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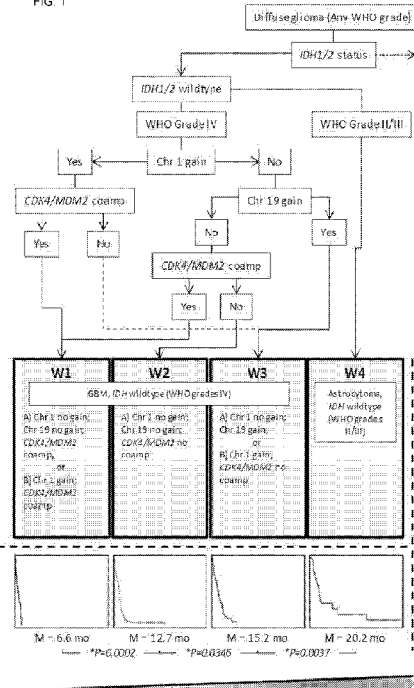
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- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

(54) Title: GENETIC PANEL TO MOLECULARLY CLASSIFY DIFFUSE GLIOMAS

FIG 1



(57) Abstract: A genetic panel to molecularly classify diffuse gliomas is described. The genetic panel includes assessments of chromosome copy number alterations and genetic sequences associated with cell cycle progression. The genetic panel distinguishes between eight distinct forms of glioma.

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GENETIC PANEL TO MOLECULARLY CLASSIFY DIFFUSE GLIOMAS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to US Provisional Patent Application No. 62/506,398 filed May 15, 2017, the entire content of which is incorporated by reference herein.

STATEMENT REGARDING SEQUENCE LISTING

[0002] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is 17-074-WO-PCT_ST25.txt. The text file is 67 KB, was created on May 11, 2018, and is being submitted electronically via EFS-Web.

FIELD OF THE DISCLOSURE

[0003] The current disclosure provides a genetic panel to molecularly classify diffuse gliomas. The genetic panel includes assessments of (i) chromosome copy number alterations, and (ii) genetic amplifications associated with cell cycle progression. The genetic panel distinguishes between eight distinct forms of glioma.

BACKGROUND OF THE DISCLOSURE

[0004] Cancer is characterized by the increase in the number of abnormal, or neoplastic, cells derived from a normal tissue which proliferate to form a tumor mass, the invasion of adjacent tissues by these neoplastic tumor cells, and the generation of malignant cells which eventually spread via the blood or lymphatic system to regional lymph nodes and to distant sites via a process called metastasis. In a cancerous state, a cell proliferates under conditions in which normal cells would not grow. Cancer manifests itself in a wide variety of forms, characterized by different degrees of invasiveness and aggressiveness.

[0005] Gliomas are the most common type of primary brain cancers. They arise from normal glial cells of the brain and/or glial precursor cells. For example, astrocytomas are glioma tumors that arise from astrocytes, a type of glial cell. Astrocytes support neuronal function. Types of astrocytomas include diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma. Astrocytomas can occur anywhere in the brain and spinal cord, however the majority are located in the cerebral hemispheres. Oligodendrogliomas are also gliomas. They arise from a different type of glial cell, oligodendrocytes. Normal oligodendrocytes provide myelin, a fatty substance that covers nerve axons in the brain and spinal cord and allows nerves to conduct electrical impulses more efficiently.

[0006] The grading of tumors is often critical to an accurate diagnosis and prognosis of disease progression, and gliomas are no exception. Decades of experience has led to a system of diagnosis of gliomas based on histology. For nearly a century, classification of primary brain tumors has been based solely upon histomorphologic characteristics and presumed histogenesis of neoplastic cell types. Early classification systems for diffuse gliomas relied upon evaluating the histological subtype as either astrocytoma or oligodendroglioma, with further histological parameters such as nuclear atypia, mitotic figures, microvascular proliferation, and necrosis, to indicate aggressiveness, or higher-grades of gliomas.

[0007] Today the most commonly used standard criteria for classifying gliomas is set forth by the World Health Organization (WHO). Originally presented in 1979, the WHO classification of central nervous system (CNS) tumors has been revised in 1993, 2000, 2007, and most recently in 2016. Prior to the 2016 classification system, WHO glioma classification was based solely upon histopathological criteria, which contains an inherent amount of interobserver variability in interpretation, leading to less predictive clinical outcomes. More recently, large scale genomic efforts such as those from The Genome Cancer Atlas (TCGA) have led to a considerable increase in the identification and understanding of recurrent genetic and epigenetic alterations found in diffuse gliomas, WHO grades II-IV, and have helped to define molecular and prognostic subclasses of these tumors. Such molecular alterations include mutations in the *isocitrate dehydrogenase (IDH) 1* and *2* genes, codeletion of chromosome arms 1p and 19q, or hypermethylation of the gene encoding O-6-methylguanine DNA methyltransferase (*MGMT*). To reflect the understanding of genetic and genomic contributions to glioma biology, the 2016 WHO classification introduced revised classification criteria to incorporate traditional histopathology and molecular signatures into 'integrated' diagnostic entities. Special attention has also been made in this new version to conceptually restructure glioma classification to consider all diffuse gliomas (astrocytomas and oligodendrogliomas) under the common header of "diffuse astrocytic and oligodendroglial tumors". Within this category, molecular alterations help to drive WHO grade II and III diagnoses, and diagnostic entities include diffuse astrocytoma designated as IDH-mutant or IDH-wildtype; anaplastic astrocytoma designated as IDH-mutant or IDH-wildtype; Oligodendroglioma, IDH-mutant, and 1p/19q-codeleted; and anaplastic oligodendroglioma, IDH-mutant, and 1p/19q-codeleted. Not otherwise specified (NOS) categories of these entities are also present, but should be reserved for cases where molecular testing is not possible or where the results are not conclusive. Another change in the WHO grade II and III diffuse glioma category is the discouragement of an oligoastrocytoma diagnosis. In most instances, oligoastrocytoma can be refined into either the astrocytoma or oligodendroglioma category

based upon molecular information. Glioblastoma, WHO grade IV, is now also classified according to IDH status into either glioblastoma, IDH-mutant or glioblastoma, IDH-wildtype. Histological variants of glioblastoma, IDH-wildtype include gliosarcoma, giant cell glioblastoma and epithelioid glioblastoma. Again, a NOS designation can be applied in cases of insufficient molecular information concerning the IDH mutation status.

[0008] A recent analysis of molecular signatures of TCGA diffuse glioma datasets by multidimensional scaling (MDS) showed that there are distinct groups of tumors that cluster together in 2-Dimensional (2D) space. This genomic analysis incorporated data from DNA methylation, DNA copy number alterations (CNAs), and DNA single nucleotide alterations (SNAs). Major genomic factors influencing non-biased large clustering of diffuse gliomas included IDH mutational status, CpG island methylator phenotype (CIMP), polysomy of chromosome 7, monosomy of chromosome 10, and codeletion of chromosome arms 1p and 19q. Regional grouping within larger clusters is also seen in association with specific genetic alterations, such as those in the genes *NRAS*, *HER2*, and *TP53*.

[0009] While this background describes advancements in the ability to distinguish between different glioma types based on histology and genetic signatures, there is still room for significant improvement in the ability to provide prognostic and diagnostic information to patients. This is especially the case given the radically different responses to treatments and survival rates associated with different forms of gliomas. While some forms of glioma can be survived, others, unfortunately and currently, cannot. An ability to reliably and accurately distinguish between different forms of the disease would allow patients and their caretakers to make better-informed decisions regarding treatment options and expectations for success.

SUMMARY OF THE DISCLOSURE

[0010] The current disclosure provides a genetic panel that reliably and accurately distinguishes between eight types of gliomas, each with a distinct survival rate. The genetic panel provides clarity of diagnosis and prognosis early, allowing patients and their caretakers to make better-informed decisions regarding treatment options and expectations of success.

[0011] In particular embodiments, the genetic panel does not require the use of histology to diagnose glioma type. Removing histology from the diagnostic process significantly improves the reliability and accuracy of diagnoses and resulting prognoses. In fact, each molecular subtype disclosed herein shows variable heterogeneity of histopathological subtypes of diffuse gliomas. This observation reinforces the concept of interobserver variability of diagnoses based upon histology alone, and highlights why integrating molecular alterations in diffuse gliomas

increases diagnostic accuracy. While particular embodiments can exclude use of histology, others can include evaluation for Grade II/III or Grade IV WHO classifications. In particular embodiments, the genetic panel utilizes histology to classify 1, 2, 3, or 4 types of glioma.

[0012] In particular embodiments, the genetic panel does not rely on levels of change within a subject, but instead can be run on a binary yes/no basis. This feature similarly improves the reliability and accuracy of diagnoses and resulting prognoses.

[0013] In particular embodiments, the genetic panel assesses changes based on chromosome and cell cycle gene copy number alterations (CNAs) in biopsied cells.

[0014] In particular embodiments, the genetic panel assesses and defines eight molecular subtypes of glioma by the following CNAs: chromosome 1 gain, chromosome 19 gain, chromosome 14 loss, and 1p19q presence or codeletion and genetic sequences associated with IDH1/2 status; CDK4 amplification and/or CDKN2A deletion; and/or CDK4 and MDM2 coamplification.

[0015] In particular embodiments, the genetic panel identifies a W1 glioma based on: IDH1/2 wildtype; chromosome 1 gain; and CDK4/MDM2 co-amplification. In particular embodiments, the genetic panel identifies a W1 glioma based on: IDH1/2 wildtype; no chromosome 1 gain; no chromosome 19 gain; and CDK4/MDM2 co-amplification. In particular embodiments, a WHO Grade IV is also confirmed. A W1 glioma has an average survival prediction of 7 months.

[0016] In particular embodiments, the genetic panel identifies a W2 glioma based on: IDH1/2 wildtype; no chromosome 1 gain; no chromosome 19 gain; and no CDK4/MDM2 co-amplification. In particular embodiments, a WHO Grade IV is also confirmed. A W2 glioma has an average survival prediction of 13 months.

[0017] In particular embodiments, the genetic panel identifies a W3 glioma based on: IDH1/2 wildtype; no chromosome 1 gain; and chromosome 19 gain. In particular embodiments, the genetic panel identifies a W3 glioma based on: IDH1/2 wildtype; chromosome 1 gain; and lack of CDK4/MDM2 coamplification. In particular embodiments, a WHO Grade IV is also confirmed. A W3 glioma has an average survival prediction of 15 months.

[0018] The W1, W2, and W3 gliomas are most similar to WHO Grade IV glioblastoma/IDH wildtype. The current genetic panel, however, provides significantly improved diagnostic and prognostic clarity.

[0019] In particular embodiments, the genetic panel identifies a W4 glioma based on: IDH1/2 wildtype; and a WHO/Grade II/III histology score. The W4 glioma is most similar to WHO Grade II/III astrocytoma/IDH wildtype. W4 reflects one of the few aspects of the genetic panel where

histology is determinative in final diagnostic outcome. A W4 glioma has an average survival prediction of 20 months.

[0020] In particular embodiments, the genetic panel identifies an M1 glioma based on: IDH1/2 mutant; no 1p19q codeletion; CDK4 amplification and/or CDKN2A deletion; and loss of chromosome 14. An M1 glioma has an average survival prediction of 23 months

[0021] In particular embodiments, the genetic panel identifies an M2 glioma based on: IDH1/2 mutant; no 1p19q codeletion; CDK4 amplification and/or CDKN2A deletion; and no loss of chromosome 14. In particular embodiments, the genetic panel identifies an M2 glioma based on: IDH1/2 mutant; no 1p19q codeletion; no CDK4 amplification and/or CDKN2A deletion; and loss of chromosome 14. An M2 glioma has an average survival prediction of 63 months.

[0022] In particular embodiments, the genetic panel identifies an M3 glioma based on: IDH1/2 mutant; no 1p19q codeletion; no CDK4 amplification and/or CDKN2A deletion; and no loss of chromosome 14. An M3 glioma has a median survival of 94.5 months.

[0023] The M1, M2, and M3 gliomas are most similar to WHO Grade II-IV astrocytoma/glioblastoma, IDH mutant. The current genetic panel, however, provides improved diagnostic and prognostic clarity.

[0024] In particular embodiments, the genetic panel identifies an O glioma based on: IDH1/2 mutant; with 1p19q codeletion. The O glioma is most similar to WHO Grade II/III oligodendroglioma, IDH mutant, 1p19q codeletion. An O glioma has a median survival of 132.6 months.

[0025] In particular embodiments, the genetic panel can include a series of yes/no biological queries that sequentially direct a sample into one of the eight described glioma types.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0026] Certain drawings submitted herein are better understood in color, which is not available in patent application publications at the time of filing. Applicants consider the color versions of the drawings as part of the original submission and reserve the right to present color images of the drawings in later proceedings.

[0027] FIG. 1. Summary/flowchart of binary decisions leading to identification of one of eight molecularly defined glioma subtypes defined by the genetic panel disclosed herein.

[0028] FIG. 2. TCGA identifiers for (i) astrocytoma/GBM, IDH mutant; (ii) astrocytoma/GBM, IDH wildtype; and (iii) oligodendroglioma, IDH mutant, 1p/19q codeleted.

[0029] FIGs. 3A-3D. 2D multidimensional scaling plots of TCGA diffuse glioma patients based on genomic data. (FIG. 3A) Multidimensional scaling shows that there are three main clusters.

(FIG. 3B) 2007 WHO histopathological classification across the three main clusters (number of cases for each cluster is listed). (FIG. 3C) WHO grades are shown across clusters (number of cases for each cluster is listed). (FIG. 3D) 3-Dimensional representation of WHO grading, reflecting progression of each cluster.

[0030] FIGs. 4A-4D. 2D diffuse glioma plots with accompanying chromosomal ideograms. (FIG. 4A) The two main clusters on the right contain mutations in the *IDH1* and *IDH2* genes, as shown by the edges connecting the gene with corresponding patients. The IDH-mutant upper right cluster also carries the majority of (FIG. 4B) TP53 and (FIG. 4C) ATRX gene mutations. (FIG. 4D) The IDH-mutant lower right cluster contains gliomas that harbor the oligodendroglioma-specific 1p/19q codeletion, as demonstrated by edges connecting low level copy loss chromosomal regions with corresponding affected patients. This cluster also contains a majority of the IDH2 mutations.

[0031] FIG. 5. 2D visualization of the revised 2016 WHO classification of diffuse gliomas. Multidimensional scaling demonstrates three major clusters of diffuse gliomas. The 2007 WHO histopathologic classifiers are heterogeneous and non-specific with regards to the three main clusters. The 2016 WHO classification aligns well with the three major clusters and can be divided into: 1) oligodendroglial tumors, IDH-mutant and 1p/19q codeleted (WHO grades II-III); 2) astrocytic gliomas/glioblastomas, IDH-mutant (WHO grades II-IV); and 3) astrocytic gliomas/glioblastomas, IDH-wildtype (WHO grades II-IV).

[0032] FIGs. 6A-6H. Clinical characteristics of TCGA diffuse glioma clusters. (FIG. 6A) Survival curves from 2007 WHO histopathological classification criteria (A=diffuse astrocytoma, O=oligodendroglioma, OA=oligoastrocytoma, AA=anaplastic astrocytoma, AO=anaplastic oligodendroglioma, AOA=anaplastic oligoastrocytoma, GBM=glioblastoma). (FIG. 6B) Survival comparison of three main 2D molecular clusters. (FIGs. 6C-6E) Age at diagnosis distribution for each cluster. Patients with astrocytic glioma/glioblastoma are older at presentation in the (FIG. 6C) IDH-wildtype cluster than in the (FIG. 6D) IDH mutant cluster. (FIG. 6E) An apparent bimodal adult age distribution is seen in the oligodendroglioma cluster, with median age of 45 years. (FIG. 6F) Survival of patients with tumors of the oligodendroglioma cluster stratified by age (<45 versus ≥45 years) is significantly different ($p=0.0033$), and comparable to survival stratified by WHO grade (FIG. 6G, FIG. 6H). P values determined using Cox proportional hazard regression.

[0033] FIG. 7. Genomic copy number alteration frequency among molecular clusters. Oligodendrogliomas are defined by 1p/19q codeletion and the second most frequent alteration is loss of chromosome 4. The IDH-mutant astrocytic glioma/glioblastoma cluster has several low

level copy number alterations, including known astrocytoma-associated alterations such as 9p loss and 19q loss. IDH-wildtype diffuse gliomas have frequent polysomy chromosome 7, chromosome 10 loss, and 9p loss. The IDH-wildtype cluster can be further divided into 3 subgroups (A-C). Subgroup A is separated from B and C by either the presence of polysomy chromosome 1 or TP53 mutations. Subgroups B and C are further separated by the presence or absence of polysomy chromosome 19.

[0034] FIGs. 8A-8D. Glioblastoma (GBM), IDH wildtype, WHO grade IV subgroups have differential outcomes with respect to CDK4/MDM2 co-amplification. (FIG. 8A) The WHO grade IV GBMs show a statistically significant survival difference between subgroup B and subgroup C ($p=0.034$), which on a copy number level, can be distinguished by chromosome 19 gain. (FIG. 8B) The presence of CDK4/MDM2 co-amplification in subgroup A shows a trend for poor survival. (FIG. 8C) Subgroup B does not show statistically significant or trending survival differences with CDK4/MDM2 co-amplification. (FIG. 8D) Subgroup C does show a significant ($p=0.033$) decrease in survival in the presence of CDK4/MDM2 co-amplification. P values determined using Cox proportional hazard regression.

[0035] FIGs. 9A-9C. Multidimensional scale mapping derived copy number alterations forms unique prognostic molecular subtypes. (FIG. 9A) Glioblastoma, IDH-wildtype, WHO grade IV can be divided into three subtypes (W1-3). (FIG. 9B) The IDH-mutant astrocytic glioma/glioblastoma cluster can be divided into three molecular subtypes. These molecular subtypes are reflective of overall survival, and independent of WHO grade. (FIG. 9C) Dividing the molecular subtypes into either poor (M1/M2) or favorable (M3) groups is significantly associated with survival (Hazard ratio [HR] 3.28, 95% confidence interval [CI] 1.62-6.62, $p=0.001$). This Hazard ratio is slightly larger, but comparable to dividing this cluster into WHO grade II versus WHO grade III/IV (HR 2.01, 95% CI 1.06-4.02, $p=0.036$). P values determined using Cox proportional hazard regression.

[0036] FIGs. 10A, 10B. Prognostic validation of The Cancer Genome Atlas (TCGA) cluster-derived molecular subtypes in a large cohort from the German Glioma Network (GGN). (FIG. 10A) Bar graph showing normalized median overall survival (OS) compared to baseline with similar trends for TCGA and GGN datasets. (FIG. 10B) Linear regression analysis demonstrating equivalent ratio of normalized molecular subtype OS between TCGA and GGN data sets.

[0037] FIGs. 11A-11D. Distribution of molecular groups across glioblastoma, IDH-wildtype cohorts. (FIGs. 11A, 11B) Overall survival of glioblastoma, copy number subtypes in combined TCGA and GGN population-based datasets (FIG. 11A). Distribution shift of increasing

percentage of better-performing copy number subtypes in paired initial/recurrent glioblastoma and elderly clinical trial cohorts, when compared to TCGA and GGN datasets (FIG. 11B). (FIG. 11C) Multidimensional scaling (MDS) analysis based on whole exome sequencing and copy number alterations shows that mapping of the paired initial/recurrent glioblastoma cohort has uneven spatial distribution when overlaid on the TCGA reference dataset (TCGA cohort = dots; Paired initial glioma = square; Paired recurrent glioma = triangle). (FIG. 11D) Edges (lines) show the connections of individual paired initial and recurrent gliomas. Star symbols highlight a tightly clustered area of which 51% of TCGA glioblastomas exist, but only one initial glioma from the paired initial/recurrent glioblastoma dataset.

[0038] FIG. 12. Exemplary supporting sequences.

DETAILED DESCRIPTION

[0039] Gliomas are the most common type of primary brain cancers. They arise from normal glial cells of the brain and/or glial precursor cells. For example, astrocytomas are glioma tumors that arise from astrocytes, a type of glial cell. Astrocytes support neuronal function. Types of astrocytomas include diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma. Astrocytomas can occur anywhere in the brain and spinal cord, however the majority are located in the cerebral hemispheres. Oligodendrogliomas are also gliomas. They arise from a different type of glial cell, oligodendrocytes. Normal oligodendrocytes provide myelin, a fatty substance that covers nerve axons in the brain and spinal cord and allows nerves to conduct electrical impulses more efficiently.

[0040] The grading of tumors is often critical to an accurate diagnosis and prognosis of disease progression, and gliomas are no exception. Decades of experience has led to a system of diagnosis of gliomas based on histology. For nearly a century, traditional classification of brain tumors has been based primarily upon histomorphologic characteristics, and presumed histogenesis of neoplastic cell types. Early classification systems for diffuse gliomas relied upon evaluating the histological subtype as either Astrocytoma or Oligodendroglioma, with further histological parameters such as nuclear atypia, mitotic figures, endothelial cell proliferation, and necrosis, to indicate aggressiveness, or higher-grades of gliomas. Today the most commonly used standard criteria for classifying gliomas is set forth by the World Health Organization (WHO).

[0041] Originally presented in 1979, the WHO classification of central nervous system (CNS) tumors has been revised in 1993, 2000, 2007, and 2016. Prior to the recent 2016 revised classification system, WHO glioma classification was based solely upon histopathological

criteria, which contains an inherent amount of interobserver variability in interpretation, leading to less predictive clinical outcomes. Over the past few years, large scale genomic efforts such as those from The Genome Cancer Atlas (TCGA) have led to a considerable increase in the identification and understanding of recurrent genetic and epigenetic alterations found in diffuse gliomas, WHO grades II-IV, and have helped to define molecular subclasses of diffuse glioma. Furthermore, recurrent molecular alterations identified in diffuse gliomas, such as mutations in the Isocitrate Dehydrogenase (IDH) 1 and 2 genes, have been found to be better predictors of clinical outcome than histopathology alone.

[0042] To reflect the understanding of genetic and genomic contributions to glioma biology, in 2016 the WHO revised its classification criteria to incorporate traditional histopathology and molecular signatures into 'integrated' diagnostic entities. This integrated diagnostic approach is the first such type of WHO classification scheme for solid tumors in the genomic era. Special attention has also been made in this new version to conceptually restructure glioma classification to consider all diffuse gliomas (astrocytomas and oligodendrogliomas) as similar entities. Within the category of diffuse gliomas, molecular alterations help to drive WHO grade II and III diagnoses, and diagnostic entities include Diffuse Astrocytoma designated as IDH mutant or IDH wildtype; Anaplastic Astrocytoma designated as IDH mutant or IDH wildtype; Oligodendroglioma, IDH mutant, 1p/19q codeleted; and Anaplastic Oligodendroglioma, IDH mutant, 1p/19q codeleted. A not otherwise specified (NOS) category of these entities is also present, but should be reserved for cases where molecular testing is not possible or where the results are not conclusive.

[0043] Another change in the WHO grade II and III diffuse glioma category is the discouragement of the category Oligoastrocytoma. In most instances Oligoastrocytoma can be refined into either the Astrocytoma or Oligodendroglioma category based upon molecular information (Louis et al., WHO Classification of Tumours of the Central Nervous System. 4th edition. Lyon, France: International Agency for Research on Cancer, 2016; Smith et al., *Oncogene* 1999;18;4144-52). Glioblastoma (GBM), WHO grade IV, is also now classified upon IDH status into either GBM, IDH mutant and GBM, IDH wildtype. Some less common forms of GBM, IDH wildtype include Gliosarcoma, Giant Cell GBM, and Epithelioid GBM. Again, a NOS designation can be applied in cases of insufficient molecular information.

[0044] While this discussion describes advancements in the ability to distinguish between different glioma types based on histology and genetic signatures, there is still room for significant improvement in the ability to provide prognostic and diagnostic information to patients. This is especially the case given the radically different responses to treatments and

survival rates associated with different forms of gliomas. While some forms of glioma can be survived, others, unfortunately and currently, cannot. An ability to reliably and accurately distinguish between different forms of the disease would allow patients and their caretakers to make better-informed decisions regarding treatment options and expectations for success.

[0045] The current disclosure provides a genetic panel that reliably and accurately distinguishes between eight types of gliomas, each with a distinct survival rate. The genetic panel provides clarity of diagnosis and prognosis early, allowing patients and their caretakers to make better-informed decisions regarding treatment options and expectations of success.

[0046] Reliable relates to the validation of the disclosed molecular groups as prognostic. The prognostic value of these molecular groups was validated in a large independent glioma cohort from the German Glioma Network (GGN). Accurate can refer to the binary output of the disclosed genetic tests (yes or no) that can be more accurate than WHO histologic grading that has known interobserver variability.

[0047] In particular embodiments, the genetic panel does not require the use of histology to diagnose glioma type. Removing histology from the diagnostic process significantly improves the reliability and accuracy of diagnoses and resulting prognoses. In fact, each molecular subtype disclosed herein shows variable heterogeneity of histopathological subtypes of diffuse gliomas. This observation reinforces the concept of interobserver variability of diagnoses based upon histology alone, and highlights why integrating molecular alterations in diffuse gliomas decrease diagnostic discrepancy.

[0048] In particular embodiments, the genetic panel does not rely on levels of change within a subject, but instead can be run on a binary yes/no basis. This feature similarly improves the reliability and accuracy of diagnoses and resulting prognoses.

[0049] In particular embodiments, the genetic panel assesses changes based on chromosome copy number alterations (CNAs) and genetic sequences associated with cell cycle progression in biopsied cells. The order in which CNAs/genetic sequences are assessed in a technical platform do not matter (i.e. they can all be done at the same time, or in any order, or in batches). The order in which CNAs are used in the algorithm (e.g., illustrated in FIG. 1) does matter for determining classification of gliomas, as described herein.

[0050] In particular embodiments, the genetic panel assesses and defines eight molecular subtypes of glioma by the following CNAs: chromosome 1 gain, chromosome 19 gain, chromosome 14 loss, and 1p19q presence or codeletion; and genetic sequences associated with IDH1/2 status; CDK4 amplification and/or CDKN2A deletion; CDK4 and MDM2 coamplification.

[0051] Gains and Losses of Chromosomes. In particular embodiments, gains and losses of chromosomes (*i.e.*, 1, 14, 19) can be called using thresholds at -0.1 and 0.1 on a log₂-scale as cutoffs when GISTIC scores are applied.

[0052] In particular embodiments, the genetic alterations include gain of chromosome 1 and/or 19. In particular embodiments, chromosome gain can refer to the presence of additional copies of a chromosome. Chromosome gain can also refer to the presence of additional copies of one or more regions of the chromosome. For diploid cells (most mammalian cells except gametes), chromosome 1 or 19 gain can refer to the presence of more than two copies of the chromosome (or a region of the chromosome). Chromosome gain can be caused by chromosomal translocation, wherein a region of chromosome becomes linked to a non-homologous chromosome during a rearrangement event.

[0053] Loss of chromosome 14. In particular embodiments, the genetic alterations include chromosome 14 loss. In particular embodiments, chromosome 14 loss can refer to partial or complete deletion of chromosome 14. Chromosome loss can also be referred to as loss of heterozygosity. In particular embodiments, chromosome loss can include loss of the short arm (p region) and/or loss of the long arm (p region) of a chromosome. Chromosome 14 loss can be due to a chromosomal translocation, wherein a region of chromosome 14 becomes replaced by a portion of a non-homologous chromosome during a rearrangement event.

[0054] In particular embodiments, for assessment of chromosomes 1, 14, and 19, any region of the chromosomes can be assessed, as they are generally lost or gained as whole chromosomes.

[0055] 1p19q co-deletion. In particular embodiments, the genetic alterations include 1p19q codeletion. 1p19q refers to the p region of chromosome 1 and the q region of chromosome 19. Each chromosome has a shorter arm (p region) and longer arm (q region), separated by a centromere. 1p deletion refers to partial or complete loss of the p region of a copy of chromosome 1, and 19q deletion refers to partial or complete loss of the q region of a copy of chromosome 19. In particular embodiments, loss of 1p and/or 19q results from a chromosomal translocation t(1;19).

[0056] IDH1/2 wildtype or mutant. In particular embodiments, the genetic alterations include IDH1/2 mutation. IDH1/2 wildtype refers to the genes encoding the enzymes isocitrate dehydrogenase 1 (IDH1, UniProt ID O75874, SEQ ID NOs: 1 and 2) and isocitrate dehydrogenase 2 (IDH2, UniProt ID P48735, SEQ ID NOs: 3 and 4). Isocitrate dehydrogenases catalyze the decarboxylation of isocitrate to 2-oxoglutarate. In particular embodiments, IDH1/2 mutant can refer to mutation of IDH1 and/or IDH2. In particular embodiments, IDH1/2 can refer

to mutations that alter enzymatic activity of the encoded proteins. In particular embodiments, IDH1 and IDH2 mutations can include missense mutations that alter the sequence of the enzymatic active site. The IDH1 active site includes R132. The most common IDH1 mutation in glioma is R132H. The IDH2 active site includes the residues R140 and R172. The most common IDH2 mutation in gliomas is R172K. In particular embodiments, the mutations are found in exon 4 of IDH1 and exon 4 of IDH2.

[0057] *CDK4 amplification.* In particular embodiments, the genetic alterations include cyclin-dependent kinase 4 (CDK4) amplification. CDK4 (SEQ ID NO: 5) is a gene on chromosome 12 that encodes the CDK4 protein (UniProt ID P11802, SEQ ID NO: 6), which, as part of the cyclin D-CDK4 complex, phosphorylates and inhibits the retinoblastoma protein. CDK4 amplification can refer to the presence of more than two copies of the gene within a human diploid cell.

