AL: "The t(4;14) translocation and FGFR3 overexpression in multiple myeloma: prognostic implications and current clinical strategies", BLOOD CANCER JOURNAL, vol. 2, no. 9, 7 September 2012 (2012-09-07), page e89, XP055093344, DOI: 10.1038/bcj.2012.37 abstract page 5, column 1, paragraph 2 - paragraph 3 page 2, column 2, paragraph 1 - page 3, column 1, paragraph 2 -----	1,3,5-8
X	AVET-LOISEAU HERVÉ ET AL: "Molecular heterogeneity of multiple myeloma: pathogenesis, prognosis, and therapeutic implications", JOURNAL OF CLINICAL ONCOLOGY, LIPPINCOTT WILLIAMS & WILKINS, USA, vol. 29, no. 14, 10 May 2011 (2011-05-10), pages 1893-1897, XP009175165, ISSN: 1527-7755, DOI: 10.1200/JCO.2010.32.8435 abstract page 1895, column 1, paragraph 1 - column 2, paragraph 2 -----	1,3,5-8
X	US 2004/156854 A1 (MULLIGAN GEORGE [US] ET AL) 12 August 2004 (2004-08-12) abstract; claims 1-20 paragraph [0005] - paragraph [0029] paragraph [0231] - paragraph [0258] paragraph [0293] page 33; table 1 -----	1,3,5-8
X	OTSUKA M ET AL: "Constitutively active FGFR3 with Lys650Glu mutation enhances bortezomib sensitivity in plasma cell malignancy", ANTICANCER RESEARCH, 1 January 2011 (2011-01-01), pages 113-122, XP055093424, Retrieved from the Internet: URL:http://ar.iiarjournals.org/content/31/1/113.full.pdf [retrieved on 2013-12-13] the whole document ----- -/--	1,3,5-8

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2013/070149

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TAJIMA E ET AL: "Global real-time quantification/reverse transcription-polymerase chain reaction for detecting proto-oncogenes associated with 14q32 chromosomal translocation in multiple myeloma", Haematologica, 1 April 2005 (2005-04-01), pages 559-562, XP055093710, Retrieved from the Internet: URL:http://www.haematologica.org/content/90/4/559.full.pdf [retrieved on 2013-12-16] abstract; table 1 page 560, column 1, paragraph 1 -----	1,3,5-8
A	ZHAN FENGHUANG ET AL: "The molecular classification of multiple myeloma", BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 108, no. 6, 1 September 2006 (2006-09-01), pages 2020-2028, XP002444356, ISSN: 0006-4971, DOI: 10.1182/BLOOD-2005-11-013458 abstract page 2020, column 2, paragraph 1 - page 2021; figure 4; table 1 page 2024, column 2 -----	1,3,5-8
A	LONIAL S. ET AL: "Treatment Options for Relapsed and Refractory Multiple Myeloma", CLINICAL CANCER RESEARCH, vol. 17, no. 6, 15 March 2011 (2011-03-15), pages 1264-1277, XP055093359, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-10-1805 page 1270, column 2 -----	1,3,5-8
A	KARLIN LIONEL ET AL: "Clinical and biological features of t(4;14) multiple myeloma: a prospective study", LEUKEMIA & LYMPHOMA, INFORMA HEALTHCARE, UK, vol. 52, no. 2, 1 February 2011 (2011-02-01), pages 238-246, XP009175174, ISSN: 1029-2403, DOI: 10.3109/10428194.2010.537795 abstract page 239, column 1, paragraph 3 - column 2, paragraph 2 ----- -/--	1,3,5-8

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2013/070149

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MULLIGAN G ET AL: "Pharmacogenomics (PGx) research in the APEX randomized multicenter international phase 3 trial comparing bortezomib and high-dose dexamethasone (Dex)", BLOOD; 47TH ANNUAL MEETING OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY; ATLANTA, GA,USA; DECEMBER 10-13, 2005, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 106, no. 11, Part 1, 1 November 2005 (2005-11-01), page 975A, XP002558148, ISSN: 0006-4971 the whole document -----	1,3,5-8

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2013/070149

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2004156854	A1	12-08-2004	
		AU 2003298873 A1	30-06-2004
		CA 2508348 A1	24-06-2004
		EP 1581629 A2	05-10-2005
		JP 2006517093 A	20-07-2006
		JP 2010207230 A	24-09-2010
		MX PA05005923 A	21-09-2005
		US 2004156854 A1	12-08-2004
		US 2009181393 A1	16-07-2009
		WO 2004053066 A2	24-06-2004

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 3, 5-8(all partially)

Methods to identify or determine if a multiple myeloma patient (that) would benefit from a treatment comprising bortezomib, comprising detecting elevated gene expression levels of FGFR3 or WHSC1/MMSET alone or in combination with other genes of claim 1, wherein an elevated level indicates the presence of a t(4;14) translocation of the 14q32 region, wherein the presence or absence of a 14q32 translocation guides the treatment decision, and preferably indicates that the subject would benefit from said treatment;
Methods of treating multiple myeloma with either bortezomib or alternative therapies, depending on the outcome of said gene expression analysis.