[0058] *CDKN2A deletion.* In particular embodiments, the genetic alterations include cyclin-dependent kinase inhibitor 2A (CDKN2A) deletion. CDKN2A (SEQ ID NO: 7) is a gene locus that encodes the proteins p16INK4a (UniProt ID P42771-1, SEQ ID NO: 8) and p14ARF (UniProt ID Q8N726-1, SEQ ID NO: 9), which are both tumor suppressors. CDKN2A deletion can refer to loss of genetic material from the CDKN2A locus. In particular embodiments, CDKN2A deletion refers to loss of genetic material that encodes p16INK4a and/or p14ARF. In particular embodiments, CDKN2A deletion can be a deletion of the chromosomal segment 9p21, which includes the CDKN2A locus.

[0059] *MDM2 amplification.* In particular embodiments, the genetic alterations include Mouse double minute 2 homolog (MDM2) amplification. MDM2 (SEQ ID NO: 10) is a gene on chromosome 12 that encodes the protein E3 ubiquitin-protein ligase MDM2 (UniProt ID Q00987, SEQ ID NO: 11), which mediates ubiquitination of p53/TP53. MDM2 amplification can refer to the presence more than two copies of the gene within a human diploid cell.

[0060] In particular embodiments, calling of amplifications and homozygous deletions in genes of interest can be thresholds at 0.6 and -0.6 when GISTIC scores are applied.

[0061] Referring to FIG. 1, in particular embodiments, the genetic panel identifies a W1 glioma based on: IDH1/2 wildtype; chromosome 1 gain; and CDK4/MDM2 co-amplification. In particular embodiments, the genetic panel identifies a W1 glioma based on: IDH1/2 wildtype; no chromosome 1 gain; no chromosome 19 gain; and CDK4/MDM2 co-amplification. In particular embodiments, a WHO Grade IV is also confirmed. A W1 glioma has a survival prediction of 7 months or less and is not responsive to treatments.

[0062] In particular embodiments, the genetic panel identifies a W2 glioma based on: IDH1/2 wildtype; no chromosome 1 gain; no chromosome 19 gain; and no CDK4/MDM2 co-

amplification. In particular embodiments, a WHO Grade IV is also confirmed. A W2 glioma has a survival prediction of 13 months or less.

[0063] In particular embodiments, the genetic panel identifies a W3 glioma based on: IDH1/2 wildtype; no chromosome 1 gain; and chromosome 19 gain. In particular embodiments, a WHO Grade IV is also confirmed. A W3 glioma has a survival prediction of 16 months or less.

[0064] The W1, W2, and W3 gliomas are most similar to WHO Grade IV glioblastoma/IDH wildtype. The current genetic panel, however, provides significantly improved diagnostic and prognostic clarity.

[0065] In particular embodiments, the genetic panel identifies a W4 glioma based on: IDH1/2 wildtype; and a WHO/Grade II/III histology score. The W4 glioma is most similar to WHO Grade II/III astrocytoma/IDH wildtype. W4 reflects one of the few aspects of the genetic panel where histology is determinative in final diagnostic outcome. A W4 glioma has a survival prediction of 21 months or less.

[0066] In particular embodiments, the genetic panel identifies an M1 glioma based on: IDH1/2 mutant; no 1p19q codeletion; CDK4 amplification and/or CDKN2A deletion; and loss of chromosome 14. An M1 glioma has a survival prediction of 24 months or less.

[0067] In particular embodiments, the genetic panel identifies an M2 glioma based on: IDH1/2 mutant; no 1p19q codeletion; CDK4 amplification and/or CDKN2A deletion; and no loss of chromosome 14. In particular embodiments, the genetic panel identifies an M2 glioma based on: IDH1/2 mutant; no 1p19q codeletion; no CDK4 amplification and/or CDKN2A deletion; and loss of chromosome 14. An M2 glioma has a survival prediction of 64 months or less.

[0068] In particular embodiments, the genetic panel identifies an M3 glioma based on: IDH1/2 mutant; no 1p19q codeletion; no CDK4 amplification and/or CDKN2A deletion; and no loss of chromosome 14. An M3 glioma has a median survival of 94.5.

[0069] The M1, M2, and M3 gliomas are most similar to WHO Grade II-IV astrocytoma/glioblastoma, IDH mutant. The current genetic panel, however, provides improved diagnostic and prognostic clarity.

[0070] In particular embodiments, the genetic panel identifies an O glioma based on: IDH1/2 mutant; with 1p19q codeletion. The O glioma is most similar to WHO Grade II/III oligodendroglioma, IDH mutant, 1p19q codeletion. An O glioma has a median survival of 132.6 months and can be responsive to treatment.

[0071] Methods to detect genetic alterations. In particular embodiments, copy number alterations and 1p19q codeletion can be assessed using GISTIC2.0 scores and R software with the copy number package. CNA alterations and 1p19q codeletion can also be detected by

comparing allele intensities to referenced baselines using, for example, the Partek Genomics Suite. Genetic alterations can also be detected by comparative genomic hybridization (CGH). CGH is useful for identifying copy number variation, chromosomal translocation, and/or large chromosomal insertions or deletions (e.g., 40kb or more). For array-based CGH, target sequences (e.g., genomic fragments from a reference genome) can be present on a tile array, and hybridized to sample DNA that has been labelled with a fluorophore (control DNA can be labelled with a different fluorophore). Imaging techniques can be used to detect fluorescent signals, and copy number can be relatively compared between sample DNA and control DNA.

[0072] In particular embodiments, genetic alterations can be detected by multiplex ligation-dependent probe amplification (MLPA). MLPA is a PCR technique that can be used to simultaneously detect alterations in copy number (such as chromosome loss or gain, or gene amplification or deletion), point mutations, and/or indels. The technique uses a forward and reverse primer that recognize adjacent target sites on DNA. When both primers bind to the target site, they can become ligated to form a probe. Next, the ligated probes can be amplified by PCR. If each probe that detects different target sites is designed to be a unique size, the amplified probes can be resolved by size and quantified by label detection (e.g. fluorescent tag). The quantified probes can be compared to a reference, such as a control sample with a known target copy number.

[0073] In particular embodiments, genetic alterations can be detected by fluorescent in situ hybridization (FISH). Probes that bind to target nucleic acids can be linked to a primary label (e.g., biotin). Next, the probes can be hybridized to the target DNA/RNA in a sample (e.g., a tissue section or cell monolayer), and binding of the probe to the target can be detected by measuring a signal from a secondary label (e.g., a fluorescently-tagged antibody specific for the primary label).

[0074] In particular embodiments, genetic alterations can be detected by gene sequencing. Examples of genetic alterations that can be detected by gene sequencing include point mutations, indels, and chromosomal translocation. Gene sequencing techniques that generate long reads can be useful for detecting gene amplifications and genetic alterations in repeat-rich regions of the genome. Examples of sequencing techniques that can be used for detecting genetic alterations include Sanger sequencing and next generation sequencing, such as pyrosequencing or Illumina® (Illumina, Inc., San Diego, CA) sequencing by synthesis. Gene sequencing techniques can be useful for detecting heterogenous genetic alterations (e.g., IDH1 mutation, which is most commonly R132H but can include other mutations).

[0075] In particular embodiments, genetic alterations can be detected by PCR amplification. Genetic alterations such as point mutations and/or short indels (e.g. 1-5 nucleotides) can be detected by allele specific PCR. Genetic alterations such as large deletions, insertions, and/or translocation can be detected by PCR by designing primers that bind to the break points of the indel or translocation. In particular embodiments genetic alterations that change an RNA sequence or the expression level of an RNA can be detected by rt-PCR.

[0076] Exemplary SEQ ID NOs: 1-11 are provided for reference and convenience. Those of ordinary skill in the art can access additional supporting sequences from publicly available databases. Further, respective binding primers and probes can be generated based on these sequences and publicly available programs, as well as exemplars provided herein.

[0077] As representative examples of publicly-available sequences to support the disclosure, Horbinski *et al.*, J. Mol. Diagn. 2010 Jul; 12(4): 487-492 describe methods to detect IDH1 and IDH2 mutations by fluorescence melting curve analyses. For *IDH1* mutation detection, forward primer (5'-ACGGTCTTCAGAGAAGC-3' (SEQ ID NO: 12)), reverse primer (5'-GGTGTAGATACCAAAAGATAAGAAT-3' (SEQ ID NO: 13)), and two probes (5'-LC640-ATGATAGGTTTTACCCATCCACTCACAAGC-3' (SEQ ID NO: 14; it is noted that other labels could be used) and 5'-ATCCCCATAAGCATGACGACCTA-FL-3' (SEQ ID NO: 15)) were used to generate a 202-bp PCR product. Similarly, for *IDH2* detection, forward primer (5'-TGCAGTGGGACCACTATTATC-3' (SEQ ID NO: 16)), reverse primer (5'-CTTGACACCACTGCCATC-3' (SEQ ID NO: 17)), and two probes (5'-LC640-TGATGGGCTTGGTCCAGCCAGGG-3' (SEQ ID NO: 18; it is noted that other labels could be used) and 5'-TGGGCGTGCCTGCCAATG-FL-3' (SEQ ID NO: 19)) were used to generate a 360-bp PCR product. All primers and probes were obtained from TIB Molbiol (Berlin, Germany).

[0078] An *et al.*, Am J. Pathol. 1999 Jan.; 154(1): 113-118 describe methods to detect CDK4 amplification and overexpression. Primer sequences for *CDK4* and the control loci were as follows: 5'-CATGTAGACCAGGACCTAAGG (sense; SEQ ID NO: 20) and 5'-AACTGGCGCATCAGATCCTAG (antisense; SEQ ID NO: 21) for *CDK4* resulting in a 206-bp PCR product, 5'-F-AACGTGTCAGTGGTGGACCTG (sense; SEQ ID NO: 22; F=fluorescein, though it is noted that other labels could be used) and 5'-AGTGGGTGTCGCTGTTGAAGT (antisense; SEQ ID NO: 23) for *GAPDH* generating a 160-bp PCR product, and 5'-TGGGAAAGCTGTTTACTGCG (sense; SEQ ID NO: 24) and 5'-CAGGGAACACATTCCTTTGC (antisense; SEQ ID NO: 25) for *APRT* generating a 134-bp PCR product. An additional forward and reverse primer to detect CDK4 are described in Sasaki *et al.*, BMC Cancer, 2014,14: 468,

and include 5'-CTTCTGCAGTCCACATATGCAACA-3' (SEQ ID NO: 26) and 5'-CAACTGGTCGGCTTCAGAGTTTC-3' (SEQ ID NO: 27), respectively.

[0079] The following, described in Aveyard and Knowles, J. Mol. Diagn. 2004 Nov; 6(4): 356-365, include exemplary primers and probes that can be used to detect CDKN2A presence or deletion. It is noted that other labels could be used.

Gene: p15: forward primer: CCCTCGACACTCACCATGAA (SEQ ID NO: 28);

reverse primer: CGACCCCTGGAATGTCACAC (SEQ ID NO: 29);

probe: AAATATCCCTGGAAATCCGCTTCTCTGTGTT-FAM (SEQ ID NO: 30).

Gene: p14ARF: forward primer: TGATGCTACTGAGGAGCCAGC (SEQ ID NO: 31);

reverse primer: AGGGCCTTTCCTACCTGGTC (SEQ ID NO: 32);

probe: TCTAGGGCAGCAGCCGCTTCCTAGA-FAM (SEQ ID NO: 33).

Gene: p16 exon 2 forward primer: TTTCCGTCATGCCGGC (SEQ ID NO: 34);

reverse primer: TCATCATGACCTGCCAGAGAGA (SEQ ID NO: 35);

probe: CCCACCCTGGCTCTGACCATTCTG-FAM (SEQ ID NO: 36).

Gene: ALDOB forward primer: TGACAGGAAAGCCCTGGC (SEQ ID NO: 37);

reverse primer: TTCCCATGGTACCTATGGTG (SEQ ID NO: 38);

probe: CTCCTTATGCTGCCCTTGGCCCTC-FAM (SEQ ID NO: 39).

Gene: PFKL forward primer: GATGCTGGCACAATACCGC (SEQ ID NO: 40);

reverse primer: GGGTCACGTGCTCCAGCT (SEQ ID NO: 41);

probe: TCAGTATGGCCGCCTACGTGTCAGG-FAM (SEQ ID NO: 42).

[0080] Sasaki *et al.*, BMC Cancer, 2014,14: 468, also describe methods to detect MDM2 expression. Exemplary forward and reverse primers to detect MDM2 expression include 5'-TGGGCAGCT TGAAGCAGTTG-3' (SEQ ID NO: 43) and 5'-CAGGCTGCCATGTGACCTAAGA-3' (SEQ ID NO: 44), respectively.

[0081] In particular embodiments, genetic alterations can be detected by immunostaining techniques. Genetic alterations that change the sequence of a protein (e.g., IDH1/2 mutation) or alter the expression of a protein can be detected using binding domains (e.g., antibodies) that specifically bind to a protein (e.g., a mutant protein). Examples of immunostaining techniques that can be used to detect proteins include enzyme linked immunosorbent assay, flow cytometry, Western blotting, and immunohistochemistry.

[0082] Binding domains can particularly include any peptide that specifically binds an epitope on a protein of interest. Sources of binding domains include antibody variable regions from various species (which can be in the form of antibodies, sFvs, scFvs, Fabs, scFv-based grababody, or soluble VH domain or domain antibodies). These antibodies can form antigen-binding regions

using only a heavy chain variable region, *i.e.*, these functional antibodies are homodimers of heavy chains only (referred to as "heavy chain antibodies") (Jespers *et al.*, Nat. Biotechnol. 22:1161, 2004; Cortez-Retamozo *et al.*, Cancer Res. 64:2853, 2004; Baral *et al.*, Nature Med. 12:580, 2006; and Barthelemy *et al.*, J. Biol. Chem. 283:3639, 2008).

[0083] Phage display libraries of partially or fully synthetic antibodies are available and can be screened for an antibody or fragment thereof that can bind a selected epitope. For example, binding domains may be identified by screening a Fab phage library for Fab fragments that specifically bind to an epitope on a protein of interest (see Hoet *et al.*, Nat. Biotechnol. 23:344, 2005). Phage display libraries of human antibodies are also available. Additionally, traditional strategies for hybridoma development using a target of interest as an immunogen in convenient systems (*e.g.*, mice, HuMAb mouse®, TC mouse™, KM-mouse®, llamas, chicken, rats, hamsters, rabbits) can be used to develop binding domains. In particular embodiments, binding domains specifically bind to selected epitopes and do not cross react with nonspecific components or unrelated targets. Once identified, the amino acid sequence or polynucleotide sequence coding for the CDR within a binding domain can be isolated and/or determined.

[0084] In particular embodiments, an antibody fragment is used as one or more binding domains. An "antibody fragment" denotes a portion of a complete or full length antibody that retains the ability to bind to an epitope. Examples of antibody fragments include Fv, scFv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; and linear antibodies. Antibody fragments can be made by various techniques, including proteolytic digestion of an intact antibody as well as production by recombinant host-cells (*e.g.*, human suspension cell lines, *E. coli* or phage). Antibody fragments can be screened for their binding properties in the same manner as intact antibodies.

[0085] Numerous relevant antibodies that bind proteins of interest are also commercially available. "Bind" means that the binding domain associates with its target epitope with a dissociation constant (1(D) of 10⁻⁸ M or less, in particular embodiments of from 10⁻⁵ M to 10⁻¹³ M, in particular embodiments of from 10⁻⁵ M to 10⁻¹⁰ M, in particular embodiments of from 10⁻⁵ M to 10⁻⁷ M, in particular embodiments of from 10⁻⁸ M to 10⁻¹³ M, or in particular embodiments of from 10⁻⁹ M to 10⁻¹³ M. The term can be further used to indicate that the binding domain does not bind to other biomolecules present, (*e.g.*, it binds to other biomolecules with a dissociation constant (KD) of 10⁻⁴ M or more, in particular embodiments of from 10⁻⁴ M to 1 M).

[0086] Methods of diagnosis. The genetic panels, kits, and methods described herein can be used to diagnose what molecular subtype of glioma a subject has. For instance, the scheme in FIG. 1 can be used to determine which subtype of glioma corresponds to a sample from a subject with glioma.

[0087] By way of further non-limiting example, a method of diagnosing whether a subject with glioma has type W1, W2, W3, W4, M1, M2, M3 or O glioma involves (a) obtaining a glioma sample from the subject; (b) contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype; if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain; if the glioma sample exhibits chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and if the glioma sample exhibits CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma; (c) if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain; if the glioma sample exhibits chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W3 glioma; (d) if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain; if the glioma sample does not exhibit chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between chromosome 19 and the primer indicative of presence or absence of chromosome 19 gain; if the glioma sample exhibits chromosome 19 gain, identifying the glioma sample as a W3 glioma; (e) if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain; if the glioma sample does not exhibit chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between chromosome 19 and the primer indicative of presence or absence of chromosome 19 gain; if the glioma sample does not exhibit chromosome 19 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and if the glioma sample does exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma; (f) if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain; if the glioma sample does not exhibit chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between chromosome

19 and the primer indicative of presence or absence of chromosome 19 gain; if the glioma sample does not exhibit chromosome 19 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W2 glioma; (g) if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as a O glioma; (h) if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma; (i) if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma; (j) if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample does exhibit chromosome 14 loss,

identifying the glioma sample as an M2 glioma; or (k) if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma; thereby diagnosing whether a subject with glioma has type W1, W2, W3, W4, M1, M2, M3 or O glioma.

[0088] Also provided are histology-free method of diagnosing whether a subject with IDH1/2 mutant glioma has type M1, M2, M3 or O glioma, which methods include: (a) obtaining an IDH1/2 mutant glioma sample from the subject; (b) contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as an O glioma, (c) if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma; (d) if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma; (e) if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample

with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma; or (f) if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma; thereby diagnosing whether a subject with IDH1/2 mutant glioma has type M1, M2, M3 or O glioma without use of histology.

[0089] Assessment to modify sets. The data described in Example 2 suggest that analysis of recurrent glioblastoma samples is missing the tumors with the most common DNA structure found in the natural population. Further, it suggests that this global DNA pattern is associated with clinical characteristics that lead surgeons across North America and Asia to not operate on these first recurrent glioblastoma patients. It also seems likely that patients with this global DNA pattern are underrepresented in clinical trials, particularly in those focused on first recurrence of glioblastoma. Given the ability described herein for DNA analysis of tumors to predict outcomes across large datasets, targeted copy number analysis with or without exome sequencing will be useful to balance clinical trial arms with patients having predictable outcomes. Current failure to do so may contribute to discordance between phase 2 and phase 3 clinical trial outcomes, for instance.

[0090] Thus, there are enabled herein methods for assessing glioma samples from subjects, such as prospective or actual research study subjects (e.g., subjects of a clinical trial), using the genetic panels and methods described herein, in order to alter the balance of glioma subtypes in a set of subjects (such as subjects in a research study, or within groups in a study) or a set of samples. Such selection may include balancing the representation of some or all glioma subtypes in a group; balancing or skewing to over- or under-represent one or more specific glioma subtype(s) (such as any one or more of molecular subtype W1, W2, W3, W4, M1, M2, M3 and/or O); and so forth. By way of non-limiting example, by classifying glioma samples from subjects selected for a clinical trial (or other research group), a group can be assembled that is weighted for glioma patients with a relatively poor survival rate, or those with a relatively good survival rate, or balanced to remove significant bias to any one or more level or rate of survival.

[0091] Kits. Kits to practice the genetic panel are also provided. Kits can include one or more containers including for example, primer pairs, probe sequences, binding proteins (e.g., antibodies) and/or reagents or supplies to assess one or more of chromosome 1 gain, chromosome 19 gain, chromosome 14 loss; 1p19q presence or codeletion; IDH1/2 wildtype or mutant; CDK4 amplification and/or CDKN2A deletion; and/or CDK4 and MDM2 coamplification. In particular embodiments, the kits include one or more containers including primer pairs, probe sequences, and/or binding proteins (e.g., antibodies) and/or reagents or supplies to assess chromosome 1 gain, chromosome 19 gain, chromosome 14 loss; 1p19q presence or codeletion; IDH1/2 wildtype or mutant; CDK4 amplification and/or CDKN2A deletion; and CDK4 and MDM2 coamplification.

[0092] Components of the kit can be prepared for storage and later use. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use, or sale of the kit, which notice reflects approval by the agency of manufacture, use, or sale, when required.

[0093] Optionally, the kits further include instructions for using the kit in the methods. In various embodiments, the instructions can include appropriate instructions to interpret results associated with using the kit; proper disposal of the related waste; and the like. The instructions can be in the form of printed instructions provided within the kit or the instructions can be printed on a portion of the kit itself. Instructions may be in the form of a sheet, pamphlet, brochure, CD-ROM, or computer-readable device, or can provide directions to instructions at a remote location, such as a website.

[0094] The genetic panels described herein can be used to guide treatment regimens for patients, understanding that actual treatment will take into account personalized factors for individual patients.

[0095] Exemplary Embodiments.

1. A genetic panel to diagnose glioma, wherein the genetic panel assesses and defines eight molecular subtypes of glioma by : the following CNAs: chromosome 1 gain, chromosome 19 gain, chromosome 14 loss, and 1p19q presence or codeletion; and genetic sequences associated with IDH1/2 status; and CDK4 amplification and/or CDKN2A deletion; and/or CDK4 and MDM2 coamplification.
2. A genetic panel to diagnose glioma, wherein the genetic panel assesses: (i) chromosome copy number alterations (CNAs) and (ii) genetic amplifications associated with cell cycle progression in biopsied cells.

3. The genetic panel of embodiment 1 or 2 wherein the genetic panel identifies a W1 glioma based on: IDH1/2 wildtype; chromosome 1 gain; and CDK4/MDM2 co-amplification.
4. The genetic panel of embodiment 1 or 2 wherein the genetic panel identifies a W1 glioma based on: IDH1/2 wildtype; no chromosome 1 gain; no chromosome 19 gain; and CDK4/MDM2 co-amplification.
5. The genetic panel of embodiment 3 or 4 wherein a WHO Grade IV is confirmed.
6. The genetic panel of embodiment 1 or 2 wherein the genetic panel identifies a W2 glioma based on: IDH1/2 wildtype; no chromosome 1 gain; no chromosome 19 gain; and no CDK4/MDM2 co-amplification.
7. The genetic panel of embodiment 6 wherein a WHO Grade IV is also confirmed.
8. The genetic panel of embodiment 1 or 2 wherein the genetic panel identifies a W3 glioma based on: IDH1/2 wildtype; no chromosome 1 gain; and chromosome 19 gain.
9. The genetic panel of embodiment 6 wherein a WHO Grade IV is also confirmed.
10. The genetic panel of embodiment 1 or 2 wherein the genetic panel identifies a W4 glioma based on: IDH1/2 wildtype; and a WHO/Grade II/III histology score.
11. The genetic panel of embodiment 1 or 2 wherein the genetic panel identifies an M1 glioma based on: IDH1/2 mutant; no 1p19q codeletion; CDK4 amplification and/or CDKN2A deletion; and loss of chromosome 14.
12. The genetic panel of embodiment 1 or 2 wherein the genetic panel identifies an M2 glioma based on: IDH1/2 mutant; no 1p19q codeletion; CDK4 amplification and/or CDKN2A deletion; and no loss of chromosome 14.
13. The genetic panel of embodiment 1 or 2 wherein the genetic panel identifies an M2 glioma based on: IDH1/2 mutant; no 1p19q codeletion; no CDK4 amplification and/or CDKN2A deletion; and loss of chromosome 14.
14. The genetic panel of embodiment 1 or 2 wherein the genetic panel identifies an M3 glioma based on: IDH1/2 mutant; no 1p19q codeletion; no CDK4 amplification and/or CDKN2A deletion; and no loss of chromosome 14.
15. The genetic panel of embodiment 1 or 2 wherein the genetic panel identifies an O glioma based on: IDH1/2 mutant; with 1p19q codeletion.
16. A genetic panel that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by:
 - (a) assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant;

- (b) if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain; if the glioma sample exhibits chromosome 1 gain, assessing whether the glioma sample exhibits CDK4/MDM2 coamplification; and if the glioma sample exhibits CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma;
- (c) if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain; if the glioma sample exhibits chromosome 1 gain, assessing whether the glioma sample exhibits CDK4/MDM2 coamplification; and if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W3 glioma;
- (d) if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain; if the glioma sample does not exhibit chromosome 1 gain, assessing whether the glioma sample exhibits chromosome 19 gain; if the glioma sample exhibits chromosome 19 gain, identifying the glioma sample as a W3 glioma;
- (e) if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain; if the glioma sample does not exhibit chromosome 1 gain, assessing whether the glioma sample exhibits chromosome 19 gain; if the glioma sample does not exhibit chromosome 19 gain, assessing whether the glioma sample exhibits CDK4/MDM2 coamplification; and if the glioma sample does exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma;
- (f) if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain; if the glioma sample does not exhibit chromosome 1 gain, assessing whether the glioma sample exhibits chromosome 19 gain; if the glioma sample does not exhibit chromosome 19 gain, assessing whether the glioma sample exhibits CDK4/MDM2 coamplification; and if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W2 glioma;
- (g) if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion; if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as a O glioma;
- (h) if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma;

- (i) if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma;
- (j) if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma;
- (k) if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma; thereby distinguishing between W1, W2, W3, W4, M1, M2, M3 and O glioma types.
17. The genetic panel of embodiment 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by: assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant; if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain; if the glioma sample exhibits chromosome 1 gain, assessing whether the glioma sample exhibits CDK4/MDM2 coamplification; and if the glioma sample exhibits CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma, thereby distinguishing between W1, W2, W3, W4, M1, M2, M3 and O glioma types.
18. The genetic panel of embodiment 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by: assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant; if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain; if the glioma sample exhibits chromosome 1 gain, assessing whether the glioma sample exhibits CDK4/MDM2 coamplification; and if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W3 glioma, thereby distinguishing between W1, W2, W3, W4, M1, M2, M3 and O glioma types.

19. The genetic panel of embodiment 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by: assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant; if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain; if the glioma sample does not exhibit chromosome 1 gain, assessing whether the glioma sample exhibits chromosome 19 gain; if the glioma sample exhibits chromosome 19 gain, identifying the glioma sample as a W3 glioma, thereby distinguishing between W1, W2, W3, W4, M1, M2, M3 and O glioma types.
20. The genetic panel of embodiment 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by: assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant; if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain; if the glioma sample does not exhibit chromosome 1 gain, assessing whether the glioma sample exhibits chromosome 19 gain; if the glioma sample does not exhibit chromosome 19 gain, assessing whether the glioma sample exhibits CDK4/MDM2 coamplification; and if the glioma sample does exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma, thereby distinguishing between W1, W2, W3, W4, M1, M2, M3 and O glioma types.
21. The genetic panel of embodiment 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by: assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant; if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain; if the glioma sample does not exhibit chromosome 1 gain, assessing whether the glioma sample exhibits chromosome 19 gain; if the glioma sample does not exhibit chromosome 19 gain, assessing whether the glioma sample exhibits CDK4/MDM2 coamplification; and if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W2 glioma, thereby distinguishing between W1, W2, W3, W4, M1, M2, M3 and O glioma types.
22. The genetic panel of embodiment 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by: assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant; if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion; if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as a O glioma, thereby distinguishing between W1, W2, W3, W4, M1, M2, M3 and O glioma types.
23. The genetic panel of embodiment 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by: assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant; if the IDH1/2 is IDH1/2 mutant, assessing whether the

glioma sample exhibits 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma, thereby distinguishing between W1, W2, W3, W4, M1, M2, M3 and O glioma types.

24. The genetic panel of embodiment 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by: assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant; if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma, thereby distinguishing between W1, W2, W3, W4, M1, M2, M3 and O glioma types.
25. The genetic panel of embodiment 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by: assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant; if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma, thereby distinguishing between W1, W2, W3, W4, M1, M2, M3 and O glioma types.
26. The genetic panel of embodiment 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by: assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant; if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma, thereby distinguishing between W1, W2, W3, W4, M1, M2, M3 and O glioma types.

27. A method of diagnosing whether a subject with glioma has type W1, W2, W3, W4, M1, M2, M3 or O glioma, the method including:
- (a) obtaining a glioma sample from the subject;
 - (b) contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype; if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain; if the glioma sample exhibits chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and if the glioma sample exhibits CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma;
 - (c) if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain; if the glioma sample exhibits chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W3 glioma;
 - (d) if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain; if the glioma sample does not exhibit chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between chromosome 19 and the primer indicative of presence or absence of chromosome 19 gain; if the glioma sample exhibits chromosome 19 gain, identifying the glioma sample as a W3 glioma;
 - (e) if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain; if the glioma sample does not exhibit chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between chromosome 19 and the primer indicative of presence or absence of chromosome 19 gain; if the glioma sample does not exhibit chromosome 19 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and if the glioma sample does exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma;

- (f) if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain; if the glioma sample does not exhibit chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between chromosome 19 and the primer indicative of presence or absence of chromosome 19 gain; if the glioma sample does not exhibit chromosome 19 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W2 glioma;
- (g) if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as a O glioma;
- (h) if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma;
- (i) if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma;

(j) if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma; or

(k) if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma;

diagnosing whether a subject with glioma has type W1, W2, W3, W4, M1, M2, M3 or O glioma.

28. The method of embodiment 27, which diagnoses whether the subject with glioma has type W1, W2, W3, W4, M1, M2, M3 or O glioma by: contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype; if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain; if the glioma sample exhibits chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and if the glioma sample exhibits CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma.

29. The method of embodiment 27, which diagnoses whether the subject with glioma has type W1, W2, W3, W4, M1, M2, M3 or O glioma by: contacting the glioma sample with a IDH1/2

mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype; if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain; if the glioma sample exhibits chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W3 glioma.

30. The method of embodiment 27, which diagnoses whether the subject with glioma has type W1, W2, W3, W4, M1, M2, M3 or O glioma by: contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype; if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain; if the glioma sample does not exhibit chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between chromosome 19 and the primer indicative of presence or absence of chromosome 19 gain; if the glioma sample exhibits chromosome 19 gain, identifying the glioma sample as a W3 glioma.
31. The method of embodiment 27, which diagnoses whether the subject with glioma has type W1, W2, W3, W4, M1, M2, M3 or O glioma by: contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype; if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain; if the glioma sample does not exhibit chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between chromosome 19 and the primer indicative of presence or absence of chromosome 19 gain; if the glioma sample does not exhibit chromosome 19 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and if the glioma sample does exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma.
32. The method of embodiment 27, which diagnoses whether the subject with glioma has type W1, W2, W3, W4, M1, M2, M3 or O glioma by: contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2

mutant or wildtype; if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain; if the glioma sample does not exhibit chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between chromosome 19 and the primer indicative of presence or absence of chromosome 19 gain; if the glioma sample does not exhibit chromosome 19 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W2 glioma.

33. The method of embodiment 27, which diagnoses whether the subject with glioma has type W1, W2, W3, W4, M1, M2, M3 or O glioma by: contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype; if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as a O glioma.
34. The method of embodiment 27, which diagnoses whether the subject with glioma has type W1, W2, W3, W4, M1, M2, M3 or O glioma by: contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype; if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma.
35. The method of embodiment 27, which diagnoses whether the subject with glioma has type W1, W2, W3, W4, M1, M2, M3 or O glioma by: contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype; if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and

detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma.

36. The method of embodiment 27, which diagnoses whether the subject with glioma has type W1, W2, W3, W4, M1, M2, M3 or O glioma by: contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype; if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma.
37. The method of embodiment 27, which diagnoses whether the subject with glioma has type W1, W2, W3, W4, M1, M2, M3 or O glioma by: contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype; if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of

chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma.

38. A genetic panel that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types according to the scheme depicted in FIG. 1.
39. A method to risk-stratify glioma subjects according to the scheme depicted in FIG. 1.
40. A histology-free genetic panel that distinguishes between M1, M2, M3, and O glioma types by: (a) assessing an IDH1/2 mutant glioma sample for 1p19q codeletion; (b) if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as an O glioma, if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma; (c) if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma; (d) if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma; or (e) if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma; thereby distinguishing between M1, M2, M3 and O glioma types without use of histology.
41. The histology-free genetic panel of embodiment 40, that distinguishes between M1, M2, M3 and O glioma types by: assessing an IDH1/2 mutant glioma sample for 1p19q codeletion; if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as an O glioma, thereby distinguishing between M1, M2, M3 and O glioma types without use of histology.

42. The histology-free genetic panel of embodiment 40, that distinguishes between M1, M2, M3 and O glioma types by: assessing an IDH1/2 mutant glioma sample for 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma, thereby distinguishing between M1, M2, M3 and O glioma types without use of histology.
43. The histology-free genetic panel of embodiment 40, that distinguishes between M1, M2, M3 and O glioma types by: assessing an IDH1/2 mutant glioma sample for 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma, thereby distinguishing between M1, M2, M3 and O glioma types without use of histology.
44. The histology-free genetic panel of embodiment 40, that distinguishes between M1, M2, M3 and O glioma types by: assessing an IDH1/2 mutant glioma sample for 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma, thereby distinguishing between M1, M2, M3 and O glioma types without use of histology.
45. A The histology-free genetic panel of embodiment 40, that distinguishes between M1, M2, M3 and O glioma types by: assessing an IDH1/2 mutant glioma sample for 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma, thereby distinguishing between M1, M2, M3 and O glioma types without use of histology.
46. A histology-free method of diagnosing whether a subject with IDH1/2 mutant glioma has type M1, M2, M3 or O glioma, the method including:

- (a) obtaining an IDH1/2 mutant glioma sample from the subject;
- (b) contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as an O glioma,
- (c) if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma;
- (d) if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma;
- (e) if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma; or
- (f) if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss;

and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma;

thereby diagnosing whether a subject with IDH1/2 mutant glioma has type M1, M2, M3 or O glioma without use of histology.

47. The histology-free method of embodiment 46, wherein diagnosing whether a subject with glioma IDH1/2 mutant has type M1, M2, M3 or O glioma includes: contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as an O glioma.
48. The histology-free method of embodiment 46, wherein diagnosing whether a subject with glioma IDH1/2 mutant has type M1, M2, M3 or O glioma includes: contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma.
49. The histology-free method of embodiment 46, wherein diagnosing whether a subject with glioma IDH1/2 mutant has type M1, M2, M3 or O glioma includes: contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma.
50. The histology-free method of embodiment 46, wherein diagnosing whether a subject with glioma IDH1/2 mutant has type M1, M2, M3 or O glioma includes: contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative

of presence or absence of 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma.

51. The histology-free method of embodiment 46, wherein diagnosing whether a subject with glioma IDH1/2 mutant has type M1, M2, M3 or O glioma includes: contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma.
52. A method of detecting in a glioma sample (i) IDH1/2 mutant or IDH1/2 wildtype; (ii) presence or absence of chromosome 1 gain; (iii) presence or absence of CDK4/MDM2 coamplification; and (iv) presence or absence of chromosome 19 gain, the method including: obtaining a glioma sample; contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype; contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain; contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and contacting the sample with a primer and detecting binding or lack thereof between chromosome 19 and the primer indicative of presence or absence of chromosome 19 gain.
53. A method of detecting in a glioma sample (i) IDH1/2 mutant or IDH1/2 wildtype; (ii) presence or absence of 1p19q codeletion; (iii) presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; and (iv) presence or absence of chromosome 14

loss, the method including: obtaining a glioma sample; contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype; contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion; contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; and contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss.

54. A method of embodiment 53 wherein the method does not utilize histology.

55. A kit including primers, probes, and/or stains to detect (i) IDH1/2 mutant or IDH1/2 wildtype; (ii) chromosome 1 gain; (iii) CDK4/MDM2 coamplification; and (iv) chromosome 19 gain.

56. A kit including primers, probes, and/or stains to detect (i) IDH1/2 mutant or IDH1/2 wildtype; (ii) 1p19q codeletion; (iii) CDK4 amplification and/or CDKN2A deletion; and (iv) chromosome 14 loss.

57. A kit including primers, probes, and/or stains to detect (i) IDH1/2 mutant or IDH1/2 wildtype; (ii) chromosome 1 gain; (iii) CDK4/MDM2 coamplification; (iv) chromosome 19 gain; (v) 1p19q codeletion; (vi) CDK4 amplification and/or CDKN2A deletion; and (vii) chromosome 14 loss.

[0096] Example 1. Multidimensional Scaling of Diffuse Gliomas: Application to the 2016 World Health Organization Classification System with Prognostically Relevant Molecular Subtype Discovery.

[0097] This example describes the development of a genetic panel that reliably and accurately distinguishes between types of gliomas, each with a distinct survival rate. At least some of the material in this example was published on May 22, 2017, as Cimino *et al.*, *Acta Neuropathol Commun* 5:39, 2017 (14 pages).

[0098] Methods. Oncoscape and TCGA Data Visualization. TCGA point mutation and copy number data for glioblastomas as well as WHO grade II and III astrocytic and oligodendroglial tumors were downloaded from the University of California Santa Cruz cancer browser. Multidimensional scaling (MDS) of molecular data was performed as previously described (Bolouri *et al.*, *Proc Natl Acad Sci U S A.* 2016;113:5394-9). Clinical data were obtained from the Genomic Data Commons (GDC) Data Portal from the National Institutes of Health (NIH) (Grossman *et al.*, *N Engl J Med.* 2016;375:1109-12). Data were visualized and analyzed using the interactive browser-based platform, Oncoscape. Diffuse gliomas from the 'gliomas (TCGA)'

dataset were visualized in 2D space with available analysis tools. Relevant genetic and genomic alterations used for 2016 WHO diffuse glioma classification were queried in Oncoscape (Louis *et al.*, WHO Classification of Tumours of the Central Nervous System. 4th ed. Lyon, France: International Agency for Research on Cancer; 2016). Also of interest for comparison, were previous 2007 histopathologic classifications and WHO grades of TCGA datasets (Louis *et al.*, Acta Neuropathol. 2007;114:97-109). Three main clusters were identified by MDS, and individual patients were assigned to each based upon *IDH1/2* mutational status and the presence or absence of 1p/19q codeletion (FIG. 2).

[0099] *TCGA Copy Number Frequency.* Using GISTIC2.0 scores, copy number frequencies of TCGA gliomas were plotted using R software (Version 3.3.2, RProject for Statistical Computing) applying the 'copynumber' package using 0.5 and -0.5 as thresholds.

[0100] *German Glioma Network Validation Set.* Glioblastoma sample molecular data (n=284) from the German Glioma Network were collected along with survival and gene methylation data as previously described (Sturm *et al.*, Cancer Cell. 2012;22:425-37). Copy number alterations of individual CpG sites were evaluated based on the R package 'conumee' applying an adapted algorithm for baseline-correction. For evaluation of chromosomal segments, the median of the states of the corresponding probes was computed. Gains and losses were called using thresholds at -0.1 and 0.1 on a log2-scale as cutoff. For calling of amplifications and homozygous deletions in genes of interest thresholds at 0.6 and -0.6 were used. Segment start- and end-positions refer to reference genome GRCh37/hg19. Results are restricted to chromosomes 1, 14 and 19 as well as *CDKN2A*, *CDK4*, and *MDM2*.

[0101] *Plots and Statistics.* Statistical analyses were performed using R software (Version 3.3.2, RProject for Statistical Computing). Kaplan-Meier analysis for overall survival was performed using the 'survival' package with *P*-values determined by Cox proportional hazards regression. Multivariate Cox proportional hazards models including indicated variables were applied utilizing SPSS statistical software (Version 22.0, IBM). Linear regression was performed using GraphPad Prism software (Version 7.02).

[0102] Results. Visualizing WHO Diffuse Glioma Classification. Initially, the diffuse glioma TCGA data were visualized in relation to 2007 WHO classification criteria, including histopathology and WHO grade (FIGs. 3A-3D). For each charted patient point, the dot diameter increases with increased genetic alterations (FIG. 3A). Histopathologic diagnoses as defined by the outdated 2007 WHO classification of primary brain tumors (oligodendroglioma, astrocytoma, oligoastrocytoma, glioblastoma) are not molecular cluster-specific, as each cluster contains a variable amount of histopathologic heterogeneity (FIG. 3B). This highlights the issue addressed

by the current 2016 WHO classification, *i.e.* that histopathologic criteria alone are not entirely representative of genetic alterations in diffuse gliomas, and that the new WHO classification of integrating histopathology with molecular studies can be more reproducible for diagnostic purposes.

[0103] Therefore, patient clusters were also queried for genetic changes corresponding to those used for the 'integrated' 2016 WHO classification of diffuse gliomas (FIGs. 4A-4D). Presence of mutated *IDH1/2* characterizes two main clusters, and is absent from the third cluster (FIG. 4A). Mutated *IDH1* is more common and more evenly dispersed than mutated *IDH2*. *TP53* and *ATRX* mutations occur mostly in one of the IDH-mutant clusters (FIG. 4B, 4C). The other IDH-mutant cluster exclusively harbors 1p/19q codeletion (FIG. 4D). Mutations in *IDH2* are seen more frequently in the 1p/19q-codeleted cluster (FIG. 4B), and appear to have regional grouping as well, indicating a unique type of DNA structure for these types of gliomas. Consistent with prior reports, 1p chromosomal deletion was more cluster specific than 19q chromosomal deletion. WHO grades II-IV are seen in both clusters without 1p/19q codeletion, consistent with the concept that there are no WHO grade IV oligodendrogliomas. This observation also supports the removal of glioblastoma with oligodendroglial component as a distinct diagnostic entity. Taken together, these three clusters can be designated based upon the 2016 WHO classification criteria as follows: 1) Oligodendroglial tumors, IDHmutant, and 1p/19q-codeleted (WHO grades II-III) (n=176); 2) Astrocytic gliomas/glioblastoma, IDH-mutant (WHO II-IV) (n=251); and 3) Astrocytic gliomas/glioblastoma, IDH-wildtype (WHO grades II-IV) (n=351) (FIG. 5).

[0104] *Cluster Demographics.* Comparison of survival between and within the major diffuse glioma molecular clusters reflects the improved and revised 2016 WHO classification system (FIGs. 6A, 6B). Comparison of the three molecular clusters defined by MDS demonstrates and confirms prognostic effect of IDH mutations, which is further stratified by 1p/19q codeletion status (FIG. 6B). When looking at WHO grade IV glioblastomas, the tumors within the IDH-mutant cluster are associated with longer survival than tumors within the IDHwildtype cluster, again consistent with prior studies of glioblastoma and the new WHO classifications. There are some caveats in trying to interpret survival studies from this TCGA data, especially for the WHO grade II and III diffuse gliomas, as availability of outcome data was not a main factor in selecting cases. WHO grade II and III diffuse glioma patients have longer survival than glioblastoma patients, and therefore require longer clinical follow up. In addition to survival, age at diagnosis was shown to vary between clusters (FIGs. 6C-6E). The astrocytic glioma/glioblastoma, IDH-wildtype cluster has the oldest age distribution peak, occurring at 56-63 years (FIG. 6C). By

contrast, the astrocytic glioma/glioblastoma, IDH-mutant cluster has the youngest age distribution peak, occurring at 26-32 years (FIG. 6D). The oligodendroglial tumor cluster shows a distribution in adults with two peak ages at diagnosis at 35-41 and 53-59 year age ranges, with a median age of 45 years (FIG. 6E). The association of age within the 2016 WHO definition of IDH-mutant and 1p/19q-codeleted oligodendroglial tumors has not been described. To investigate associations of this bimodal age distribution with overall survival, the oligodendroglial tumor cluster was subdivided into two groups utilizing median age at diagnosis (45 years) as a cut-off (FIG. 6F). Kaplan-Meier survival analysis demonstrated similar survival curves when divided either by age or WHO grade (FIGs. 6F, 6H). Additionally, hazard ratios were prognostic in a Cox regression model containing age <45 versus ≥ 45 (hazard ratio [HR] 0.137, 95% confidence interval [CI] 0.024-0.774, $p=0.024$), WHO grade II versus grade III (hazard ratio [HR] 0.200, 95% confidence interval [CI] 0.051-0.791, $p=0.022$) and Karnofsky performance score 90-100% versus <90% (hazard ratio [HR] 0.167, 95% confidence interval [CI] 0.042-0.660, $p=0.011$).

[0105] *Global Copy Number Alterations of MDS Clusters.* To add to the current WHO genetic classifiers of IDH mutation and 1p/19q codeletion, global copy number alteration (CNA) frequencies were analyzed across the molecular clusters defined by MDS to confirm known CNAs as well as identify new cluster-associated CNAs (FIG. 7). The oligodendroglial tumor cluster is defined by the presence of 1p/19q codeletion and the second most frequent alteration (25%) is loss of chromosome 4. Other CNAs across oligodendrogliomas include low-level gains (chromosomes 7, 8, 11, 16, 17, 20, 21, and 22) and low-level losses (chromosomes 6, 9, 10, 12, 13, 14, 15, 18, and 22) of uncertain significance. The astrocytic glioma/glioblastoma, IDH-mutant cluster appears more heterogeneous with respect to CNA than the other clusters. It has several low- to mid-level CNAs, but unlike the other clusters, no alteration was present in >50% of the cluster. Some of the mid-level CNAs include known astrocytoma associated alterations such as 9p loss, 10q loss, and 19q loss. Gain of chromosome 7 is also present in tumors of the astrocytic glioma/glioblastoma, IDH-mutant cluster, but not as frequently as in the corresponding astrocytic glioma/glioblastoma, IDH-wildtype cluster. Regardless of IDH status, astrocytic glioma/glioblastoma patients have low-frequency amplifications of 12q in the region of *CDK4* and *MDM2*. Of note, these alterations were not limited to glioblastomas. The IDH-wildtype diffuse gliomas have frequent chromosome 7 gain, chromosome 10 loss, and 9p loss, among other CNAs. Additional MDS in three dimensions demonstrates that the IDH-wildtype cluster can be divided spatially into three subgroups (designated as A-C in FIG. 7). The IDH-wildtype subgroup A is separated from B and C by chromosome 1 gain and/or *TP53* point mutations.

Furthermore, subgroups B and C can be simply distinguished by chromosome 19 gain. Co-gain of chromosomes 19 and 20, which has been described in a small series of long-term glioblastoma survivors, is frequently observed in the IDH-wildtype subgroup B. There is a significant survival difference ($p=0.034$, Cox proportional hazards regression) between subgroups B and C, which are distinguished by gain of chromosome 19 (FIG. 8). Co-amplification of *CDK4* and *MDM2* further augments survival in a molecular subgroup-specific manner (FIG. 8).

[0106] Identification and Characterization of Cluster-Derived Molecular Subtypes. After evaluation of global CNA frequency across the astrocytic glioma/glioblastoma clusters, a small number of cluster-derived CNAs were interrogated for survival prediction and possible risk-stratification. CNA molecular subtypes are defined by chromosome 1 gain, chromosome 19 gain, and *CDK4/MDM2* co-amplification for the astrocytic glioma/glioblastoma, IDH-wildtype cluster (W1-W4), as well as *CDK4* amplification, *CDKN2A* deletion, and chromosome 14 gain for the astrocytic glioma/glioblastoma, IDH-mutant cluster (M1-M3) (FIG. 1). For the most common type of diffuse glioma, *i.e.* glioblastoma, IDH-wildtype, WHO Grade IV, there is a difference in overall survival across the W1-W3 molecular subtypes ($p=0.002$, Cox proportional hazards regression, FIG. 9A), with median overall survival of 6.6 months (W1), 12.7 months (W2) and 15.2 months (W3), respectively. As these are all WHO grade IV tumors, these wildtype molecular subtypes are independent of grading. For the astrocytic glioma/glioblastoma, IDH-mutant cluster, independent of WHO grade, there is a significant overall survival difference across the M1-M3 molecular subtypes ($p<0.001$, Cox proportional hazards regression, FIG. 6B), with median survivals of 23.3 months (M1), 63.0 months (M2) and 94.5 months (M3). Segregation by WHO grade was prognostic within the astrocytic glioma/glioblastoma, IDH-mutant cluster, yielding a median overall survival of 34.1 months (WHO Grade IV), 68.4 months (WHO Grade III) and 95.8 months (WHO Grade II), respectively ($p=0.007$, Cox proportional hazards regression, FIG. 9B). In patients within the astrocytic glioma/glioblastoma, IDH-mutant glioma cluster, median overall survival with WHO grade III/IV versus WHO grade II was 63.0 versus 95.8 months ($p=0.007$, Cox proportional hazards regression, FIG. 9C). Segregation of these patients by M1/2 versus M3 molecular subgroups yielded similar survival proportions of 51.2 versus 94.5 months ($p<0.001$, Cox proportional hazards regression, FIG. 9C). The association with overall survival was retained for M1/2 versus M3 upon adjustment for WHO grade (Hazard ratio [HR] 3.28, 95% confidence interval [CI] 1.62-6.62, $p=0.001$), and vice versa for WHO grade III/IV versus grade II upon adjustment for molecular subgroup (HR 2.01, 95% CI 1.06-4.02, $p=0.036$, FIG. 9C). Survival curves within the astrocytic glioma/glioblastoma,

IDHmutant cluster based on molecular subtypes (M1-3) or WHO grade (II-IV) are somewhat comparable, with a slightly stronger association with overall survival for molecular subtyping. However, molecular classification may be more reliable than grading between pathologists as the exact criteria for defining a WHO grade II diffuse astrocytoma versus WHO grade III anaplastic astrocytoma are not well-defined for resection material and may be associated with interobserver variability (Louis *et al.*, WHO Classification of Tumours of the Central Nervous System. 4th ed. Lyon, France: International Agency for Research on Cancer; 2016; van den Bent, *Acta Neuropathol.* 2010;120:297-304; Louis *et al.*, *Acta Neuropathol.* 2016;131:803-20; Mittler *et al.*, *J Neurosurg.* 1996;85:1091-4; Aldape *et al.*, *Cancer.* 2000;88:2342-9).

[0107] Validation of Cluster-Derived Molecular Subtypes. To test the potential clinical utility of cluster-derived molecular subtypes, an independent large validation data set (n=284) of glioblastomas from the German Glioma Network (GGN) was evaluated. Comparing WHO grade IV glioblastomas, median overall survival of the GGN cohort (18.9 months) versus the TCGA cohort (13.5 months) was longer ($p<0.001$, Cox proportional hazards regression). A caveat to this comparison is that the TCGA gliomas are WHO grade II-IV while the GGN gliomas are all WHO grade IV, which should not be a significant confounder as the molecular subtypes appear to be grade independent (FIGs. 9A-9C) and in addition, the GGN data set has longer overall survival. Overall survival data in the GGN cohort were normalized for validation to account for the difference in median survival between the GGN and TCGA cohorts. In the GGN cohort the W1 and W2 subtypes did not show difference ($p=0.913$, Cox proportional hazards regression) in survival so their combined median overall survival was used for baseline normalization. Normalized median overall survival for each molecular subtype showed similar trends for TCGA and GGN data sets (FIG. 10A). Additionally, linear regression shows a nearly 1:1 overall ratio (slope=0.958, R=0.899) comparing TCGA and GGN overall survival by molecular subtypes (FIG. 10B). In summary, overall survival times of the cluster derived molecular subtypes identified in the TCGA discovery set were comparable in the GGN validation set.

[0108] Discussion. In addition to illustrating the inherent interobserver variability of histopathologic only classification of diffuse gliomas, the current disclosure reflects why the diagnosis of (mixed) oligoastrocytoma is discouraged in the new WHO system and how 'oligoastrocytoma' easily resolves into either oligodendroglioma or astrocytoma entities. If oligoastrocytoma truly existed as a specific biological entity, these cases would be expected to exist between the IDH-mutant oligodendroglial and astrocytic glioma clusters shown in FIG. 3B; however, no such cases are seen. Furthermore, histopathological 'oligoastrocytoma' cases are predominantly evenly distributed and completely embedded within the oligodendroglial tumor

and astrocytic glioma/glioblastoma, IDH-mutant clusters, further arguing against oligoastrocytoma as a distinct entity. However, it is noted that there are reported rare cases of true oligoastrocytomas with distinct regions of either molecular oligodendroglioma or astrocytoma features, and the WHO does allow for designation of these gliomas as oligoastrocytoma, NOS. Where such rare molecular biphenotypic cases fall within the subtypes of the current disclosure has yet to be determined.

[0109] Molecular alterations are strong drivers of clinical behavior, and can be considered as a first stratifier, as IDH-mutant diffuse gliomas clinically behave better than IDH-wildtype diffuse gliomas across all grades. For example, determination of IDH mutational status, may be considered as baseline diagnostic criteria. After the baseline diagnosis is established, cluster-specific molecular grading is warranted. The data shown in this Example support prognostic heterogeneity within major diffuse glioma clusters, which in some aspects is identified by conventional grading, but is even better identified by an additional set of molecular markers. These results provide evidence of the utility for 'molecular grading' within major subgroups of diffuse gliomas.

[0110] Along with reflecting changes in WHO classification of diffuse gliomas, some patterns of genetic alterations become readily apparent, as was the case for the bimodal age distribution of the IDH-mutant, and 1p/19q-codeleted Oligodendroglioma cluster (FIG. 6) and cluster-specific gene amplifications (FIG. 7). For example, the size of dots, representing the amount of genetic alterations in a single sample as shown in FIG. 3, demonstrates the amount of genetic variation within clusters. The IDH-mutant, and 1p/19q codeleted oligodendroglial tumor cluster is enriched for the smallest points, indicating a genomically stable group, while the astrocytic glioma/glioblastoma, IDH-wildtype cluster has the largest points, representing more frequent mutations and copy number aberrations in this more aggressive type of glioma. The group with an intermediate clinical outcome, the astrocytic glioma/glioblastoma, IDH-mutant cluster, has the most variability in dot size, indicating a genomically more heterogeneous group. Perhaps in diffuse gliomas, the IDH mutational pathway of oncogenesis leads to some genomic stability with 1p/19q codeletion further potentiating relative genomic stability.

[0111] In summary, cluster-derived molecular subtypes such as the ones described in this Example (W1-4, M1-3, O), provide a beneficial approach to risk-stratify patients in a time-sensitive and cost-effective manner.

[0112] Example 2. Copy number profiling across glioblastoma populations has implications for clinical trial design.

[0113] Copy number alterations predict patient survival in diffuse gliomas, and subtypes with clear differences in median survival can be derived from copy number profiling (Example 1; Cimino *et al.*, 2017. *Acta Neuropathol Commun* 5: 39). Within the classification of diffuse glioma, IDH-wildtype glioblastoma can be further stratified into three distinct prognostic DNA copy number subtypes: W1 (worst survival), W2 (intermediate survival), and W3 (best survival) (FIG. 11A). The distribution of these subtypes is similar across two large data sets of initial glioblastoma across North America (The Cancer Genome Atlas [TCGA]) and in Europe (German Glioma Network [GGN]), suggesting that this is the natural distribution in the population (FIG. 11A).

[0114] Selection bias exists towards a longer-lived glioblastoma subgroup when enrolling patients healthy enough to enter clinical trials or when deciding that patients are well enough for a resection at recurrence. Given the better overall survival of these types of pre-selected patients, whether there was evidence of a skew in the population with respect to distribution of the copy number subtypes defined above was considered. In fact, there were two such cohorts for analysis (FIG. 11B). The first cohort is the phase II ARTE trial of hypofractionated radiotherapy with or without bevacizumab in elderly patients with newly diagnosed glioblastoma (Wirsching *et al.*, *Annals of Oncology* DOI: 10.1093/annonc/mdy120, April 10, 2018). When compared to the elderly TCGA and GGN general glioblastoma populations, there was a distribution skew toward the better prognostic W3 copy number subtype. The second prospective cohort is a multi-institutional paired initial/recurrence glioma group (Wang *et al.*, 2016. *Nat Genet* 48: 768-776), which investigated glioblastoma patients of all ages that were healthy enough to have a resection at first recurrence. This paired initial/recurrence glioma cohort also showed a skew towards the better prognostic W3 copy number subtype.

[0115] A more granular method to characterize DNA alterations is through multidimensional scaling (MDS) analysis by combining copy number with whole exome sequencing (Bolouri *et al.*, 2016. *Proc Natl Acad Sci U S A* 113: 5394-5399; Cimino *et al.*, 2017. *Acta Neuropathol Commun* 5: 39). MDS defines distinct glioma groups with differences in survival as well. To investigate a potential distribution skew with respect to MDS molecular signatures, the paired initial/recurrent glioblastoma data cohort were overlaid onto the TCGA reference MDS map. As shown in FIGs. 11C & 11D, 51% of glioblastomas from TCGA exhibit a similar DNA structure that does not correspond to those appropriate for surgical resection at recurrence. This MDS group of IDH-wildtype glioblastoma largely includes, but is not limited to, glioblastomas that

have the poorest survival among MDS regions and is characterized by relatively few regions of chromosomal alterations with the exception of whole chromosome 7 gain, whole chromosome 10 loss, and loss of chromosome 9p (Cimino *et al.*, 2017. *Acta Neuropathol Commun* 5: 39). Essentially all patients who do well-enough to have a second surgical resection at recurrence arise from the other half of glioblastomas.

[0116] The data suggest that analysis of recurrent glioblastoma samples is missing the tumors with the most common DNA structure found in the natural population. Further, it suggests that this global DNA pattern is associated with clinical characteristics that lead surgeons across North America and Asia to not operate on these first recurrent glioblastoma patients. It also seems likely that patients with this global DNA pattern are underrepresented in clinical trials, particularly in those focused on first recurrence of glioblastoma. Given the ability described herein for DNA analysis of tumors at initial resection to predict outcomes across large datasets, targeted copy number analysis with or without exome sequencing will be critical to evenly balance clinical trial arms with patients having predictable outcomes. Failure to do so may contribute to occasional discordance between phase 2 and phase 3 clinical trial outcomes.

[0117] As will be understood by one of ordinary skill in the art, each embodiment disclosed herein can comprise, consist essentially of or consist of its particular stated element, step, ingredient or component. Thus, the terms “include” or “including” should be interpreted to recite: “comprise, consist of, or consist essentially of.” The transition term “comprise” or “comprises” means includes, but is not limited to, and allows for the inclusion of unspecified elements, steps, ingredients, or components, even in major amounts. The transitional phrase “consisting of” excludes any element, step, ingredient or component not specified. The transition phrase “consisting essentially of” limits the scope of the embodiment to the specified elements, steps, ingredients or components and to those that do not materially affect the embodiment. A material effect would cause a statistically-significant reduction in the ability to accurately classify a glioma as W1, W2, W3, W4, M1, M2, M3, or O.