2. claims: 1, 3, 5-8(all partially)

Methods to identify or determine if a multiple myeloma patient (that) would benefit from a treatment comprising bortezomib, comprising detecting elevated gene expression levels of CCND1 alone or in combination with other genes of claim 1, wherein elevated level indicates the presence of a t(11;14) translocation of the 14q32 region, wherein the presence or absence of a 14q32 translocation guides the treatment decision, and preferably indicates that the subject would benefit from said treatment;
Methods of treating multiple myeloma with either bortezomib or alternative therapies, depending on the outcome of said gene expression analysis.

3. claims: 1, 3, 5-8(all partially)

Methods to identify or determine if a multiple myeloma patient (that) would benefit from a treatment comprising bortezomib, comprising detecting elevated gene expression levels of CCND3 alone or in combination with other genes of claim 1, wherein an elevated level indicates the presence of a t(6;14) translocation of the 14q32 region, wherein the presence or absence of a 14q32 translocation guides the treatment decision, and preferably indicates that the subject would benefit from said treatment;
Methods of treating multiple myeloma with either bortezomib or alternative therapies, depending on the outcome of said gene expression analysis.

4. claims: 1, 4-6, 9, 10(all partially)

Methods to identify or determine if a multiple myeloma patient (that) would benefit from a treatment comprising

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

bortezomib, comprising detecting elevated gene expression level of MAF alone or in combination with other genes of claim 1, wherein an elevated level indicates the presence of a t(14;16) translocation of the 14q32 region, wherein the presence or absence of a 14q32 translocation guides the treatment decision, and preferably indicates that the subject would not benefit from said treatment;
Methods of treating multiple myeloma with either bortezomib or alternative therapies, depending on the outcome of said gene expression analysis.

5. claims: 1, 3, 5-8(all partially)

Methods to identify or determine if a multiple myeloma patient (that) would benefit from a treatment comprising bortezomib, comprising detecting elevated gene expression levels of MAFB alone or in combination with other genes of claim 1, wherein an elevated level indicates the presence of a t(14;20) translocation of the 14q32 region, wherein the presence or absence of a 14q32 translocation guides the treatment decision, and preferably indicates that the subject would benefit from said treatment;
Methods of treating multiple myeloma with either bortezomib or alternative therapies, depending on the outcome of said gene expression analysis.

6. claims: 11-22(completely); 2-10(partially)

Methods to detect IgHC or IgHV translocations in a subject with a B-cell malignancy to a chromosomal region encoding a genomic locus selected from FGFR3, WHSC1/MMSET, CCND1, CCND3, MAF or MAFB, comprising performing FISH using at least a first and a second probe;
Methods thereof to identify or determine if a multiple myeloma patient (that) would benefit from a treatment comprising bortezomib, comprising determining whether a patient has a 14q32 translocation according to the above FISH methods, wherein the presence or absence of a 14q32 translocation guides the treatment decision;
Methods thereof of treating multiple myeloma with either bortezomib or alternative therapies, depending on the outcome of said methods.

7. claims: 23-30(completely); 2-10(partially)

Methods to establish a gene expression threshold value indicative of a 14q32 translocation in a subject with B-cell malignancy, comprising comparing the results of FISH using at least a first and a second probe with the results of measuring the gene expression level for a 14q32 translocation partner;
Methods thereof to predict a 14q32 translocation in a

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

suspect suspected to have multiple myeloma, comprising measuring the expression level for one or more 14q32 translocation partners and comparing to a gene expression threshold value obtained by said methods;
Methods thereof to identify or determine if a multiple myeloma patient (that) would benefit from a treatment comprising bortezomib, comprising determining whether a patient has a 14q32 translocation according to the above FISH methods, wherein the presence or absence of a 14q32 translocation guides the treatment decision;
Methods thereof of treating multiple myeloma with either bortezomib or alternative therapies, depending on the outcome of said methods.