[0118] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about.” Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying

ordinary rounding techniques. When further clarity is required, the term “about” has the meaning reasonably ascribed to it by a person skilled in the art when used in conjunction with a stated numerical value or range, *i.e.* denoting somewhat more or somewhat less than the stated value or range, to within a range of $\pm 20\%$ of the stated value; $\pm 19\%$ of the stated value; $\pm 18\%$ of the stated value; $\pm 17\%$ of the stated value; $\pm 16\%$ of the stated value; $\pm 15\%$ of the stated value; $\pm 14\%$ of the stated value; $\pm 13\%$ of the stated value; $\pm 12\%$ of the stated value; $\pm 11\%$ of the stated value; $\pm 10\%$ of the stated value; $\pm 9\%$ of the stated value; $\pm 8\%$ of the stated value; $\pm 7\%$ of the stated value; $\pm 6\%$ of the stated value; $\pm 5\%$ of the stated value; $\pm 4\%$ of the stated value; $\pm 3\%$ of the stated value; $\pm 2\%$ of the stated value; or $\pm 1\%$ of the stated value.

[0119] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0120] The terms “a,” “an,” “the” and similar referents used in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. For instance, the order in which CNAs/genetic sequences are assessed in a technical platform do not matter (*i.e.* they can all be done at the same time, or in any order, or in batches). The order in which CNAs are used in the algorithm (decision tree) illustrated in FIG. 1 matters for determining classification of gliomas, as described herein. The use of any and all examples, or exemplary language (*e.g.*, “such as”) provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0121] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted

from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0122] Certain embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0123] Furthermore, numerous references have been made to patents, printed publications, journal articles and other written text throughout this specification (referenced materials herein). Each of the referenced materials are individually incorporated herein by reference in their entirety for their referenced teaching.

[0124] In closing, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed are within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and described.

[0125] The particulars shown herein are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of various embodiments of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for the fundamental understanding of the invention, the description taken with the drawings and/or examples making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

[0126] Definitions and explanations used in the present disclosure are meant and intended to be controlling in any future construction unless clearly and unambiguously modified in the following examples or when application of the meaning renders any construction meaningless or

essentially meaningless. In cases where the construction of the term would render it meaningless or essentially meaningless, the definition should be taken from Webster's Dictionary, 3rd Edition or a dictionary known to those of ordinary skill in the art, such as the Oxford Dictionary of Biochemistry and Molecular Biology (Ed. Anthony Smith, Oxford University Press, Oxford, 2004).

CLAIMS

What is claimed is:

1. A genetic panel to diagnose glioma, wherein the genetic panel assesses and defines eight molecular subtypes of glioma by :
the following CNAs: chromosome 1 gain, chromosome 19 gain, chromosome 14 loss, and 1p19q presence or codeletion; and
genetic sequences associated with IDH1/2 status; and
CDK4 amplification and/or CDKN2A deletion; and/or
CDK4 and MDM2 coamplification.
2. A genetic panel to diagnose glioma, wherein the genetic panel assesses:
(i) chromosome copy number alterations (CNAs) and (ii) genetic amplifications associated with cell cycle progression in biopsied cells.
3. The genetic panel of claim 1 or 2 wherein the genetic panel identifies a W1 glioma based on: IDH1/2 wildtype; chromosome 1 gain; and CDK4/MDM2 co-amplification.
4. The genetic panel of claim 1 or 2 wherein the genetic panel identifies a W1 glioma based on: IDH1/2 wildtype; no chromosome 1 gain; no chromosome 19 gain; and CDK4/MDM2 co-amplification.
5. The genetic panel of claim 3 or 4 wherein a WHO Grade IV is confirmed.
6. The genetic panel of claim 1 or 2 wherein the genetic panel identifies a W2 glioma based on: IDH1/2 wildtype; no chromosome 1 gain; no chromosome 19 gain; and no CDK4/MDM2 co-amplification.
7. The genetic panel of claim 6 wherein a WHO Grade IV is also confirmed.
8. The genetic panel of claim 1 or 2 wherein the genetic panel identifies a W3 glioma based on: IDH1/2 wildtype; no chromosome 1 gain; and chromosome 19 gain.
9. The genetic panel of claim 6 wherein a WHO Grade IV is also confirmed.

10. The genetic panel of claim 1 or 2 wherein the genetic panel identifies a W4 glioma based on: IDH1/2 wildtype; and a WHO/Grade II/III histology score.
11. The genetic panel of claim 1 or 2 wherein the genetic panel identifies an M1 glioma based on: IDH1/2 mutant; no 1p19q codeletion; CDK4 amplification and/or CDKN2A deletion; and loss of chromosome 14.
12. The genetic panel of claim 1 or 2 wherein the genetic panel identifies an M2 glioma based on: IDH1/2 mutant; no 1p19q codeletion; CDK4 amplification and/or CDKN2A deletion; and no loss of chromosome 14.
13. The genetic panel of claim 1 or 2 wherein the genetic panel identifies an M2 glioma based on: IDH1/2 mutant; no 1p19q codeletion; no CDK4 amplification and/or CDKN2A deletion; and loss of chromosome 14.
14. The genetic panel of claim 1 or 2 wherein the genetic panel identifies an M3 glioma based on: IDH1/2 mutant; no 1p19q codeletion; no CDK4 amplification and/or CDKN2A deletion; and no loss of chromosome 14.
15. The genetic panel of claim 1 or 2 wherein the genetic panel identifies an O glioma based on: IDH1/2 mutant; with 1p19q codeletion.
16. A genetic panel that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by:
 - (a) assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant;
 - (b) if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain;
 - if the glioma sample exhibits chromosome 1 gain, assessing whether the glioma sample exhibits CDK4/MDM2 coamplification; and
 - if the glioma sample exhibits CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma;

(c) if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain;

if the glioma sample exhibits chromosome 1 gain, assessing whether the glioma sample exhibits CDK4/MDM2 coamplification; and

if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W3 glioma;

(d) if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain;

if the glioma sample does not exhibit chromosome 1 gain, assessing whether the glioma sample exhibits chromosome 19 gain;

if the glioma sample exhibits chromosome 19 gain, identifying the glioma sample as a W3 glioma;

(e) if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain;

if the glioma sample does not exhibit chromosome 1 gain, assessing whether the glioma sample exhibits chromosome 19 gain;

if the glioma sample does not exhibit chromosome 19 gain, assessing whether the glioma sample exhibits CDK4/MDM2 coamplification; and

if the glioma sample does exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma;

(f) if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain;

if the glioma sample does not exhibit chromosome 1 gain, assessing whether the glioma sample exhibits chromosome 19 gain;

if the glioma sample does not exhibit chromosome 19 gain, assessing whether the glioma sample exhibits CDK4/MDM2 coamplification; and

if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W2 glioma;

(g) if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion;

if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as a O glioma;

(h) if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion;

if the sample exhibits CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and

if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma;

(i) if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion;

if the sample exhibits CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and

if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma;

(j) if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion;

if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and

if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma; and/or

(k) if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion;

if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and

if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma;

thereby distinguishing between W1, W2, W3, W4, M1, M2, M3 and O glioma types.

17. The genetic panel of claim 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by:

assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant;

if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain;

if the glioma sample exhibits chromosome 1 gain, assessing whether the glioma sample exhibits CDK4/MDM2 coamplification; and

if the glioma sample exhibits CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma.

18. The genetic panel of claim 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by:

assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant;

if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain;

if the glioma sample exhibits chromosome 1 gain, assessing whether the glioma sample exhibits CDK4/MDM2 coamplification; and

if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W3 glioma.

19. The genetic panel of claim 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by:

assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant;

if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain;

if the glioma sample does not exhibit chromosome 1 gain, assessing whether the glioma sample exhibits chromosome 19 gain;

if the glioma sample exhibits chromosome 19 gain, identifying the glioma sample as a W3 glioma.

20. The genetic panel of claim 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by:

assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant;

if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain;

if the glioma sample does not exhibit chromosome 1 gain, assessing whether the glioma sample exhibits chromosome 19 gain;

if the glioma sample does not exhibit chromosome 19 gain, assessing whether the glioma sample exhibits CDK4/MDM2 coamplification; and

if the glioma sample does exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma.

21. The genetic panel of claim 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by:

assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant;

if the IDH1/2 is IDH1/2 wildtype, assessing whether the glioma sample exhibits chromosome 1 gain;

if the glioma sample does not exhibit chromosome 1 gain, assessing whether the glioma sample exhibits chromosome 19 gain;

if the glioma sample does not exhibit chromosome 19 gain, assessing whether the glioma sample exhibits CDK4/MDM2 coamplification; and

if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W2 glioma.

22. The genetic panel of claim 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by:

assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant;

if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion;

if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as a O glioma.

23. The genetic panel of claim 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by:

assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant;

if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion;

if the sample exhibits CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and

if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma.

24. The genetic panel of claim 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by:

assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant;

if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion;

if the sample exhibits CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and

if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma.

25. The genetic panel of claim 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by:

assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant;

if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion;

if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and

if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma.

26. The genetic panel of claim 16, that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types by:

assessing whether a glioma sample derived from a patient is IDH1/2 wildtype or IDH1/2 mutant;

if the IDH1/2 is IDH1/2 mutant, assessing whether the glioma sample exhibits 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion;

if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and

if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma.

27. A method of diagnosing whether a subject with glioma has type W1, W2, W3, W4, M1, M2, M3 or O glioma, the method comprising:

(a) obtaining a glioma sample from the subject;

(b) contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype;

if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain;

if the glioma sample exhibits chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and

if the glioma sample exhibits CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma;

(c) if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain;

if the glioma sample exhibits chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and

if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W3 glioma;

(d) if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain;

if the glioma sample does not exhibit chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between chromosome 19 and the primer indicative of presence or absence of chromosome 19 gain;

if the glioma sample exhibits chromosome 19 gain, identifying the glioma sample as a W3 glioma;

(e) if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain;

if the glioma sample does not exhibit chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between chromosome 19 and the primer indicative of presence or absence of chromosome 19 gain;

if the glioma sample does not exhibit chromosome 19 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and

if the glioma sample does exhibit CDK4/MDM2 coamplification,
identifying the glioma sample as a W1 glioma;

(f) if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain;

if the glioma sample does not exhibit chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between chromosome 19 and the primer indicative of presence or absence of chromosome 19 gain;

if the glioma sample does not exhibit chromosome 19 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and

if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W2 glioma;

(g) if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;

if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as a O glioma;

(h) if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion;

if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and

if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma;

(i) if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion;

if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and

if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma;

(j) if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion;

if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and

if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma; and/or

(k) if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion;

if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and

if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma;

thereby diagnosing whether the subject with glioma has type W1, W2, W3, W4, M1, M2, M3 or O glioma.

28. The method of claim 27, which diagnoses whether the subject with glioma has type W1 glioma by:

contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype;

if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain;

if the glioma sample exhibits chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and

if the glioma sample exhibits CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma.

29. The method of claim 27, which diagnoses whether the subject with glioma has type W3 glioma by:

contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype;

if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain;

if the glioma sample exhibits chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and

if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W3 glioma.

30. The method of claim 27, which diagnoses whether the subject with glioma has type W3 glioma by:

contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype;

if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain;

if the glioma sample does not exhibit chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between chromosome 19 and the primer indicative of presence or absence of chromosome 19 gain;

if the glioma sample exhibits chromosome 19 gain, identifying the glioma sample as a W3 glioma.

31. The method of claim 27, which diagnoses whether the subject with glioma has type W1 glioma by:

contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype;

if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain;

if the glioma sample does not exhibit chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between chromosome 19 and the primer indicative of presence or absence of chromosome 19 gain;

if the glioma sample does not exhibit chromosome 19 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and

if the glioma sample does exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W1 glioma.

32. The method of claim 27, which diagnoses whether the subject with glioma has type W2, glioma by:

contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype;

if the IDH1/2 is IDH1/2 wildtype, contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain;

if the glioma sample does not exhibit chromosome 1 gain, contacting the sample with a primer and detecting binding or lack thereof between chromosome 19 and the primer indicative of presence or absence of chromosome 19 gain;

if the glioma sample does not exhibit chromosome 19 gain, contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and

if the glioma sample does not exhibit CDK4/MDM2 coamplification, identifying the glioma sample as a W2 glioma.

33. The method of claim 27, which diagnoses whether the subject with glioma has type O glioma by:

contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype;

if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;

if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as a O glioma.

34. The method of claim 27, which diagnoses whether the subject with glioma has type M1 glioma by:

contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype;

if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion;

if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and

if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma.

35. The method of claim 27, which diagnoses whether the subject with glioma has type M, glioma by:

contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype;

if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion;

if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and

if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma.

36. The method of claim 27, which diagnoses whether the subject with glioma has type M2 glioma by:

contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype;

if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion;

if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and

if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma.

37. The method of claim 27, which diagnoses whether the subject with glioma has type M3 glioma by:

contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype;

if the IDH1/2 is IDH1/2 mutant, contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion;

if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and

if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma.

38. A genetic panel that distinguishes between W1, W2, W3, W4, M1, M2, M3 and O glioma types according to the scheme depicted in FIG. 1.

39. A method to risk-stratify glioma subjects according to the scheme depicted in FIG. 1.

40. A histology-free genetic panel that distinguishes between M1, M2, M3, and O glioma types by:

(a) assessing an IDH1/2 mutant glioma sample for 1p19q codeletion;

(b) if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as an O glioma,

if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion;

if the sample exhibits CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and

if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma;

(c) if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion;

if the sample exhibits CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and

if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma;

(d) if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion;

if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and

if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma; and/or

(e) if the glioma sample does not exhibit 1p19q codeletion,
assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion;
if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion,
assessing whether the glioma sample exhibits chromosome 14 loss; and
if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample
as an M3 glioma;
thereby distinguishing between M1, M2, M3 and O glioma types without use of histology.

41. The histology-free genetic panel of claim 40, that distinguishes between M1, M2, M3 and O glioma types by:

assessing an IDH1/2 mutant glioma sample for 1p19q codeletion;
if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as an O glioma, thereby distinguishing between M1, M2, M3 and O glioma types without use of histology.

42. The histology-free genetic panel of claim 40, that distinguishes between M1, M2, M3 and O glioma types by:

assessing an IDH1/2 mutant glioma sample for 1p19q codeletion;
if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion;
if the sample exhibits CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and
if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma, thereby distinguishing between M1, M2, M3 and O glioma types without use of histology.

43. The histology-free genetic panel of claim 40, that distinguishes between M1, M2, M3 and O glioma types by:

assessing an IDH1/2 mutant glioma sample for 1p19q codeletion;
if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion;
if the sample exhibits CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and

if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma, thereby distinguishing between M1, M2, M3 and O glioma types without use of histology.

44. The histology-free genetic panel of claim 40, that distinguishes between M1, M2, M3 and O glioma types by:

assessing an IDH1/2 mutant glioma sample for 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion;

if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and

if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma, thereby distinguishing between M1, M2, M3 and O glioma types without use of histology.

45. The histology-free genetic panel of claim 40, that distinguishes between M1, M2, M3 and O glioma types by:

assessing an IDH1/2 mutant glioma sample for 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, assessing whether the glioma sample exhibits CDK4 amplification and/or CDKN2A deletion;

if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, assessing whether the glioma sample exhibits chromosome 14 loss; and

if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma, thereby distinguishing between M1, M2, M3 and O glioma types without use of histology.

46. A histology-free method of diagnosing whether a subject with IDH1/2 mutant glioma has type M1, M2, M3 or O glioma, the method comprising:

(a) obtaining an IDH1/2 mutant glioma sample from the subject;

(b) contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;

if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as an O glioma,

(c) if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion;

if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and

if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma;

(d) if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion;

if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and

if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma;

(e) if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion;

if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and

if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma; and/or

(f) if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion;

if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and

if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma;
thereby diagnosing whether the subject with IDH1/2 mutant glioma has type M1, M2, M3 or O glioma without use of histology.

47. The histology-free method of claim 46, wherein diagnosing whether a subject with glioma IDH1/2 mutant has type M1, M2, M3 or O glioma comprises:

contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;

if the glioma sample exhibits 1p19q codeletion, identifying the glioma sample as an O glioma.

48. The histology-free method of claim 46, wherein diagnosing whether a subject with glioma IDH1/2 mutant has type M1, M2, M3 or O glioma comprises:

contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion;

if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and

if the glioma sample exhibits chromosome 14 loss, identifying the glioma sample as an M1 glioma.

49. The histology-free method of claim 46, wherein diagnosing whether a subject with glioma IDH1/2 mutant has type M1, M2, M3 or O glioma comprises:

contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion;

if the sample exhibits CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and

if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma.

50. The histology-free method of claim 46, wherein diagnosing whether a subject with glioma IDH1/2 mutant has type M1, M2, M3 or O glioma comprises:

contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion;

if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and

if the glioma sample does exhibit chromosome 14 loss, identifying the glioma sample as an M2 glioma.

51. The histology-free method of claim 46, wherein diagnosing whether a subject with glioma IDH1/2 mutant has type M1, M2, M3 or O glioma comprises:

contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;

if the glioma sample does not exhibit 1p19q codeletion, contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion;

if the sample does not exhibit CDK4 amplification and/or CDKN2A deletion, contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss; and

if the glioma sample does not exhibit chromosome 14 loss, identifying the glioma sample as an M3 glioma.

52. A method of detecting in a glioma sample (i) IDH1/2 mutant or IDH1/2 wildtype; (ii) presence or absence of chromosome 1 gain; (iii) presence or absence of CDK4/MDM2 coamplification; and (iv) presence or absence of chromosome 19 gain, the method comprising:

- obtaining a glioma sample;
- contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype;
- contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain;
- contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/MDM2 coamplification; and
- contacting the sample with a primer and detecting binding or lack thereof between chromosome 19 and the primer indicative of presence or absence of chromosome 19 gain.

53. A method of detecting in a glioma sample (i) IDH1/2 mutant or IDH1/2 wildtype; (ii) presence or absence of 1p19q codeletion; (iii) presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; and (iv) presence or absence of chromosome 14 loss, the method comprising:

- obtaining a glioma sample;
- contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype;
- contacting the sample with a primer and detecting binding or lack thereof between 1p19q and the primer indicative of presence or absence of 1p19q codeletion;
- contacting the sample with primers and detecting binding or lack thereof between CDK4 and CDKN2A and the primers indicative of presence or absence of CDK4 amplification and/or presence or absence of CDKN2A deletion; and
- contacting the sample with a primer and detecting binding or lack thereof between chromosome 14 and the primer indicative of presence or absence of chromosome 14 loss.

54. A method of claim 53 wherein the method does not utilize histology.

55. A kit comprising primers, probes, and/or stains to detect (i) IDH1/2 mutant or IDH1/2 wildtype; (ii) chromosome 1 gain; (iii) CDK4/MDM2 coamplification; and (iv) chromosome 19 gain.

56. A kit comprising primers, probes, and/or stains to detect (i) IDH1/2 mutant or IDH1/2 wildtype; (ii) 1p19q codeletion; (iii) CDK4 amplification and/or CDKN2A deletion; and (iv) chromosome 14 loss.

57. A kit comprising primers, probes, and/or stains to detect (i) IDH1/2 mutant or IDH1/2 wildtype; (ii) chromosome 1 gain; (iii) CDK4/MDM2 coamplification; (iv) chromosome 19 gain; (v) 1p19q codeletion; (vi) CDK4 amplification and/or CDKN2A deletion; and (vii) chromosome 14 loss.

FIG. 1

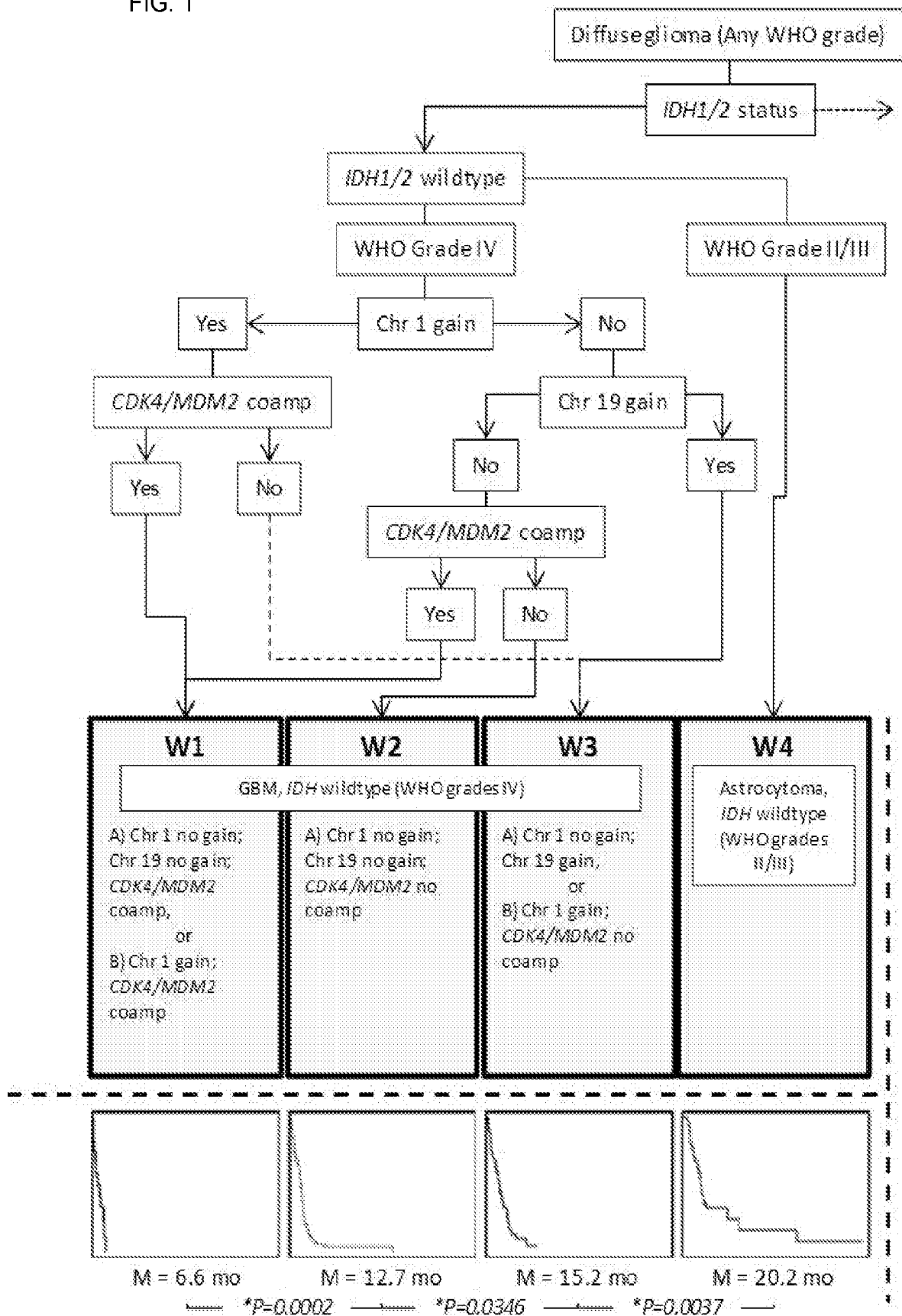


FIG. 1 cont'd

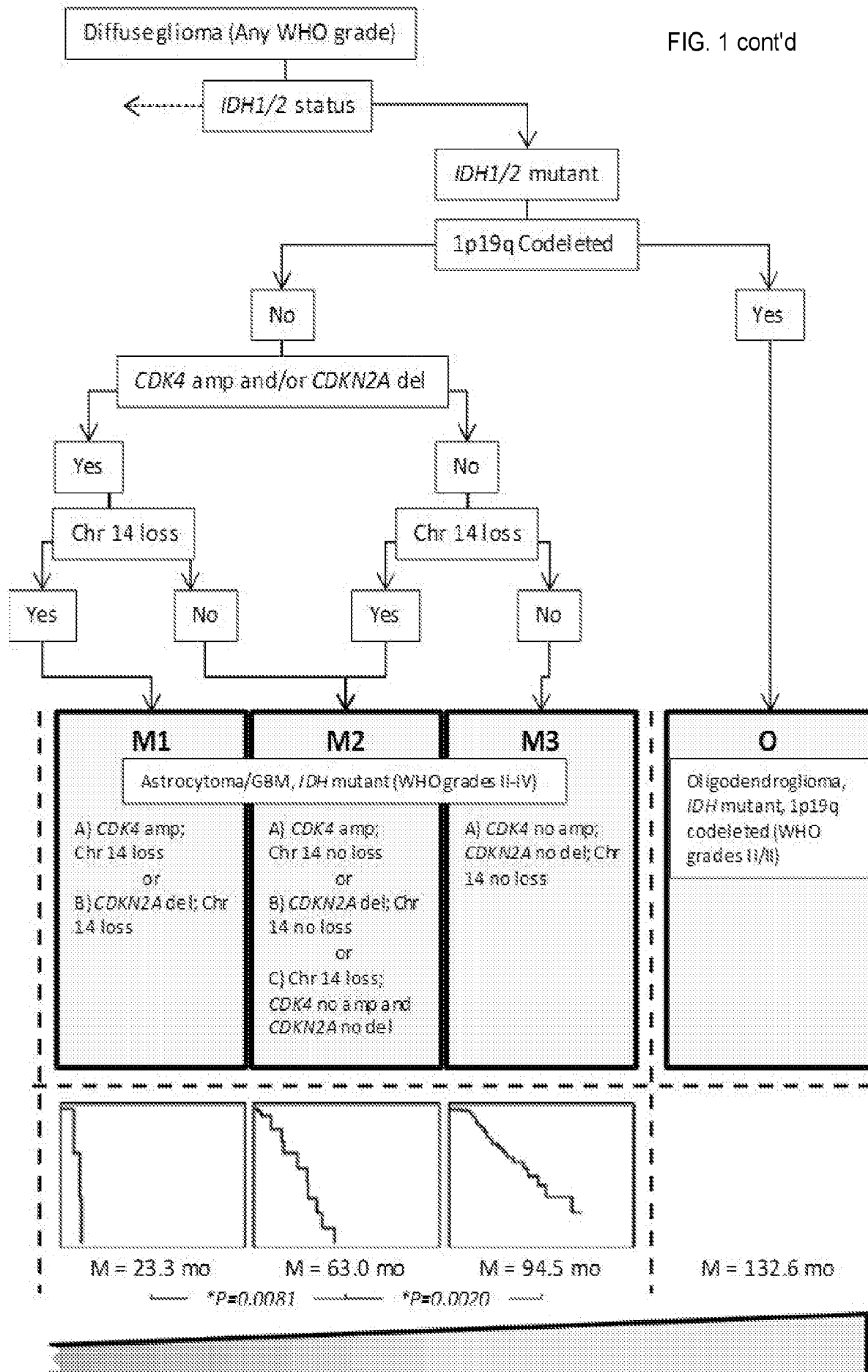


FIG. 2

Cluster	Astrocytoma/GBM, IDH mutant	Astrocytoma/GBM, IDH wildtype	Oligodendroglioma, IDH mutant, 1p/19q codeleted
TCGA Identifier	TCGA-02-2483-01, TCGA-06-0128-01, TCGA-06-0129-01, TCGA-06-2570-01, TCGA-06-6389-01, TCGA-06-6701-01, TCGA-14-1456-01, TCGA-14-4157-01, TCGA-15-1444-01, TCGA-19-2629-01, TCGA-26-1442-01, TCGA-27-2521-01, TCGA-32-4208-01, TCGA-CS-4938-01, TCGA-CS-4942-01, TCGA-CS-4943-01, TCGA-CS-4944-01, TCGA-CS-5393-01, TCGA-CS-6290-01, TCGA-CS-6665-01, TCGA-CS-6666-01, TCGA-CS-6667-01, TCGA-DB-5270-01, TCGA-DB-5273-01, TCGA-DB-5275-01, TCGA-DB-5276-01, TCGA-DB-5277-01, TCGA-DB-5280-01, TCGA-DB-5281-01, TCGA-DB-A4X9-01, TCGA-DB-A4XB-01, TCGA-DB-A4XC-01, TCGA-DB-A4XD-01, TCGA-DB-A4XE-01, TCGA-DB-A4XF-01, TCGA-DB-A64S-01, TCGA-DB-A64X-01, TCGA-DB-A75L-01, TCGA-DB-A75M-01, TCGA-DB-A75O-01, TCGA-DH-5142-01, TCGA-DH-5143-01,	TCGA-02-0003-01, TCGA-02-0033-01, TCGA-02-0047-01, TCGA-02-0055-01, TCGA-02-2470-01, TCGA-02-2485-01, TCGA-02-2486-01, TCGA-06-0119-01, TCGA-06-0122-01, TCGA-06-0124-01, TCGA-06-0126-01, TCGA-06-0130-01, TCGA-06-0132-01, TCGA-06-0137-01, TCGA-06-0139-01, TCGA-06-0140-01, TCGA-06-0141-01, TCGA-06-0142-01, TCGA-06-0145-01, TCGA-06-0151-01, TCGA-06-0152-01, TCGA-06-0154-01, TCGA-06-0155-01, TCGA-06-0157-01, TCGA-06-0158-01, TCGA-06-0165-01, TCGA-06-0166-01, TCGA-06-0168-01, TCGA-06-0169-01, TCGA-06-0173-01, TCGA-06-0174-01, TCGA-06-0178-01, TCGA-06-0184-01, TCGA-06-0185-01, TCGA-06-0188-01, TCGA-06-0189-01, TCGA-06-0192-01, TCGA-06-0195-01, TCGA-06-0209-01, TCGA-06-0213-01, TCGA-06-0214-01, TCGA-06-0216-01,	TCGA-CS-5390-01, TCGA-CS-5394-01, TCGA-CS-5396-01, TCGA-CS-6668-01, TCGA-CS-6670-01, TCGA-DB-5274-01, TCGA-DB-5278-01, TCGA-DB-5279-01, TCGA-DB-A4XA-01, TCGA-DB-A4XG-01, TCGA-DB-A4XH-01, TCGA-DB-A64L-01, TCGA-DB-A64P-01, TCGA-DB-A64Q-01, TCGA-DB-A64R-01, TCGA-DB-A64U-01, TCGA-DB-A64V-01, TCGA-DB-A64W-01, TCGA-DB-A75K-01, TCGA-DH-5141-01, TCGA-DH-5144-01, TCGA-DH-A669-01, TCGA-DH-A66F-01, TCGA-DH-A7UR-01, TCGA-DH-A7US-01, TCGA-DU-5849-01, TCGA-DU-5870-01, TCGA-DU-5874-01, TCGA-DU-6393-01, TCGA-DU-6394-01, TCGA-DU-6397-01, TCGA-DU-6400-01, TCGA-DU-6410-01, TCGA-DU-7009-01, TCGA-DU-7018-01, TCGA-DU-7294-01, TCGA-DU-7300-01, TCGA-DU-7302-01, TCGA-DU-8164-01, TCGA-DU-8168-01, TCGA-DU-A6S2-01, TCGA-DU-A6S3-01,

FIG. 2 CONT'D

Cluster	Astrocytoma/GBM, <i>IDH</i> mutant	Astrocytoma/GBM, <i>IDH</i> wildtype	Oligodendroglioma, <i>IDH</i> mutant, 1p/19q codeleted
	TCGA-DH-A66B-01, TCGA-DH-A66D-01, TCGA-DH-A66G-01, TCGA-DH-A7UT-01, TCGA-DH-A7UU-01, TCGA-DH-A7UV-01, TCGA-DU-5851-01, TCGA-DU-5853-01, TCGA-DU-5855-01, TCGA-DU-5871-01, TCGA-DU-5872-01, TCGA-DU-6395-01, TCGA-DU-6396-01, TCGA-DU-6401-01, TCGA-DU-6407-01, TCGA-DU-6408-01, TCGA-DU-6542-01, TCGA-DU-7007-01, TCGA-DU-7008-01, TCGA-DU-7010-01, TCGA-DU-7011-01, TCGA-DU-7015-01, TCGA-DU-7019-01, TCGA-DU-7298-01, TCGA-DU-7299-01, TCGA-DU-7301-01, TCGA-DU-7304-01, TCGA-DU-7306-01, TCGA-DU-7309-01, TCGA-DU-8163-01, TCGA-DU-8166-01, TCGA-DU-8167-01, TCGA-DU-A5TP-01, TCGA-DU-A5TR-01, TCGA-DU-A5TS-01, TCGA-DU-A5TU-01, TCGA-DU-A5TW-01, TCGA-DU-A6S7-01, TCGA-DU-A76O-01, TCGA-DU-A7T8-01, TCGA-DU-A7TA-01, TCGA-DU-A7TC-01, TCGA-E1-5302-01, TCGA-E1-5303-01,	TCGA-06-0219-01, TCGA-06-0237-01, TCGA-06-0238-01, TCGA-06-0240-01, TCGA-06-0241-01, TCGA-06-0644-01, TCGA-06-0645-01, TCGA-06-0646-01, TCGA-06-0648-01, TCGA-06-0649-01, TCGA-06-0650-01, TCGA-06-0686-01, TCGA-06-0743-01, TCGA-06-0744-01, TCGA-06-0745-01, TCGA-06-0747-01, TCGA-06-0749-01, TCGA-06-0750-01, TCGA-06-0875-01, TCGA-06-0876-01, TCGA-06-0877-01, TCGA-06-0878-01, TCGA-06-0879-01, TCGA-06-0881-01, TCGA-06-0882-01, TCGA-06-0939-01, TCGA-06-1804-01, TCGA-06-1806-01, TCGA-06-2557-01, TCGA-06-2558-01, TCGA-06-2559-01, TCGA-06-2561-01, TCGA-06-2562-01, TCGA-06-2563-01, TCGA-06-2564-01, TCGA-06-2565-01, TCGA-06-2567-01, TCGA-06-2569-01, TCGA-06-5408-01, TCGA-06-5410-01, TCGA-06-5411-01, TCGA-06-5412-01, TCGA-06-5413-01, TCGA-06-5414-01,	TCGA-DU-A6S6-01, TCGA-DU-A6S8-01, TCGA-DU-A76R-01, TCGA-DU-A7T6-01, TCGA-DU-A7TG-01, TCGA-E1-5311-01, TCGA-E1-5318-01, TCGA-E1-5319-01, TCGA-E1-5322-01, TCGA-E1-A7YO-01, TCGA-E1-A7YS-01, TCGA-E1-A7YY-01, TCGA-EZ-7264-01, TCGA-F6-A803-01, TCGA-FG-5962-01, TCGA-FG-5964-01, TCGA-FG-7634-01, TCGA-FG-7638-01, TCGA-FG-7641-01, TCGA-FG-8186-01, TCGA-FG-8187-01, TCGA-FG-8189-01, TCGA-FG-A60K-01, TCGA-FG-A6IZ-01, TCGA-FG-A6J1-01, TCGA-FG-A710-01, TCGA-FG-A713-01, TCGA-HT-7467-01, TCGA-HT-7468-01, TCGA-HT-7471-01, TCGA-HT-7480-01, TCGA-HT-7481-01, TCGA-HT-7605-01, TCGA-HT-7607-01, TCGA-HT-7608-01, TCGA-HT-7616-01, TCGA-HT-7620-01, TCGA-HT-7677-01, TCGA-HT-7681-01, TCGA-HT-7687-01, TCGA-HT-7692-01, TCGA-HT-7694-01, TCGA-HT-7695-01, TCGA-HT-7856-01,

FIG. 2 CONT'D

Cluster	Astrocytoma/GBM, IDH mutant	Astrocytoma/GBM, IDH wildtype	Oligodendroglioma, IDH mutant, 1p/19q codeleted
	TCGA-E1-5304-01, TCGA-E1-5305-01, TCGA-E1-5307-01, TCGA-E1-A7YE-01, TCGA-E1-A7YH-01, TCGA-E1-A7YI-01, TCGA-E1-A7YK-01, TCGA-E1-A7YU-01, TCGA-E1-A7YV-01, TCGA-E1-A7YW-01, TCGA-E1-A7Z3-01, TCGA-E1-A7Z4-01, TCGA-E1-A7Z6-01, TCGA-F6-A804-01, TCGA-FG-5965-01, TCGA-FG-6689-01, TCGA-FG-6690-01, TCGA-FG-6691-01, TCGA-FG-7636-01, TCGA-FG-8182-01, TCGA-FG-8185-01, TCGA-FG-8188-01, TCGA-FG-8191-01, TCGA-FG-A4MT-01, TCGA-FG-A4MX-01, TCGA-FG-A4MY-01, TCGA-FG-A60J-01, TCGA-FG-A60L-01, TCGA-FG-A6J3-01, TCGA-FG-A70Y-01, TCGA-FG-A711-01, TCGA-FG-A87N-01, TCGA-FN-7833-01, TCGA-HT-7470-01, TCGA-HT-7472-01, TCGA-HT-7473-01, TCGA-HT-7474-01, TCGA-HT-7475-01, TCGA-HT-7476-01, TCGA-HT-7477-01, TCGA-HT-7478-01, TCGA-HT-7479-01, TCGA-HT-7482-01, TCGA-HT-7483-01,	TCGA-06-5415-01, TCGA-06-5418-01, TCGA-06-5856-01, TCGA-06-5858-01, TCGA-06-5859-01, TCGA-06-6388-01, TCGA-06-6390-01, TCGA-06-6391-01, TCGA-06-6693-01, TCGA-06-6694-01, TCGA-06-6695-01, TCGA-06-6697-01, TCGA-06-6698-01, TCGA-06-6699-01, TCGA-06-6700-01, TCGA-08-0386-01, TCGA-12-0615-01, TCGA-12-0616-01, TCGA-12-0618-01, TCGA-12-0619-01, TCGA-12-0688-01, TCGA-12-0692-01, TCGA-12-0821-01, TCGA-12-3649-01, TCGA-12-3650-01, TCGA-12-3651-01, TCGA-12-3653-01, TCGA-12-5295-01, TCGA-12-5299-01, TCGA-12-5301-01, TCGA-14-0740-01, TCGA-14-0781-01, TCGA-14-0786-01, TCGA-14-0787-01, TCGA-14-0789-01, TCGA-14-0790-01, TCGA-14-0813-01, TCGA-14-0817-01, TCGA-14-0862-01, TCGA-14-0871-01, TCGA-14-1034-01, TCGA-14-1395-01, TCGA-14-1450-01, TCGA-14-1823-01,	TCGA-HT-7874-01, TCGA-HT-7875-01, TCGA-HT-7877-01, TCGA-HT-7881-01, TCGA-HT-8010-01, TCGA-HT-8012-01, TCGA-HT-8105-01, TCGA-HT-8109-01, TCGA-HT-8113-01, TCGA-HT-A4DV-01, TCGA-HT-A5R9-01, TCGA-HT-A615-01, TCGA-HT-A619-01, TCGA-HT-A74K-01, TCGA-HT-A74L-01, TCGA-HW-7486-01, TCGA-HW-7487-01, TCGA-HW-7491-01, TCGA-HW-8322-01, TCGA-HW-A5KJ-01, TCGA-1K-8125-01, TCGA-KT-A74X-01, TCGA-P5-A5ET-01, TCGA-P5-A5EX-01, TCGA-P5-A5F0-01, TCGA-P5-A72Z-01, TCGA-P5-A730-01, TCGA-P5-A737-01, TCGA-P5-A77W-01, TCGA-P5-A77X-01, TCGA-P5-A781-01, TCGA-QH-A85R-01, TCGA-QH-A85V-01, TCGA-QH-A85X-01, TCGA-QH-A85Z-01, TCGA-QH-A6CU-01, TCGA-QH-A6CY-01, TCGA-QH-A6CZ-01, TCGA-QH-A6X4-01, TCGA-QH-A6X5-01, TCGA-QH-A6X8-01, TCGA-QH-A86X-01, TCGA-R8-A6MK-01, TCGA-R8-A6ML-01,

FIG. 2 CONT'D

Cluster	Astrocytoma/GBM, IDH mutant	Astrocytoma/GBM, IDH wildtype	Oligodendroglioma, IDH mutant, 1p/19q codeleted
	TCGA-HT-7485-01, TCGA-HT-7601-01, TCGA-HT-7602-01, TCGA-HT-7603-01, TCGA-HT-7604-01, TCGA-HT-7606-01, TCGA-HT-7609-01, TCGA-HT-7610-01, TCGA-HT-7611-01, TCGA-HT-7676-01, TCGA-HT-7684-01, TCGA-HT-7686-01, TCGA-HT-7688-01, TCGA-HT-7689-01, TCGA-HT-7690-01, TCGA-HT-7693-01, TCGA-HT-7855-01, TCGA-HT-7858-01, TCGA-HT-7873-01, TCGA-HT-7879-01, TCGA-HT-7880-01, TCGA-HT-7884-01, TCGA-HT-7902-01, TCGA-HT-8013-01, TCGA-HT-8018-01, TCGA-HT-8106-01, TCGA-HT-8108-01, TCGA-HT-8111-01, TCGA-HT-8114-01, TCGA-HT-8563-01, TCGA-HT-A5R5-01, TCGA-HT-A5R7-01, TCGA-HT-A5RB-01, TCGA-HT-A614-01, TCGA-HT-A616-01, TCGA-HT-A618-01, TCGA-HT-A61B-01, TCGA-HT-A74J-01, TCGA-HT-A74O-01, TCGA-HW-7489-01, TCGA-HW-7490-01, TCGA-HW-8319-01, TCGA-HW-8320-01, TCGA-HW-8321-01,	TCGA-14-1825-01, TCGA-14-1829-01, TCGA-14-2554-01, TCGA-15-0742-01, TCGA-16-0846-01, TCGA-16-0861-01, TCGA-16-1045-01, TCGA-19-1390-01, TCGA-19-2619-01, TCGA-19-2620-01, TCGA-19-2623-01, TCGA-19-2624-01, TCGA-19-2625-01, TCGA-19-2631-01, TCGA-19-5947-01, TCGA-19-5950-01, TCGA-19-5951-01, TCGA-19-5952-01, TCGA-19-5953-01, TCGA-19-5954-01, TCGA-19-5955-01, TCGA-19-5958-01, TCGA-19-5959-01, TCGA-19-5960-01, TCGA-26-1439-01, TCGA-26-5132-01, TCGA-26-5133-01, TCGA-26-5134-01, TCGA-26-5135-01, TCGA-26-5136-01, TCGA-26-5139-01, TCGA-26-6173-01, TCGA-26-6174-01, TCGA-27-1830-01, TCGA-27-1831-01, TCGA-27-1832-01, TCGA-27-1833-01, TCGA-27-1834-01, TCGA-27-1835-01, TCGA-27-1836-01, TCGA-27-1837-01, TCGA-27-1838-01, TCGA-27-2518-01, TCGA-27-2519-01,	TCGA-R8-A6MO-01, TCGA-R8-A73M-01, TCGA-RY-A83X-01, TCGA-RY-A83Y-01, TCGA-RY-A840-01, TCGA-RY-A847-01, TCGA-S9-A6TW-01, TCGA-S9-A6TX-01, TCGA-S9-A6TY-01, TCGA-S9-A6U2-01, TCGA-S9-A6U5-01, TCGA-S9-A6UB-01, TCGA-S9-A6WD-01, TCGA-S9-A6WE-01, TCGA-S9-A6WH-01, TCGA-S9-A6WI-01, TCGA-S9-A6WL-01, TCGA-S9-A6WN-01, TCGA-S9-A6WP-01, TCGA-S9-A7IQ-01, TCGA-S9-A7IY-01, TCGA-S9-A7J1-01, TCGA-S9-A7J2-01, TCGA-S9-A7J3-01, TCGA-S9-A7QY-01, TCGA-S9-A7QZ-01, TCGA-S9-A7R1-01, TCGA-TM-A7C5-01, TCGA-TM-A84G-01, TCGA-TM-A84M-01, TCGA-TM-A84O-01, TCGA-TM-A84R-01, TCGA-TM-A84S-01, TCGA-TQ-A7RG-01, TCGA-TQ-A7RI-01, TCGA-TQ-A7RN-01, TCGA-TQ-A7RO-01, TCGA-TQ-A7RQ-01, TCGA-TQ-A7RS-01, TCGA-TQ-A7RU-01, TCGA-VM-A8CA-01, TCGA-VM-A8CB-01, TCGA-VM-A8CE-01, TCGA-VV-A829-01,

FIG. 2 CONT'D

Cluster	Astrocytoma/GBM, <i>IDH</i> mutant	Astrocytoma/GBM, <i>IDH</i> wildtype	Oligodendroglioma, <i>IDH</i> mutant, 1p/19q codeleted
	TCGA-HW-A5KL-01, TCGA-HW-A5KM-01, TCGA-IK-7675-01, TCGA-P5-A5EU-01, TCGA-P5-A5EV-01, TCGA-P5-A5EW-01, TCGA-P5-A5EZ-01, TCGA-P5-A5F1-01, TCGA-P5-A5F2-01, TCGA-P5-A5F4-01, TCGA-P5-A72W-01, TCGA-P5-A72X-01, TCGA-P5-A731-01, TCGA-P5-A733-01, TCGA-P5-A735-01, TCGA-P5-A736-01, TCGA-P5-A780-01, TCGA-QH-A65S-01, TCGA-QH-A6CW-01, TCGA-QH-A6X3-01, TCGA-QH-A6X9-01, TCGA-QH-A6XA-01, TCGA-QH-A870-01, TCGA-RY-A83Z-01, TCGA-RY-A843-01, TCGA-RY-A845-01, TCGA-S9-A6TS-01, TCGA-S9-A6TU-01, TCGA-S9-A6TV-01, TCGA-S9-A6TZ-01, TCGA-S9-A6U1-01, TCGA-S9-A6U6-01, TCGA-S9-A6U8-01, TCGA-S9-A6U9-01, TCGA-S9-A6WG-01, TCGA-S9-A6WO-01, TCGA-S9-A6WQ-01, TCGA-S9-A7IS-01, TCGA-S9-A7IZ-01, TCGA-S9-A7J0-01, TCGA-S9-A7QW-01, TCGA-S9-A7QX-01, TCGA-S9-A7R3-01, TCGA-S9-A7R4-01,	TCGA-27-2523-01, TCGA-27-2524-01, TCGA-27-2526-01, TCGA-27-2527-01, TCGA-27-2528-01, TCGA-28-1747-01, TCGA-28-1753-01, TCGA-28-2502-01, TCGA-28-2509-01, TCGA-28-2513-01, TCGA-28-2514-01, TCGA-28-5204-01, TCGA-28-5207-01, TCGA-28-5208-01, TCGA-28-5209-01, TCGA-28-5211-01, TCGA-28-5213-01, TCGA-28-5214-01, TCGA-28-5215-01, TCGA-28-5216-01, TCGA-28-5218-01, TCGA-28-5219-01, TCGA-28-5220-01, TCGA-28-6450-01, TCGA-32-1970-01, TCGA-32-1977-01, TCGA-32-1979-01, TCGA-32-1980-01, TCGA-32-1982-01, TCGA-32-1986-01, TCGA-32-1991-01, TCGA-32-2491-01, TCGA-32-2494-01, TCGA-32-2495-01, TCGA-32-2615-01, TCGA-32-2632-01, TCGA-32-2634-01, TCGA-32-2638-01, TCGA-32-4210-01, TCGA-32-4211-01, TCGA-32-4213-01, TCGA-32-4719-01, TCGA-32-5222-01, TCGA-41-2571-01,	TCGA-VW-A7QS-01, TCGA-W9-A837-01

FIG. 2 CONT'D

Cluster	Astrocytoma/GBM, <i>IDH</i> mutant	Astrocytoma/GBM, <i>IDH</i> wildtype	Oligodendroglioma, <i>IDH</i> mutant, 1p/19q codeleted
	TCGA-S9-A7R7-01, TCGA-S9-A7R8-01, TCGA-S9-A89Z-01, TCGA-TM-A7C4-01, TCGA-TM-A7CA-01, TCGA-TM-A7CF-01, TCGA-TM-A84F-01, TCGA-TM-A84H-01, TCGA-TM-A84I-01, TCGA-TM-A84L-01, TCGA-TM-A84Q-01, TCGA-TM-A84T-01, TCGA-TQ-A7RF-01, TCGA-TQ-A7RH-01, TCGA-TQ-A7RJ-01, TCGA-TQ-A7RK-01, TCGA-TQ-A7RM-01, TCGA-TQ-A7RR-01, TCGA-TQ-A7RV-01, TCGA-TQ-A7RW-01, TCGA-TQ-A8XE-01, TCGA-VM-A8C8-01, TCGA-VM-A8CF-01, TCGA-VM-A8CH-01, TCGA-VV-A86M-01, TCGA-WH-A86K-01, TCGA-WY-A858-01, TCGA-WY-A859-01, TCGA-WY-A85A-01, TCGA-WY-A85B-01, TCGA-WY-A85C-01, TCGA-WY-A85D-01, TCGA-WY-A85E-01	TCGA-41-2572-01, TCGA-41-2573-01, TCGA-41-2575-01, TCGA-41-3392-01, TCGA-41-3393-01, TCGA-41-3915-01, TCGA-41-4097-01, TCGA-41-5651-01, TCGA-41-6646-01, TCGA-74-6573-01, TCGA-74-6575-01, TCGA-74-6577-01, TCGA-74-6578-01, TCGA-74-6584-01, TCGA-76-4925-01, TCGA-76-4926-01, TCGA-76-4928-01, TCGA-76-4929-01, TCGA-76-4931-01, TCGA-76-4934-01, TCGA-76-4935-01, TCGA-76-6191-01, TCGA-76-6192-01, TCGA-76-6193-01, TCGA-76-6280-01, TCGA-76-6282-01, TCGA-76-6283-01, TCGA-76-6285-01, TCGA-76-6286-01, TCGA-76-6656-01, TCGA-76-6657-01, TCGA-76-6660-01, TCGA-76-6661-01, TCGA-76-6662-01, TCGA-76-6663-01, TCGA-76-6664-01, TCGA-81-5910-01, TCGA-81-5911-01, TCGA-87-5896-01, TCGA-CS-4941-01, TCGA-CS-5395-01, TCGA-CS-5397-01, TCGA-CS-6186-01, TCGA-CS-6188-01,	

FIG. 2 CONT'D

Cluster	Astrocytoma/GBM, IDH mutant	Astrocytoma/GBM, IDH wildtype	Oligodendroglioma, IDH mutant, 1p/19q codeleted
		TCGA-CS-6669-01, TCGA-DB-A640-01, TCGA-DB-A75P-01, TCGA-DH-5140-01, TCGA-DU-5847-01, TCGA-DU-5852-01, TCGA-DU-5854-01, TCGA-DU-6392-01, TCGA-DU-6399-01, TCGA-DU-6402-01, TCGA-DU-6403-01, TCGA-DU-6404-01, TCGA-DU-6405-01, TCGA-DU-6406-01, TCGA-DU-7006-01, TCGA-DU-7012-01, TCGA-DU-7013-01, TCGA-DU-7290-01, TCGA-DU-7292-01, TCGA-DU-8158-01, TCGA-DU-8161-01, TCGA-DU-8162-01, TCGA-DU-8165-01, TCGA-DU-A5TT-01, TCGA-DU-A5TY-01, TCGA-DU-A76K-01, TCGA-DU-A76L-01, TCGA-DU-A7TB-01, TCGA-DU-A7TD-01, TCGA-DU-A7TJ-01, TCGA-E1-A7YD-01, TCGA-E1-A7YJ-01, TCGA-E1-A7YL-01, TCGA-E1-A7YM-01, TCGA-E1-A7YN-01, TCGA-E1-A7YQ-01, TCGA-E1-A7Z2-01, TCGA-FG-5963-01, TCGA-FG-6688-01, TCGA-FG-6692-01, TCGA-FG-7643-01, TCGA-FG-8181-01, TCGA-FG-A4MU-01, TCGA-FG-A4MW-01,	

FIG. 2 CONT'D

Cluster	Astrocytoma/GBM, <i>IDH</i> mutant	Astrocytoma/GBM, <i>IDH</i> wildtype	Oligodendroglioma, <i>IDH</i> mutant, 1p/19q codeleted
		TCGA-FG-A70Z-01, TCGA-FG-A87Q-01, TCGA-HT-7459-01, TCGA-HT-7680-01, TCGA-HT-7691-01, TCGA-HT-7854-01, TCGA-HT-7857-01, TCGA-HT-7860-01, TCGA-HT-7882-01, TCGA-HT-8011-01, TCGA-HT-8015-01, TCGA-HT-8019-01, TCGA-HT-8104-01, TCGA-HT-8107-01, TCGA-HT-8110-01, TCGA-HT-8564-01, TCGA-HT-A4DS-01, TCGA-HT-A5RA-01, TCGA-HT-A5RC-01, TCGA-HT-A617-01, TCGA-HT-A61C-01, TCGA-HT-A74H-01, TCGA-HW-A5KK-01, TCGA-KT-A7W1-01, TCGA-P5-A5EY-01, TCGA-P5-A5F6-01, TCGA-P5-A72U-01, TCGA-QH-A6CS-01, TCGA-QH-A6CV-01, TCGA-QH-A6CX-01, TCGA-QH-A6XC-01, TCGA-S9-A6U0-01, TCGA-S9-A6UA-01, TCGA-S9-A6WM-01, TCGA-S9-A7IX-01, TCGA-S9-A7R2-01, TCGA-S9-A89V-01, TCGA-TM-A7C3-01, TCGA-TM-A84B-01, TCGA-TM-A84C-01, TCGA-TM-A84J-01, TCGA-TQ-A7RP-01, TCGA-VM-A8C9-01,	

FIG. 2 CONT'D

Cluster	Astrocytoma/GBM, <i>IDH</i> mutant	Astrocytoma/GBM, <i>IDH</i> wildtype	Oligodendroglioma, <i>IDH</i> mutant, 1p/19q codeleted
		TCGA-VM-A8CD-01, TCGA-VW-A8FI-01	

FIG. 3A

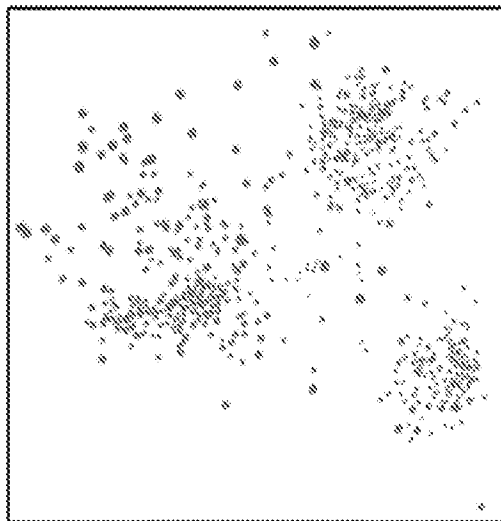


FIG. 3B

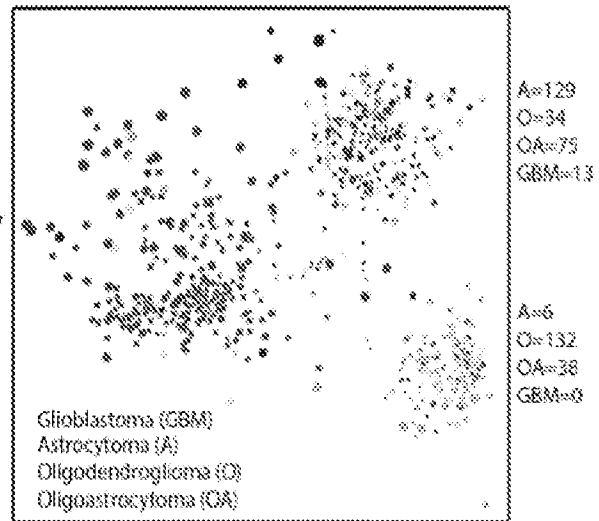


FIG. 3C

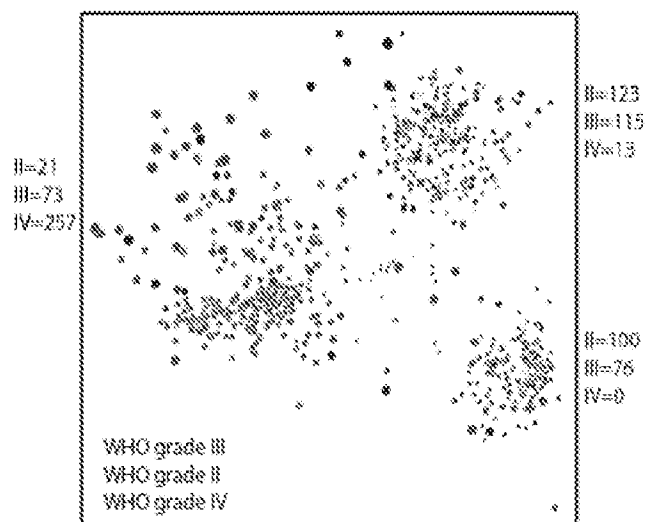


FIG. 3D

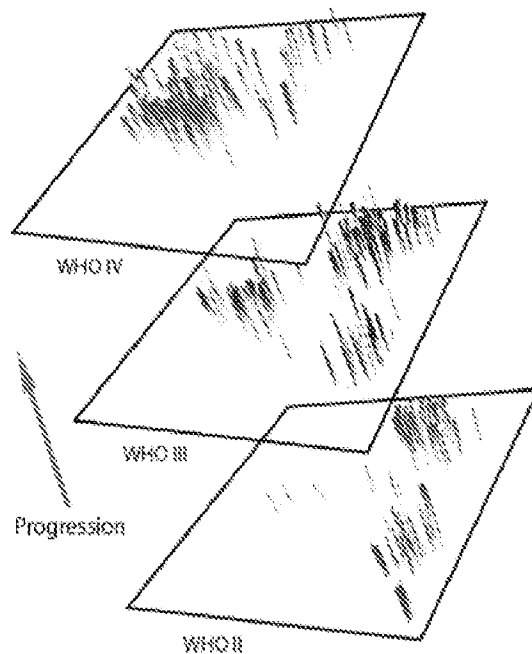


FIG. 4A

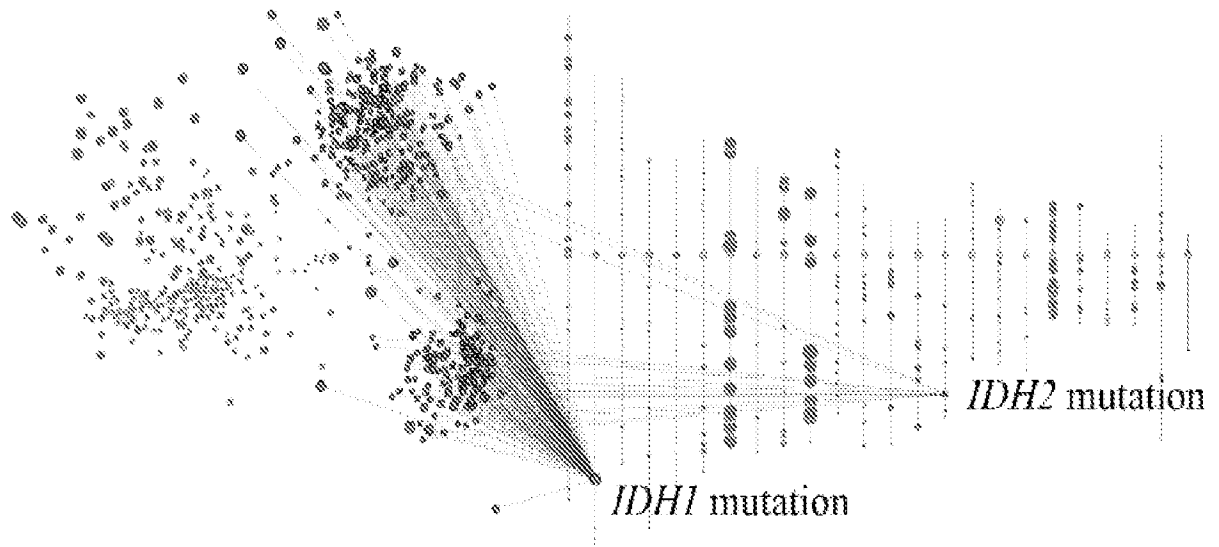


FIG. 4B

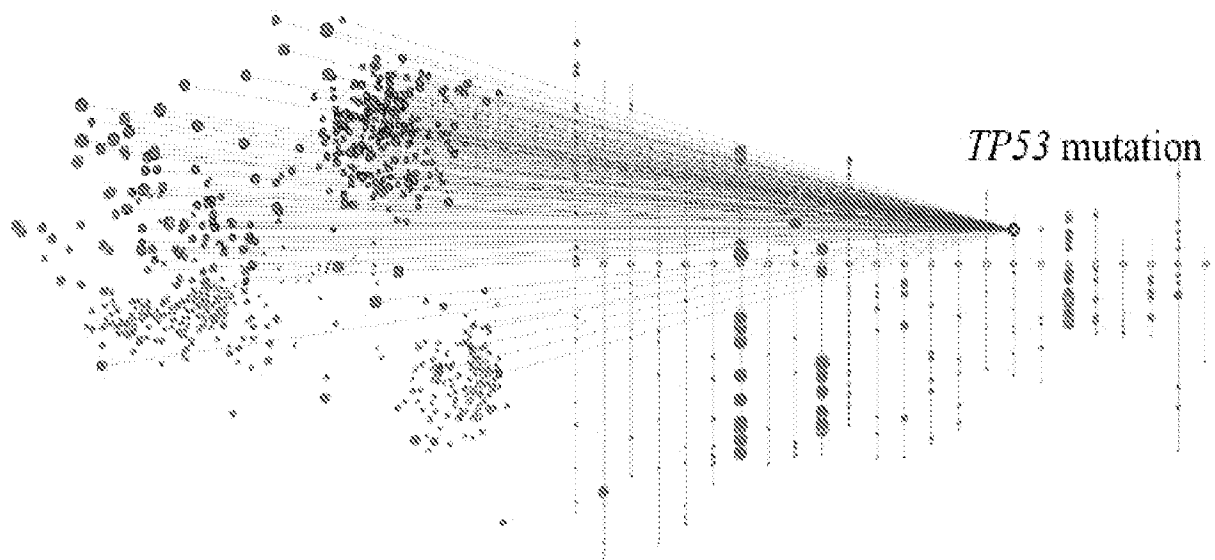


FIG. 4C

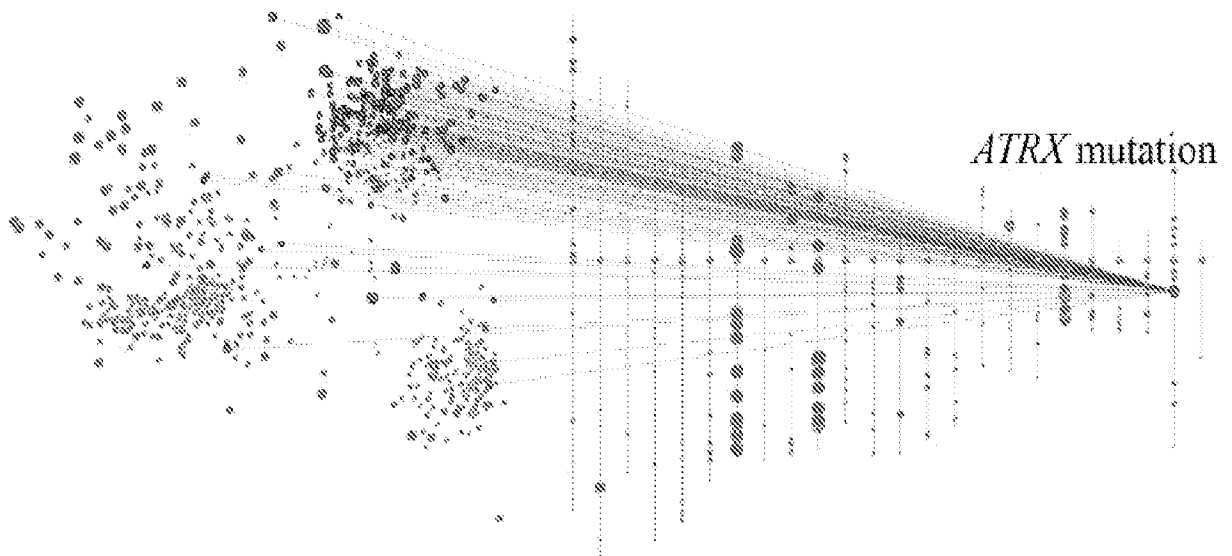
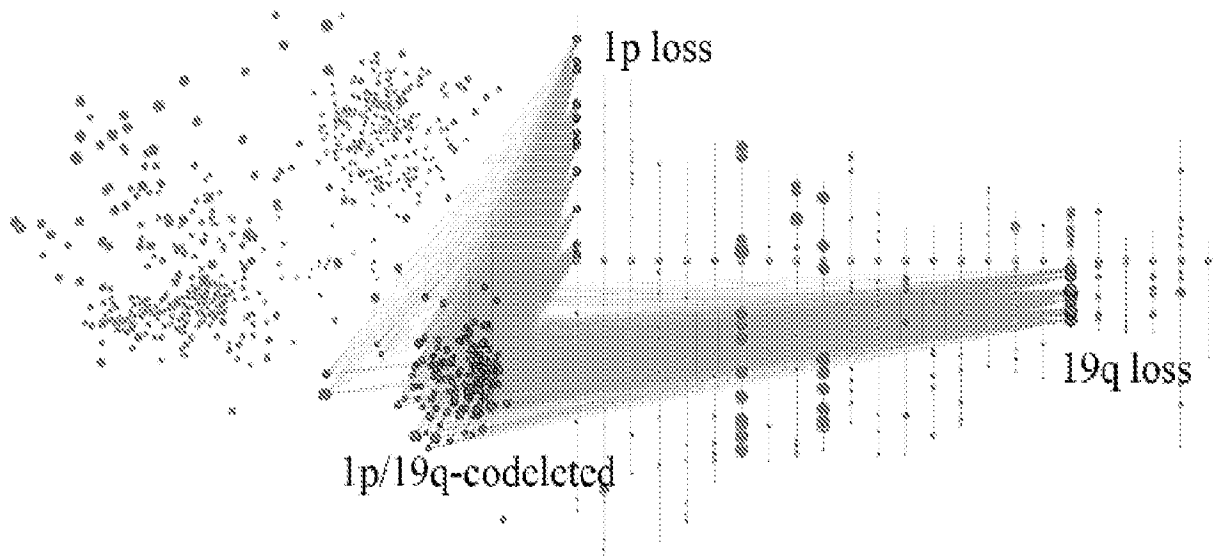
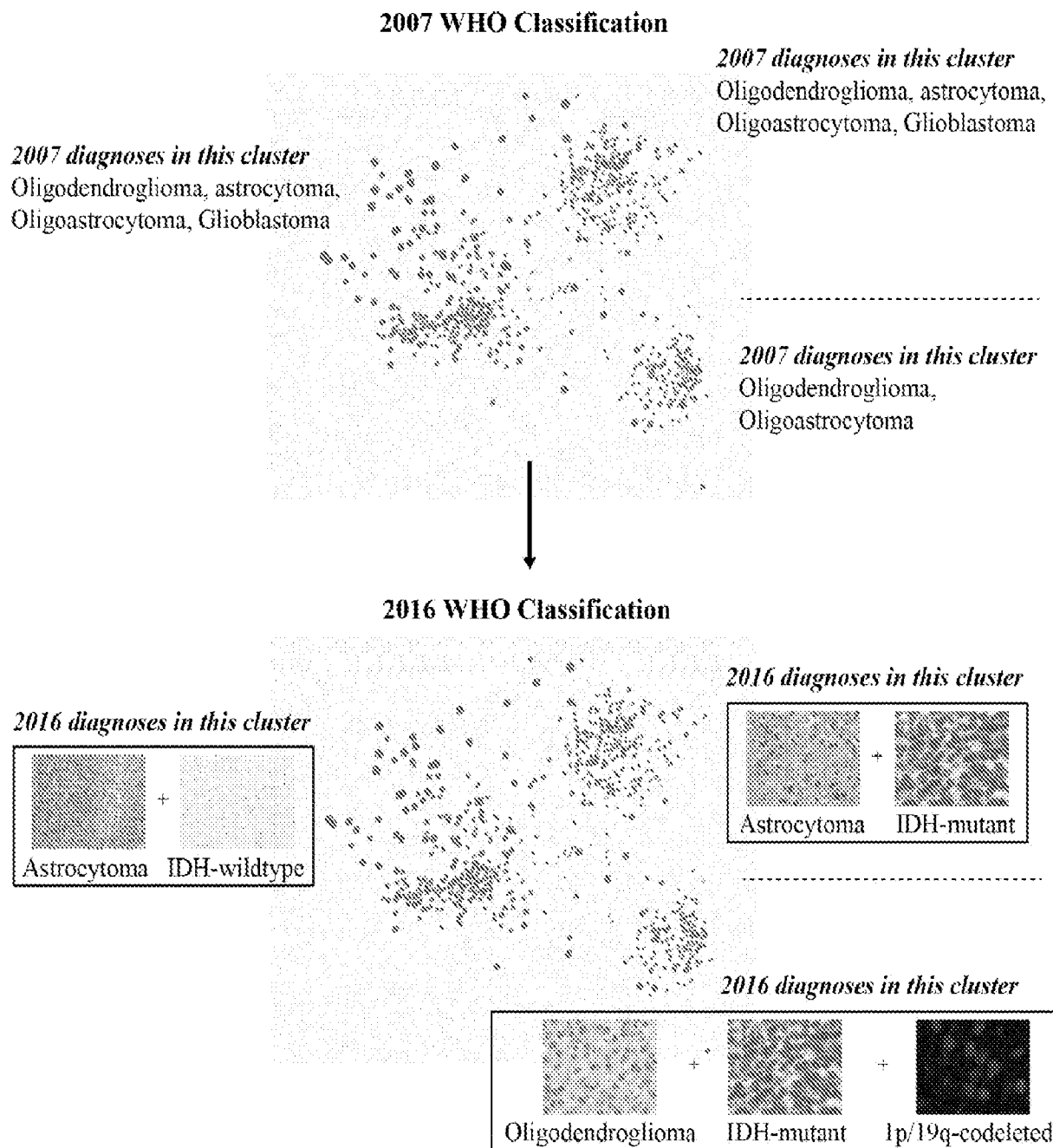


FIG. 4D



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FIG. 5



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FIG. 6A

2007 WHO histological classification

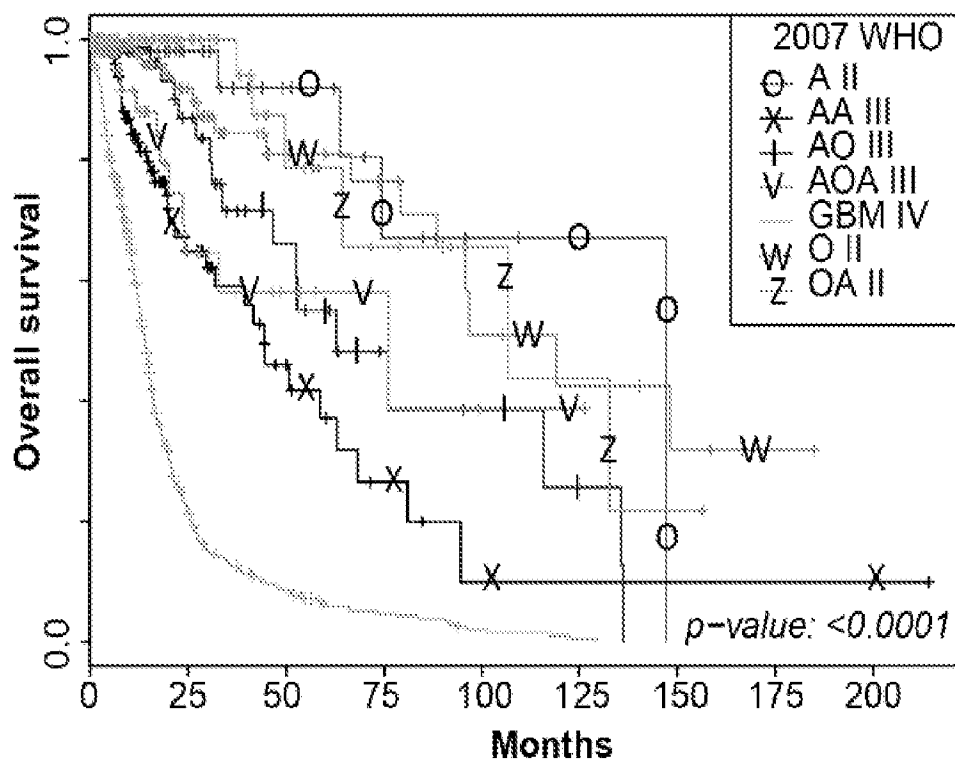
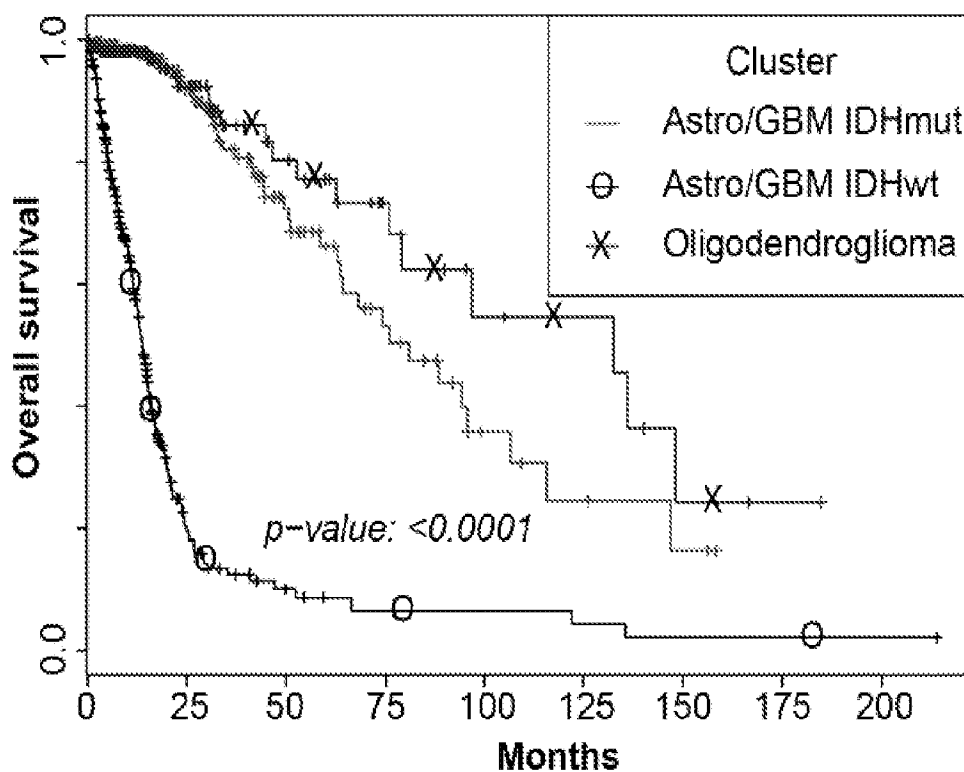


FIG. 6B

Three molecular clusters



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FIG. 6C

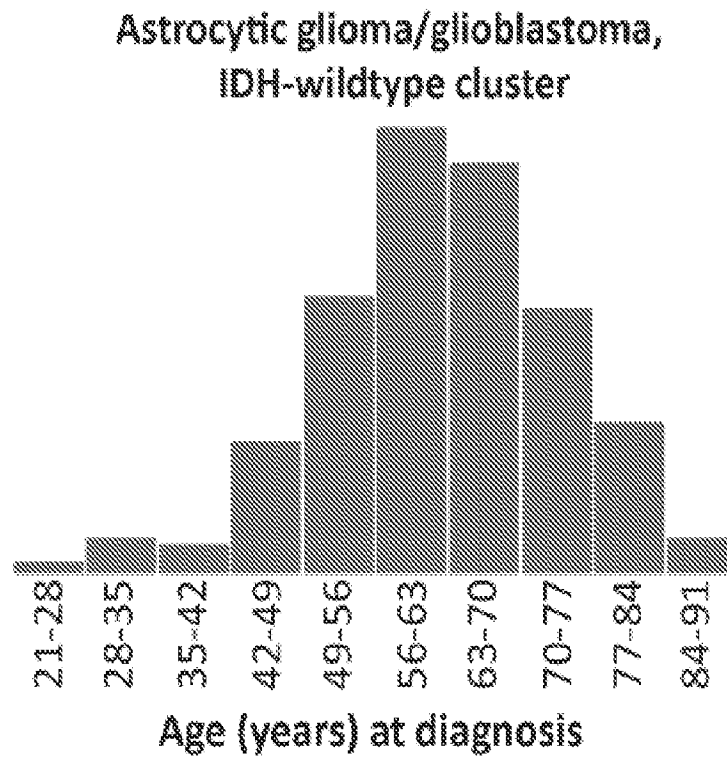
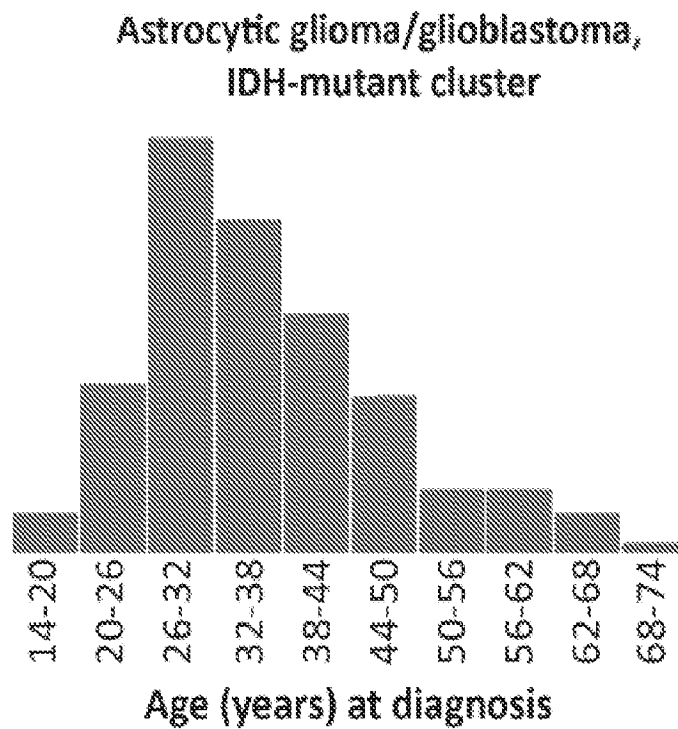


FIG. 6D



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FIG. 6E

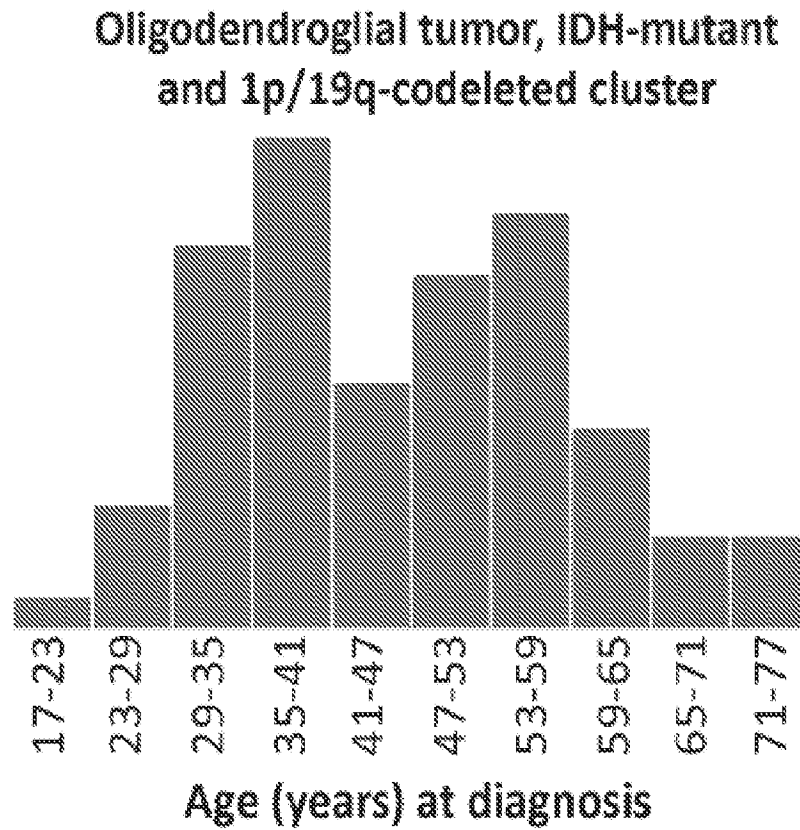
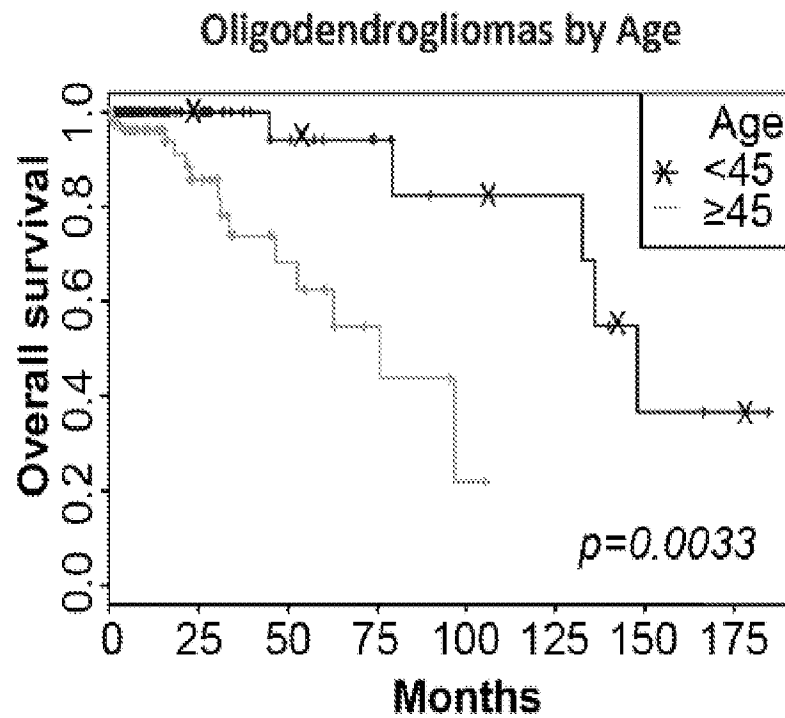


FIG. 6F



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FIG. 6G

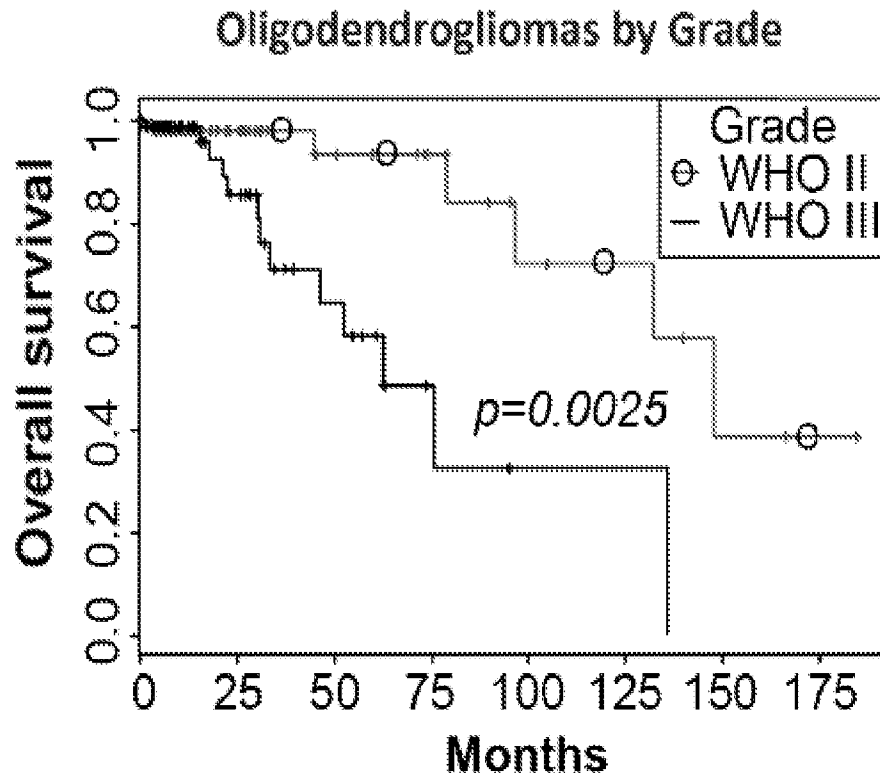
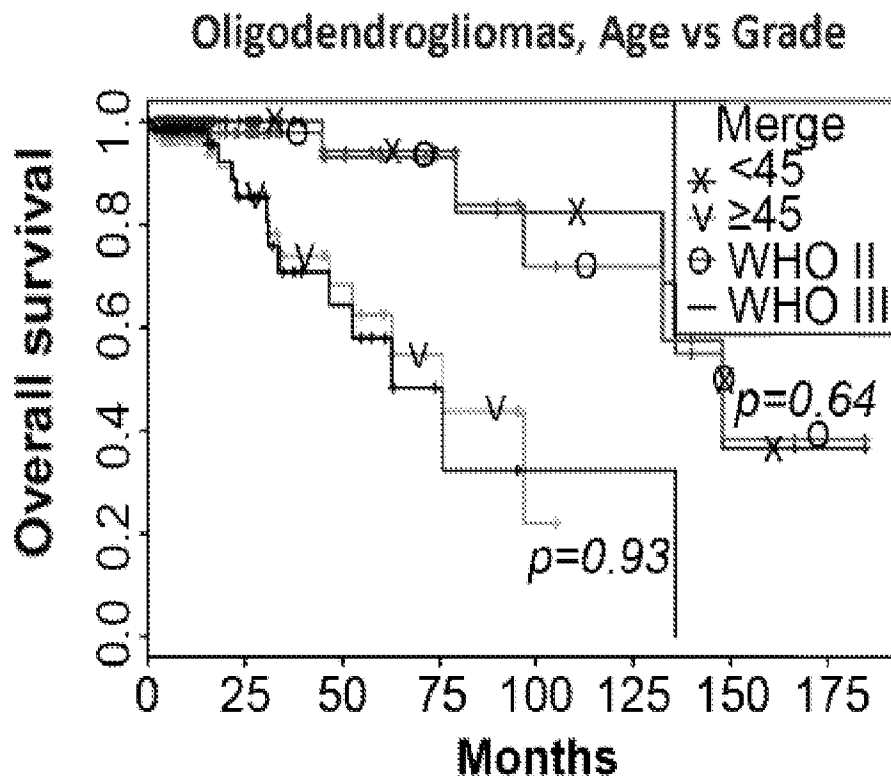
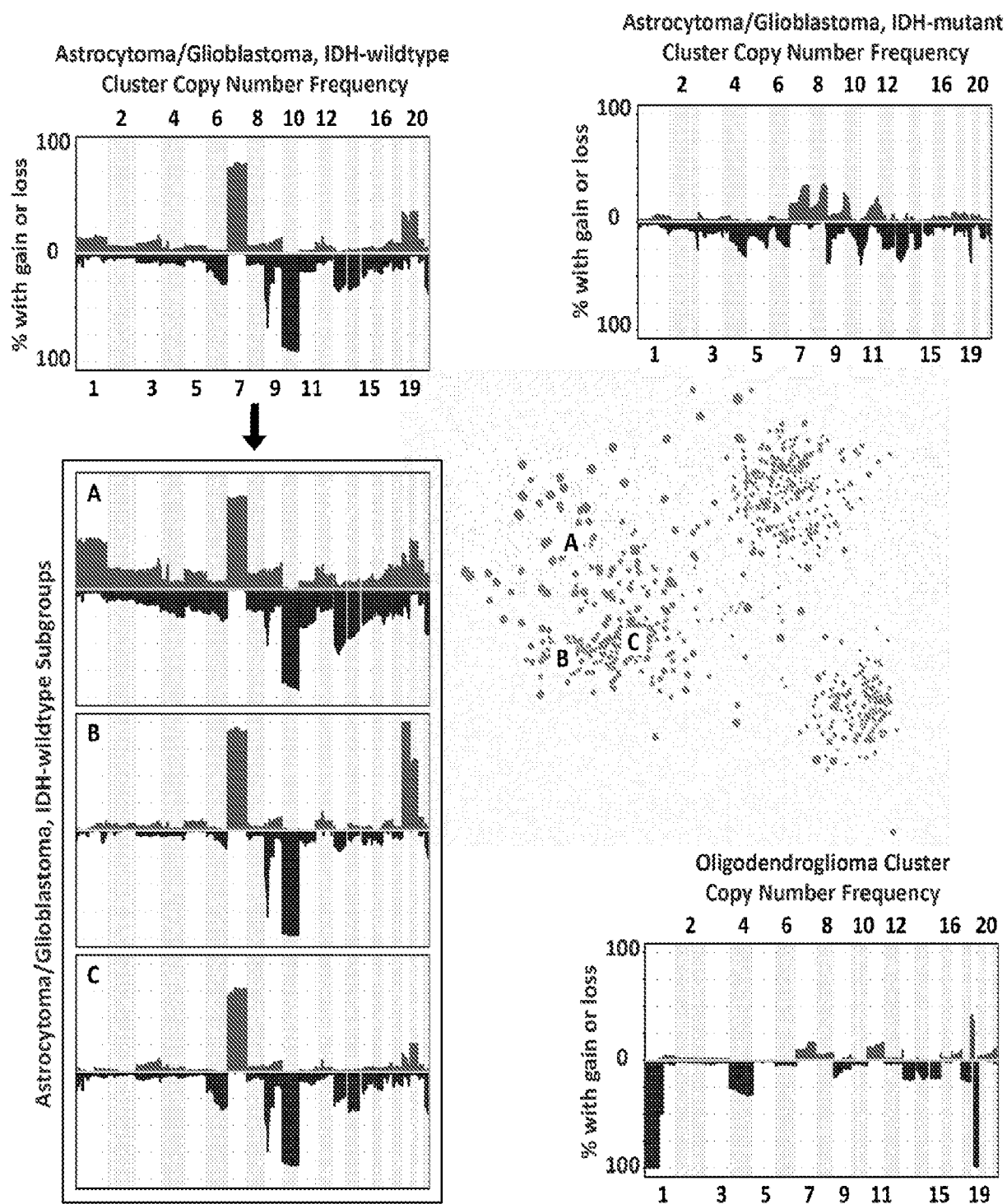


FIG. 6H



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FIG. 7



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FIG. 8A

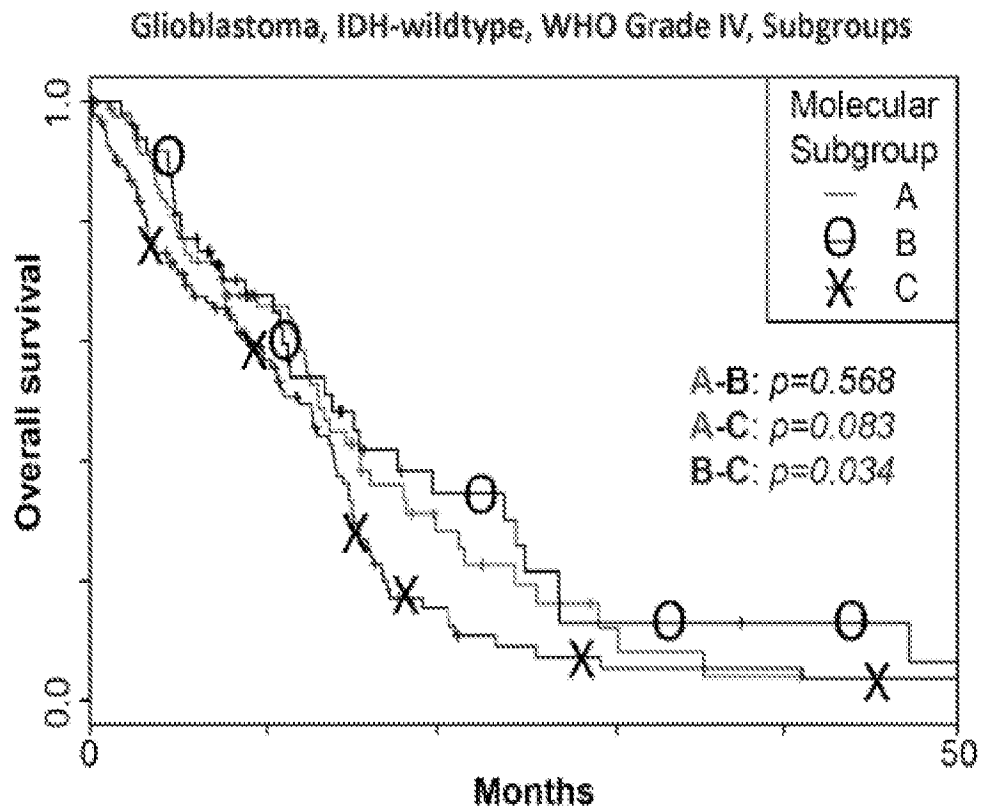
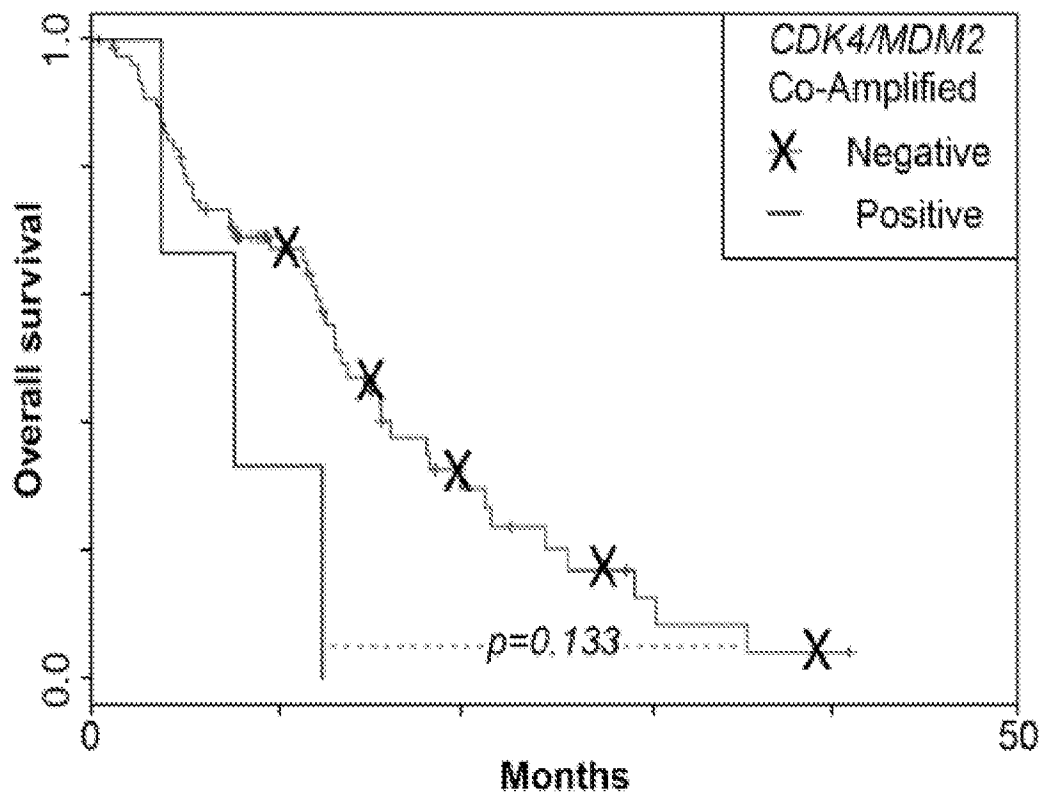


FIG. 8B
Subgroup A



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FIG. 8C

Subgroup B

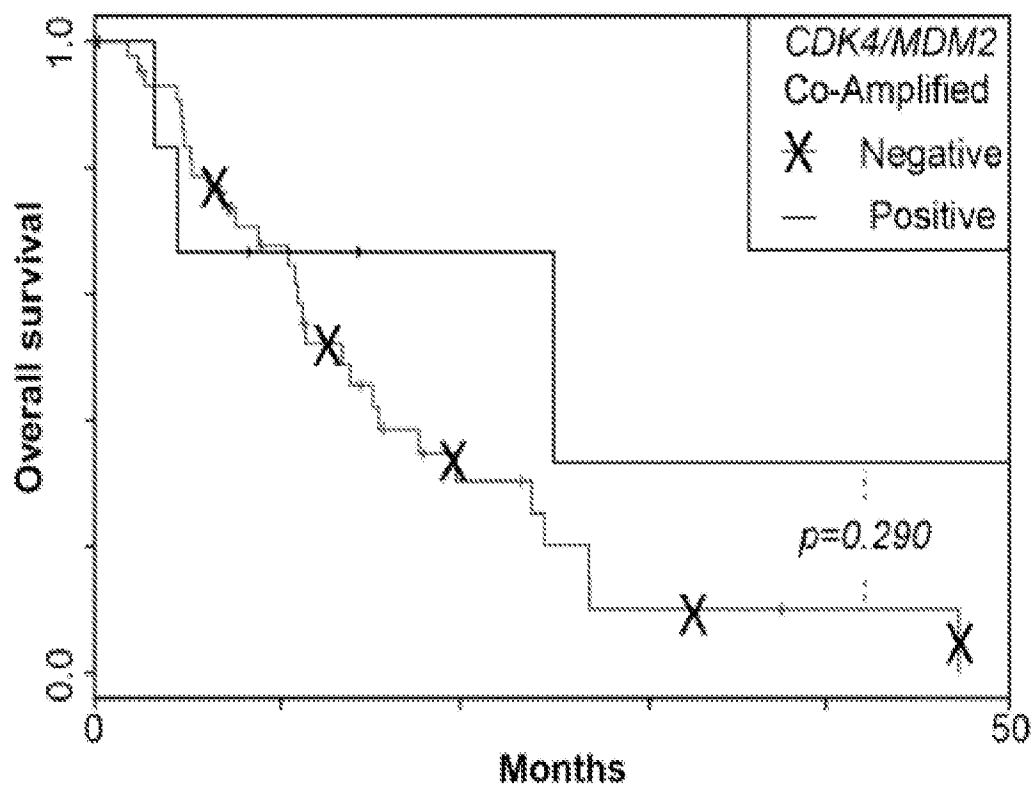


FIG. 8D

Subgroup C

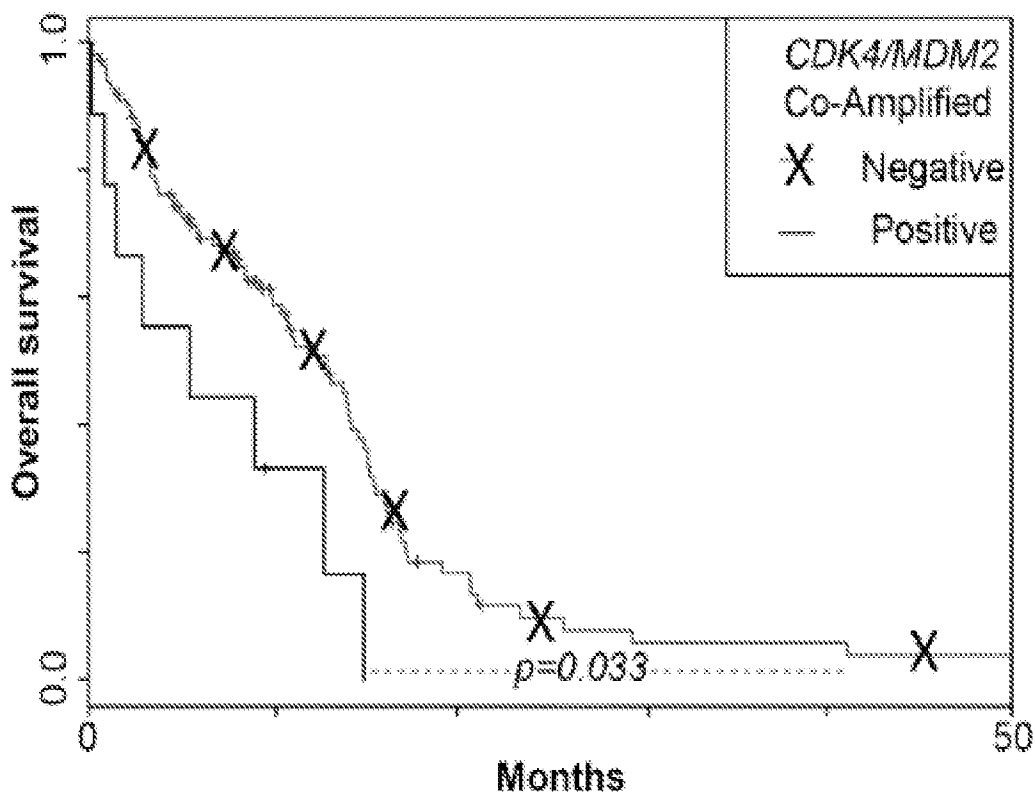


FIG. 9A

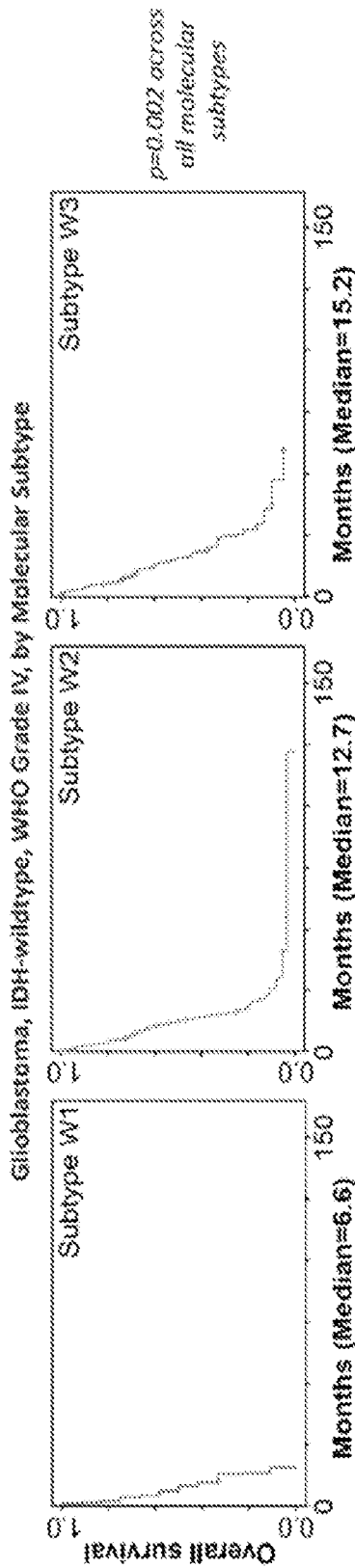


FIG. 9B

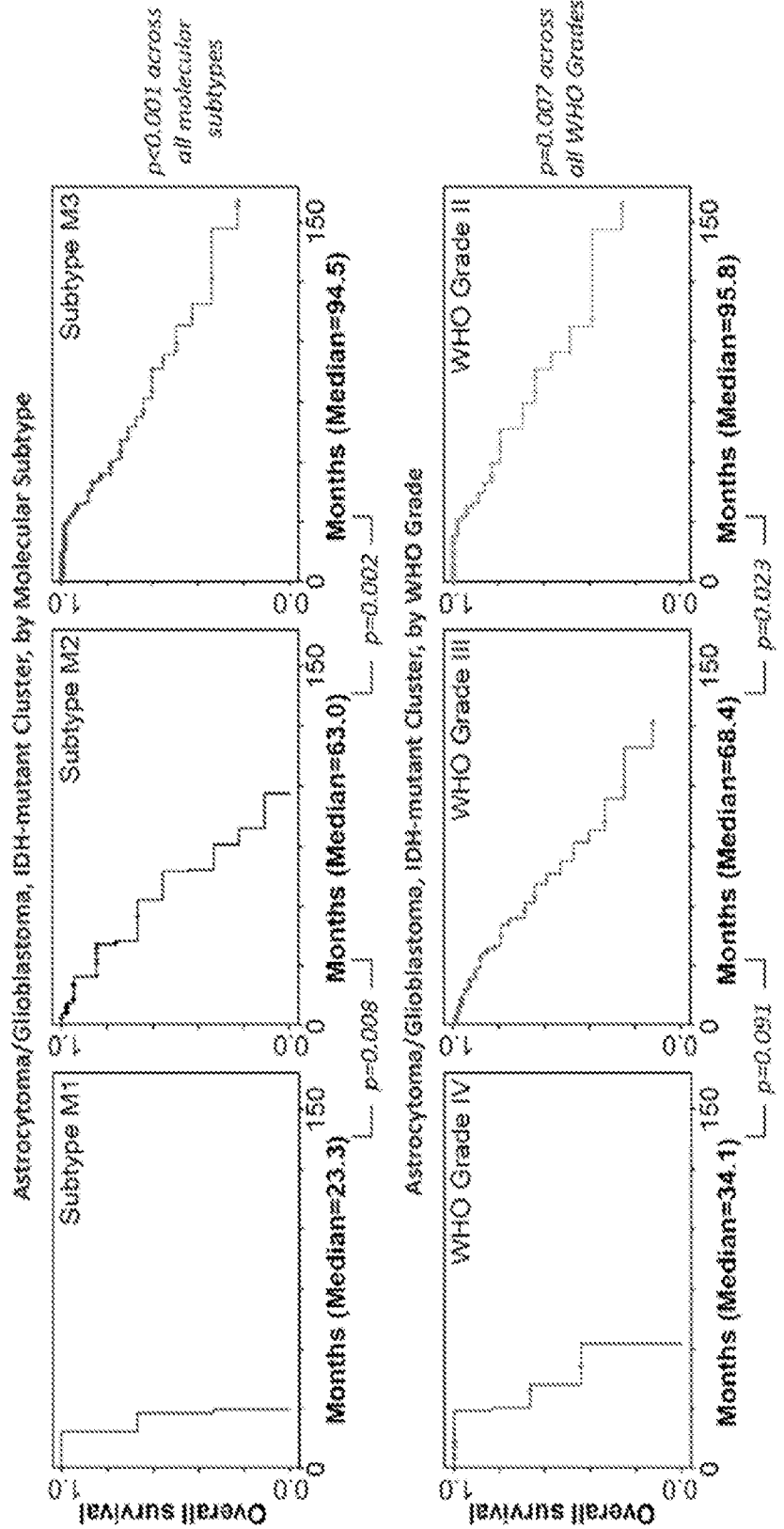
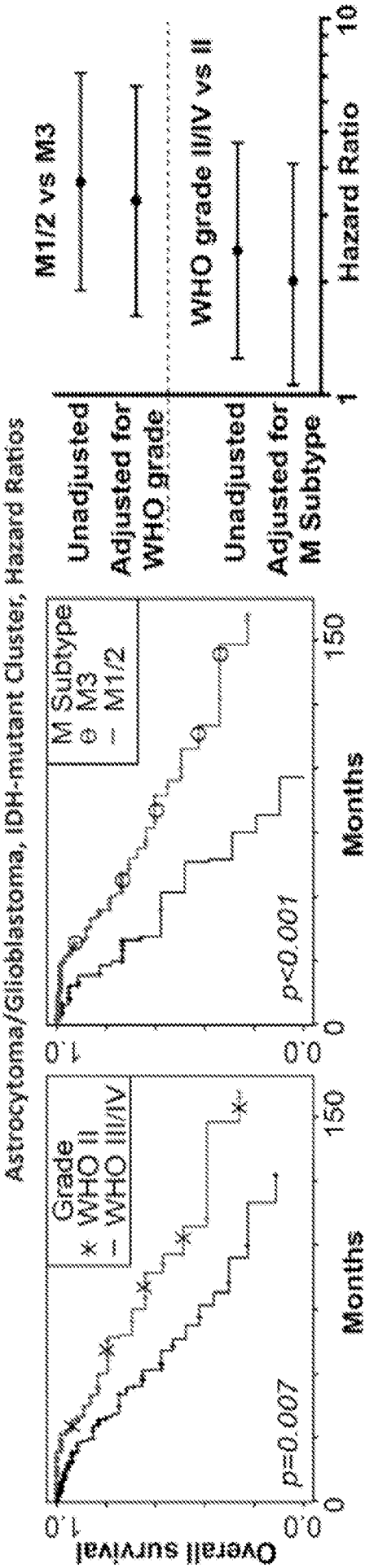


FIG. 9C



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FIG. 10A

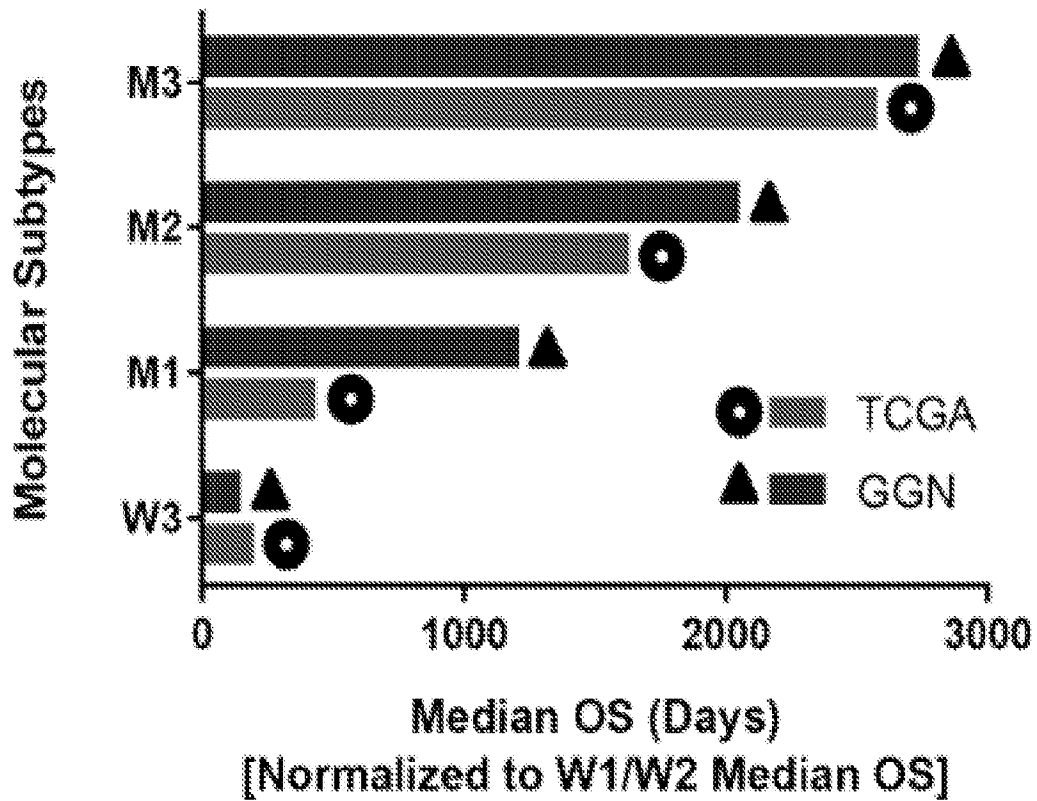
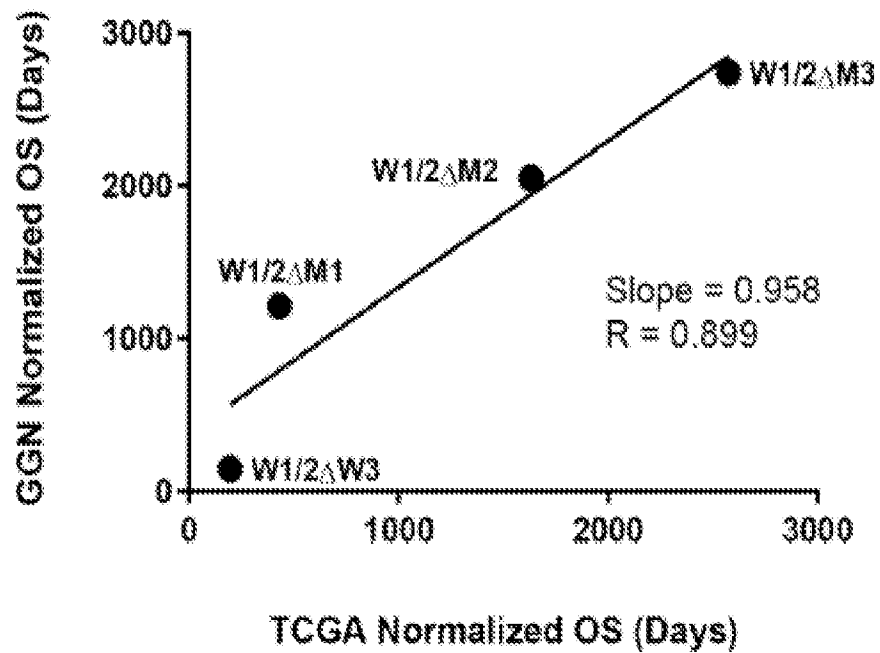


FIG. 10B



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FIG. 11A

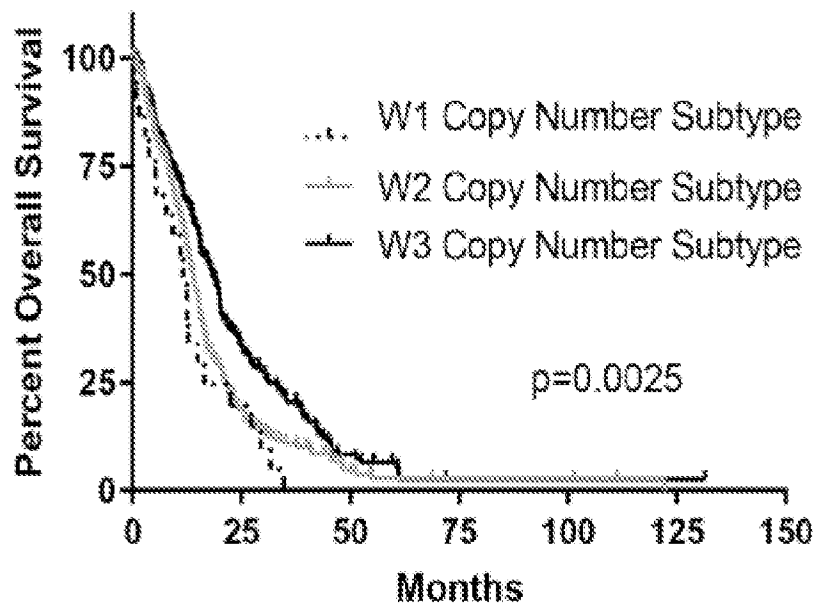
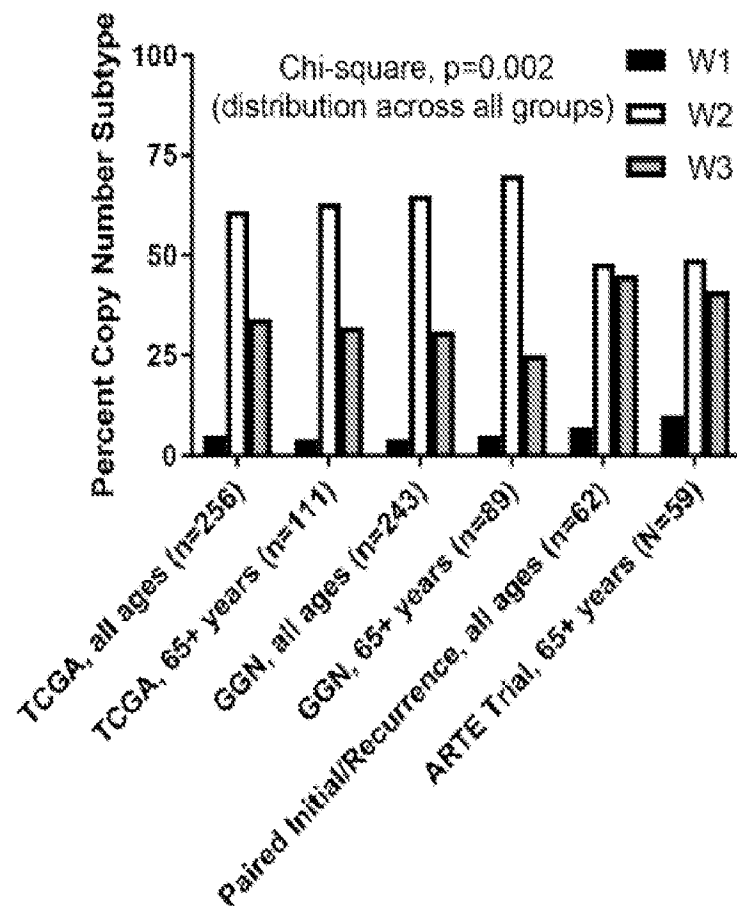
**IDH-wildtype Glioblastoma (TCGA and GGN)**

FIG. 11B



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FIG. 11C

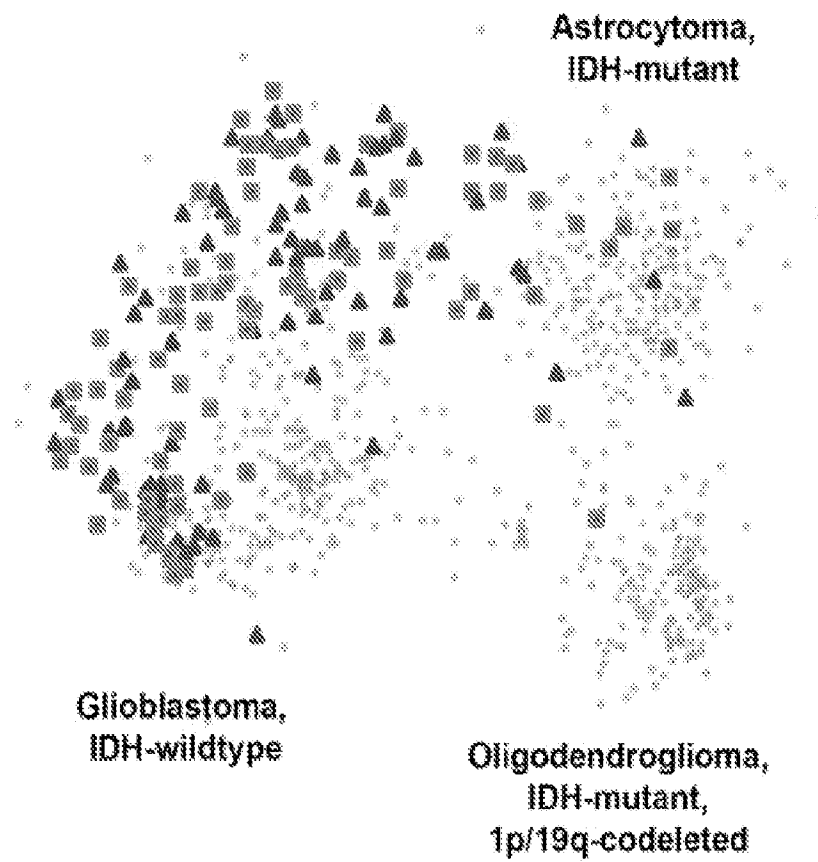
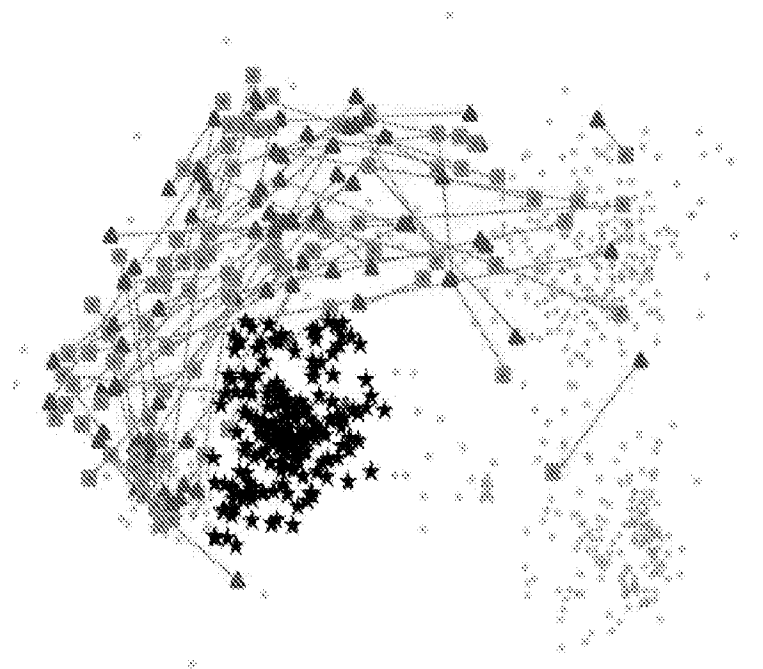


FIG. 11D



Distribution disparity of paired
initial/recurrent glioblastoma cohort

FIG. 12

IDH1 cds (SEQ ID NO: 1)

ATGTCCAAAAAATCAGTGGCGGTTCTGTGGTAGAGATGCAAGGAGATGAAATGACACGAATCA
TTTGGGAATTGATTAAAGAGAAACTCATTTTTCCCTACGTGGAATTGGATCTACATAGCTATGAT
TTAGGCATAGAGAATCGTGATGCCACCAACGACCAAGTCACCAAGGATGCTGCAGAAGCTATA
AAGAAGCATAATGTTGGCGTCAAATGTGCCACTATCACTCCTGATGAGAAGAGGGTTGAGGAGT
TCAAGTTGAAACAAATGTGGAAATCACCAAATGGCACCATACGAAATATTCTGGGTGGCACGGT
CTTCAGAGAAGCCATTATCTGCAAAAATATCCCCCGGCTTGTGAGTGGATGGGTAAAACCTATC
ATCATAGGTCGTCATGCTTATGGGGATCAATACAGAGCAACTGATTTTGTGTTCTGGGCCTG
GAAAAGTAGAGATAACCTACACACCAAGTGACGGAACCCAAAAGGTGACATACCTGGTACATAA
CTTTGAAGAAGGTGGTGGTGTGGCCATGGGGATGTATAATCAAGATAAGTCAATTGAAGATTTT
GCACACAGTTCCTTCCAAATGGCTCTGTCTAAGGGTTGGCCTTTGTATCTGAGCACCAAAAACA
CTATTCTGAAGAAATATGATGGGCGTTTTAAAGACATCTTTCAGGAGATATATGACAAGCAGTAC
AAGTCCCAGTTTGAAGCTCAAAGATCTGGTATGAGCATAGGCTCATCGACGACATGGTGGCC
CAAGCTATGAAATCAGAGGGAGGCTTCATCTGGGCCTGTAAAACTATGATGGTGACGTGCAG
TCGGACTCTGTGGCCCAAGGGTATGGCTCTCTCGGCATGATGACCAGCGTGCTGGTTTGTCCA
GATGGCAAGACAGTAGAAGCAGAGGCTGCCCACGGGACTGTAACCCGTCCTACCGCATGTAC
CAGAAAGGACAGGAGACGTCCACCAATCCCATTGCTTCCATTTTTGCCTGGACCAGAGGGTTA
GCCCACAGAGCAAAGCTTGATAACAATAAAGAGCTTGCCTTCTTTGCAAATGCTTTGGAAGAAG
TCTCTATTGAGACAATTGAGGCTGGCTTCATGACCAAGGACTTGGCTGCTTGCATTAAGGTTT
ACCCAATGTGCAACGTTCTGACTACTTGAATACATTTGAGTTCATGGATAAACTTGGAGAAAAC
TGAAGATCAAACCTAGCTCAGGCCAACTTTAA

IDH1 protein (SEQ ID NO: 2)

MSKKISGGSVVEMQGDENRIIWELIKEKLIFPYVELDLHSYDLGIENRDATNDQVTKDAAEAIKKHN
VGVKCATITPDEKRVEEFKLKQMWKSPNGTIRNILGGTVFREAIICKNIPRLVSGWVKPIIIGRHAYGD
QYRATDFVVPGPVKVEITYTPSDGTQKVTVLHNFEEGGGVAMGMYNQDKSIEDFAHSSFQMALSL
KGWPLYLSTKNILKKYDGRFKDIFQEIYDKQYKSQFEAQKIWYEHRLIDDMVAQAMKSEGGFIWAC
KNYDGDVQSDSVAQGYGSLGMMTSVLVCPDGKTVEAEAAHGTVTRHYRMYQKGQETSTNPIASIF
AWTRGLAHRAKLDNNKELAFFANALEEVSITIEAGFMTKDLAACIKGLPNVQRSYDLNTFEFMDKL
GENLKIKLAQAKL

FIG. 12 CONT'D

IDH2 cds (SEQ ID NO: 3)

ATGGCCGGCTACCTGCGGGTCGTGCGCTCGCTCTGCAGAGCCTCAGGCTCGCGGCCGGCCT
GGGCGCCGGCGGCCCTGACAGCCCCACCTCGCAAGAGCAGCCGCGGCGCCACTATGCCGA
CAAAAGGATCAAGGTGGCGAAGCCCGTGGTGGAGATGGATGGTATGAGATGACCCGTATTAT
CTGGCAGTTCATCAAGGAGAAGCTCATCCTGCCCCACGTGGACATCCAGCTAAAGTATTTTGAC
CTCGGGCTCCCAAACCGTGACCAGACTGATGACCAGGTCACCATTGACTCTGCACTGGCCACC
CAGAAGTACAGTGTGGCTGTCAAGTGTGCCACCATCACCCCTGATGAGGCCCGTGTGGAAGAG
TTCAAGCTGAAGAAGATGTGGAAGTCCCAATGGAATATCCGGAACATCCTGGGGGGGACT
GTCTTCCGGGAGCCCATCATCTGCAAAAACATCCCACGCCTAGTCCCTGGCTGGACCAAGCCC
ATCACCATTGGCAGGCACGCCCATGGCGACCAGTACAAGGCCACAGACTTTGTGGCAGACCG
GGCCGGCACTTTCAAATGGTCTTCACCCCAAAGATGGCAGTGGTGTCAAGGAGTGGGAAGT
GTACAACCTCCCCGCAGGCGGCGTGGGCATGGGCATGTACAACACCGACGAGTCCATCTCAG
GTTTTGCGCAGAGCTGCTTCCAGTATGCCATCCAGAAGAAATGGCCGCTGTACATGAGCACC
AGAACACCATACTGAAAGCCTACGATGGGCGTTTCAAGGACATCTTCCAGGAGATCTTTGACAA
GCACTATAAGACCGACTTCGACAAGAATAAGATCTGGTATGAGCACCGGCTCATTGATGACATG
GTGGCTCAGGTCCTCAAGTCTTCGGGTGGCTTTGTGTGGGCCTGCAAGAACTATGACGGAGAT
GTGCAGTCAGACATCCTGGCCCAGGGCTTTGGCTCCCTTGGCCTGATGACGTCCGTCTCTGGTC
TGCCCTGATGGGAAGACGATTGAGGCTGAGGCCGCTCATGGGACCGTCACCCGCCACTATCG
GGAGCACCAGAAGGGCCGGCCACCAGCACCAACCCCATCGCCAGCATCTTTGCCCTGGACAC
GTGGCCTGGAGCACCGGGGGAAGCTGGATGGGAACCAAGACCTCATCAGGTTTGCCAGATG
CTGGAGAAGGTGTGCGTGGAGACGGTGGAGAGTGGAGCCATGACCAAGGACCTGGCGGGCT
GCATTCACGGCCTCAGCAATGTGAAGCTGAACGAGCACTTCCTGAACACCACGGACTTCCTCG
ACACCATCAAGAGCAACCTGGACAGAGCCCTGGGCAGGCAGTAG

IDH2 protein (SEQ ID NO: 4)

MAGYLRVVRSLCRASGSRPAWAPAALTAPTSQEQPRRHYADKRIKVAKPVEMDGDDEMTRIIWQFI
KEKLILPHVDIQLKYFDLGLPNRDQTDQVTIDSLATQKYSVAVKCATITPDEARVEEFKLKKMWKS
PNGTIRNILGGTVFREPIICKNIPRLVPGWTKPITIGRHAHGDQYKATDFVADRAGTFKMVFTPKDGS
GVKEWEVYNFPAGGVGMGMYNNTDESISGFAHSCFQYAIQKKWPLYMSTKNILKAYDGRFKDIFQE
IFDKHYKTDFDKNIWYEHRLIDDMVAQVLKSSGGFVWACKNYDGDVQSDILAQQFGSLGLMTSVL
VCPDGKTIEAAHGTVTRHYREHQGRPTSTNPIASIFAWTRGLEHRGKLDGNQDLIRFAQMLEK
VCVETVESGAMTKDLAGCIHGLSNVKLNEHFLNTTDFLDTIKSNLDRALGRQ

FIG. 12 CONT'D

CDK4 cds (SEQ ID NO: 5)

ATGGCTACCTCTCGATATGAGCCAGTGGCTGAAATTGGTGTGGTGCCTATGGGACAGTGTAC
AAGGCCCGTGATCCCCACAGTGGCCACTTTGTGGCCCTCAAGAGTGTGAGAGTCCCCAATGGA
GGAGGAGGTGGAGGAGGCCTTCCCATCAGCACAGTTCGTGAGGTGGCTTTACTGAGGCGACT
GGAGGCTTTTGAGCATCCCAATGTTGTCCGGCTGATGGACGTCTGTGCCACATCCCGAACTGA
CCGGGAGATCAAGGTAACCCTGGTGTGTTGAGCATGTAGACCAGGACCTAAGGACATATCTGGA
CAAGGCACCCCCACCAGGCTTGCCAGCCGAAACGATCAAGGATCTGATGCGCCAGTTTCTAAG
AGGCCTAGATTTCTTCATGCCAATTGCATCGTTCACCGAGATCTGAAGCCAGAGAACATTCTG
GTGACAAGTGGTGGAACAGTCAAGCTGGCTGACTTTGGCCTGGCCAGAATCTACAGCTACCAG
ATGGCACTTACACCCGTGGTTGTTACACTCTGGTACCGAGCTCCCGAAGTTCTTCTGCAGTCCA
CATATGCAACACCTGTGGACATGTGGAGTGTGGCTGTATCTTTGCAGAGATGTTTCGTGAAA
GCCTCTCTTCTGTGGAACTCTGAAGCCGACCAGTTGGGCAAAATCTTTGACCTGATTGGGCTG
CCTCCAGAGGATGACTGGCCTCGAGATGTATCCCTGCCCCGTGGAGCCTTTCCCCCAGAGG
GCCCCGCCAGTGCAGTCGGTGGTACCTGAGATGGAGGAGTCGGGAGCACAGCTGCTGCTGG
AAATGCTGACTTTTAACCCACACAAGCGAATCTCTGCCTTTCGAGCTCTGCAGCACTCTTATCTA
CATAAGGATGAAGGTAATCCGGAGTGA

CDK4 protein (SEQ ID NO: 6)

MATSRYEPVAEIGVGAYGTVYKARDPHSGHFVALKSVRVPNGGGGGGGGLPISTVREVALLRRLEAF
EHPNVVRLMDVCATSRTDREIKVTLVFEHVDQDLRTYLDKAPPPGLPAETIKDLMRQFLRGLDFLHA
NCIVHRDLKPENILVTSGGTVKLADFLGARIYSYQMALTPVVVTLWYRAPEVLLQSTYATPVDMWSV
GCIFAEMFRRKPLFCGNSEADQLGKIFDLIGLPPEDDWPRDVS LPRGAFP RGRPRPVQSVPEMEE
SGAQLLLEMLTFNPHKRISAFRALQHSYLHKDEGNPE

FIG. 12 CONT'D

CDKN2A Whole genomic locus (SEQ ID NO: 7)

GGTTAAACCGAAAATAAAAATGGGCTAGACACAAAGGACTCGGTGCTTGTCCCAGCCAGGCG
CCCTCGGCGACGCGGGCAGCTGGGAGGGGAATGGGCGCCCGGACCCAGCTGGGACCCCCG
GGTGCGACTCCACCTACCTAGTCCGGCGCCAGGCCGGGTGACAGCTCCGGCAGCGCCAGC
GCCGCGCCGTGTCCAGATGTCGCGTCAGAGGCGTGCAGCGGTTTAGTTTAATTCGCTTGTTT
TCCAAATCTAGAAGAGGAGCGGAGCGGCTTTTAGTTCAAAACTGACATTCAGCCTCCTGATTGG
CGGATAGAGCAATGAGATGACCTCGCTTTCTTTCTTCTTTTTCATTTTTAAATAATCTAGTTTG
AAGAATGGAAGACTTTCGACGAGGGGAGCCAGGAATAAAATAAGGGGAATAGGGGAGCGGGG
ACGCGAGCAGCACCAGAATCCGCGGGAGCGCGGCTGTTCTTGGTAGGGCCGTGTCAGGTGAC
GGATGTAGCTAGGGGGCGAGCTGCCTGGAGTTGCGTTCCAGGCGTCCGGCCCCCTGGGCCGT
CACCGCGGGGCGCCCCGCGCTGAGGGTGGGAAGATGGTGGTGGGGGTGGGGGCGCACACAG
GGCGGGAAGTGGCGGTAGGCGGGAGGGAGAGGAACGCGGGGCCCTGAGCCGCCCGCGCGC
GCGCCTCCCTACGGGCGCCTCCGGCAGCCCTTCCCGCGTGCAGAGGGCTCAGAGCCGTTCC
GAGATCTTGGAGGTCCGGGTGGGAGTGGGGGTGGGGTGGGGGTGGGGGTGAAGGTGGGGG
GCGGGCGCGCTCAGGGAAGGCGGGTGCAGCGCTGCGGGGCGGAGATGGGCAGGGGGCGGT
GCGTGGGTCCCAGTCTGCAGTTAAGGGGGCAGGAGTGGCGCTGCTCACCTCTGGTGCCAAAG
GGCGGCGCAGCGGTGCCGAGCTCGGCCCTGGAGGCGGCGAGAACATGGTGCGCAGGTTCT
TGGTGACCCTCCGGATTCCGGCGCGCGTGCGGCCCCGCCGCGAGTGAGGGTTTTCTGGTTTAC
ATCCCGCGGCTCACGGGGGAGTGGGCAGCGCCAGGGGCGCCCCGCCGCTGTGGCCCTCGTG
TGATGCTACTGAGGAGCCAGCGTCTAGGGCAGCAGCCGCTTCTAGAAGACCAGGTAGGAAA
GGCCCTCGAAAAGTCCGGGGCGCATTGGCACTTGTTTTGTTTGGTGTGATTTCTGTAACAGAT
AATTCGTCTCTAGCCAGGCTAGGAGGAGGAGGAGATAACCGCCGGTGGAGGCTTCCCCATT
GGGTTACAACGACTTAGACATGTGGTTCTCGCAGTACCATTGAACCTGGACCTCCCTTCACACA
GCCCCCTCAATCGTGGGAACTGAGGCGAACAGAGCTTCTAAACCCACCTCAGAAGTCAGTGAG
TCCCGAATATCCTGGGTGGGAATGACTAAGACACACACACACACACACACACACACACACA
CACACACACACACAGTAGGAAAGGTGATTTCAAGCACACTTTCTTTCTCCTTGGGGAGAATTAT
TGCTAACCATCTAAGTTTTCTGGAGGCGGCCTTTTTTCTCCCAGCCTCCCGGCGGGGTACC
CTCTCCCACCTTCCAGGAGAGTGGAGGACCCGTGAGATACGGGGCACGCAGGCAGCGACTTC
CTGAAATGCTAACAAGGATCGTAGGATCAGTTACTGCTGCGAGGAGCAAGCACTTGCTTCTTGG
GGGAGTTTTGCAGCCAACAGGGAAATGGGCTTTCTTTGTGAGTTAGAGGTAGAGGTCCGGCGG
CCTGAGTGATTGAACTGCTCGGGACAATGCTCGTATGTTTAGCAAACGACAGAAGTGTAGAAC
TGTTCTGAGAAATCCCAACTGATAGTATTTTAGTCATCTCAGACGACAGTTAGCACAGTTTAAA
AATGAGGCCTACTTCTTGAAAAACAGAATCCAAGGTAGTTTTGTCTCACATTGACAAATGTTGA
CACAGCCAGTGTAATTTCTATAACCAGGAAACTGAAAGAATATATGTACAGTTAAAATATGTA
CAATGCTAATTAACCTTGTGTAATAAGTCTAAAAGTAATTTAATGAGGCTTCACTTTTATGACCG
TCCTTGTGGTATGCTTCGCCAGGAATATATAGCTTCAAAAAGCAAAGGCCAGCGGAGGGGTAAT
TATTTTTTTACTGCAATGTTAATTGTCTCTTTGACATGGAAATATAAACCTGTTAAACTATCAGT
GTTTAATTTAGTGTCTCAATTTCTATTAGCAAAAATTTATAATCTATAGGATAAATGCACATTTTAT
TTTTTACTTTTCATATTATGCAAGTTAATTTTTTTAATTTAGTCAAAGGAGCTTATAAAGGATTTCA
GGCCTGTTGCTGGATTTGATTTTAATTCATTTTGAACATTGACAAGACCCTGGTTGTTGTTTT
TTTAAACAGT

SUBSTITUTE SHEET (RULE 26)

FIG. 12 CONT'D

CACAGCACTTGGCGCCCTTCTTGGGATCTCTTCTCTCCGCCCCCACTACCCCACCCCACAAGC
ACACTCTAGTCCCCTCCAATCAATTTTCAGGCAGGTCTCGCCGCCTCCGGAGCCACGCTGGGGG
TGCAAGGGCCCTGGACCCGAAAGAGCGCCCGCCCGGCGACAAGAGATGAGATGCACGCTGCT
CCTCCACTCCTCAGCCCCCACCATCCTCCTCCTGGATCCTAACTTCCCCACTCTCTCAATTCCT
AGAGACGCTGCGGATCCCAGAGGCTTAAGTGGCAGCTGGAACGAGGTCTCCAACAAGAATTT
AGACGCTAGGTCCAATTATCACTCCACCGCGCGCACTTTCCGCAGGAGCGATGTGATCCGTTA
TCATAACTGCGGACCTGGGGTTCCACGTGGAAGACGATTGGGATTTCACTGGCCGCGGTGGG
GGTGGGAGCAGACAGAGTCTGAGTGGGGTTAGTGGACTCGAGACGAAAGGCAGGACATGACA
GAAGGCAACTCTGGGTACCTCTCCAGCTTGGAACTGGCTAGGCCTTGTTTTGGAGGGGATGG
GTAGATGAAAAGTGAGTCAGGGTTACCCGGAGGAACACGGGGAAAGTGCGCTTCTGAGACTC
TTGACAGCCATTTTCGTTCCCTTCCAAGCCAGATGGAGACCCAAGAGTGTTGAAAGGCCACGAC
TTCCCTCAGTTTCTCCATCTGGGGGTGCAGGATGGTATAGAGAGTGGCCCGTAGTATTTTTTCCA
GTGACGATGTCTCTCATTGTTTTCTTCTTATATTGCAGCTTTCCCATGTTTGAAAATTTTCTTT
TCAAATGAAATCATTGATTAGAATAAAAAAAGTAAGTAGCTATTAAAACAAGATCAATTTCCATG
ACAGTAAGCCAACCGATGGAGAAAACCTTGGGAATTAATAAATGAAGGATTTGTTTGGTAGATG
ATAAAAGGTCTTTTTAAAGGGTCTGACTCTTCCTAGAAAAACCCACCAACTTGGGACCGCAACA
GATTTACCATATCCTAATTCATGCTATTTTAATGTGTATTTCAGCAAACCCACATGTGTTTACAATT
GTCGAAGCTACCAAATGTCAATAGCGTTTTTTTTCTATTTGTTGAATGTGAATCTCTTGACGAA
GCCATATAAACAGAAGAAATTACAGGAATGATTTTAAATCACATACAAAACCAATAGTATTGCTA
GAGGAGAGTTAGTCAAGGACGGCATTATGAAGAAAGTGAGGGAGAATTTCCAAAGAGCAGAAC
GATAGGGCTTGGTGGACCAAAGAACGTTTCCATCTAAAGGGAATGGCAAATACTTAGAGTCTCT
GAACCCACTGAATCTTGGACTATTTAACTAATATTTGTAGTTCCAGATATAGCACAGTGCCTTGT
ACATAGTGGTATTTTTTAAAAATATAGTGCCTCGTAGATTTTTTTTCAACTTTTATTTAGGAGGAGA
GGGCACATGTGCAGGTTAATTACAAAGGTATATTGCACCATGCTGAGGTTTCGAGTACGACTGA
ATCTGTCACTCAAGTAGTGAGCACAGTACCCACAGTAGGTAGTATTTTCAGCCCTCGCTCATTCC
CTTTCTCCTCCATCTAGTAGTCCCCAATGTCTATTGTTCTCATATTTATGTCCAATTAGCATTGT
TTTTTAAAAAGGGTGGTTGAAGAAATTCTCAGTGCTTGTCAGTGTCTCTCAGTGCATTCAATTAA
TTCATGAGCCCTGGAATGATGGTTTCATTTGGGCAGAACTCTACAATCAAAAAGAAGTAATAAAA
GGGAAAAAAGTGAAGCCATCAACTACAGGATTGAAATTCCCAAAGCATCAGAGGTCCTTTC
AAAAAATAGTATGTTGATTTTTAATTTTTATGACTTATTGGCTTTGTTTCATGAAAATATAACATGT
TATCACAAAGGATTTTTTAATTCAACTATTTCTCAGTTTTCTCTTTCACCTTCAAAATAAAATATCA
TAAATTATTTAAATGGTTGTGAAGGCAGTAGGATTTTTTTAAGAGAGAAAAGTTTTATAGAGGTTT
AGAATTACATGAACAAAGACATGTAATCTCTTAAGCAAATTGAAACTAATAAAATCGTACAATCAA
GGTAACGTAAATAAAAAAGCCTCTGCTTCTTAATTGAATTATGTGAGTAAGTAAATTTTAAAA
GTATGGCAAAGGTTAACAACAGCATTATTACCTGGGCTGCCTTTAAAAATACATATTTCTGGGGT
TCACGTTTCAGAAAATTTGATTCAGATTTGCTGTGGGTCCCAGAAATCTGCATTTTAAATAAACAC
TTGAAGGAGATACTAATAACAAGTGGCCCATTTGGGACACAATTTGACAAATATGACCAATTTTACT
TTTTAAACCTTATTTCTGCTTCTTTATCTTTGAATTGAGGTCCAGGATTTTAGGTAAGATTTTAAG
TTTAGAGTCAGTTTACTGGATCCCAGGGAGGAGAGTCTGAGTAATCAGTGGAGGAGTTATTTCA
CCAAATGAAGGAGACCCTTTATTATTATGTGACCCTTTGTATGAATTGGAAAAGAATGTCTTGTA
GATACCA

SUBSTITUTE SHEET (RULE 26)

FIG. 12 CONT'D

GTTACTTATTTAATCAAAGTTGATTATATTTTCATCAAGAAGTTGATTCCCTTGAGTGGAGTTGAAT
CACATATCAGGTGAAGAATGTGATTTGGGGAAGAATGGTCTAACACAAGAAAATTTTCTTGCAAT
CTTTAATAATATCAGAGGGGAGATTGGCTTCAGAACTCTCCTAAGTTCAGGAAAGGACACAGAA
AATTGAACATAACAGTAAGACTATAGAGTCCCAAGAAAGCAAGCTACTTTTAAAGGATAGTTTTT
TAGAGGGGGCAAAGGGGGGACAACCATTCTCCATTTGATGAGAAAAGCTTCCATGTAGATGGTG
CCCCTGAAATTAGAGTATCCTAAACCAGTGTTAAACCTATCAGTGAAACATGAATATTAACCTC
CACTCCCAGTAGTGAAAACCGAATACATTATTATTTATCTGTGACTTTCAACATTATCTCAGAACT
CTAACAGCACATGCGTACATCAGCAGCATAAGCAGAAATGAGATATTATATATGCTTGTGTTAGC
AATTAAAAAGGACAGCATATTTGAGAGGGGAAAATCTGTCCTATCAAGAATGAAAAAGAGGGAG
GTTAGGAAAAGTAGTTTATAGAGAAAGTAAATTTTGCAATTCCTCAGTTTTAACTGTAGTTTCTCCAT
TGTACCTTCCACTTGAAATGCACTCCAAGCAGTGGAGGTGGGTAGCAATGAATGCAGAGGAAA
CACTGAACACAGTGCACTCTCCAGTGTCACTTCTCATGATTTAATGAGGGGTTTTTTTTTGAAA
TTCTTCTGTCATAACATGGGAACTTTGTTACAAAGAAGCTGTTTTTTCAGAGGGTTAGAATTCA
GAGGTAGCATCATACCTTTTAGAAGAGAATTTGCTTGTGAAACCACAGATACCTGCTAGAATGT
ACAGGAATTAATGAAAAATTACTCAAAGGACATTTATTTTGATGACCTAAATGAATAACTTCATA
GTAAATGTCATATATATTCTCAAAAAATTAAAAAGCACCATTTATTGAGAGCCTACCGTGCACCC
GGATTTTTATATATCTGACATTCTTTATTCTCACAGTAACCTTATGGGGTAAATTTTATTTCCC
CACTTTGTGAGGTGAGGAAATAAAGGCTCAGAAAGTTTACATAACTTATTCAAGCCCACAGAGC
TGGTAAATGAGAGGTCAGTTCTATCTGAGTTTAAAGACTAGGCTTGTCCCACTTGCATATGTGTC
ATTTCCAAAATTATGATTAAGGATATGGTTGGCATTTCCTGCCACCCACATTAAGTCCAATTAAG
TAGCTGTGGCCATAGAAAGAATGGAGAATGGAGAGAGGAACTGACTTCAACAGCTACAGCAAA
CATTTATTAGCTGAGTAACCATAGCTACATAGTTTCTCAATATGTACCACTCCTCCATTTTGTTAT
CTATAAATCAAATGGTGGCTTTTTTAAAAAGCAGTTTTACAATATATTCAAGAGCCTTCTACCCTT
TGAAAACTGCAATACTATTTTTAGTAGCAATTAGAAACACCTTAAATATCTGACAACAGGGACA
TCATTAAGTAAATTATAACTTTTTCCAGTGGGATGTGTTACAGCTGTTAAAAAGTAGCATTTATGAA
GTGTTTTTGGAGAAGTTTGGAAAATGCTGTAATAAGTTAGAAAAAGCTCATTTCAAATTCGATA
ATATTCACAATGTAAAGATTAAGCAAAGAAAAAGGAAGAAGTATTTCAAATGTTAATAATTATTG
CTTTGTGTGGGGTAGTTTTTCATTTTCTATGTGCAGCTAATTCCTTAATTATTTTTAAATATGTGA
GCTTTAATCAGGAAAGCAAATCATTCAAAAATGAGGGGACTGAATTAAGTGACTTTTCAGGGGAC
TTTGCGTGTCTTTGAGTTCCAAATTTCTATCACTATGTATTACTACTGAAGAATAATCATAGAAGC
ACAGTAGTTTCTGAAAATGGAGAGTCAGTAATCTTGGCCCAGGTTTTGCAACTTGCTCTAAAGC
AGAGTCCTCAAAGAAAAGGAAGCATTGATGAGTTGTCCACAATGTACTGGATAAATTATCATTAG
GAAAACATATTGTAGTAGGGAGAGTGAGGACCTCTCAAACAGAACTGAGAACCTTAAGTTTGAA
CTTTTCTTTTCTTATTACTTAAGCACTCTGAGCTTTTTTTTTTGTCTGCATTATGAAGAAAGAATA
ATACTCTCTATCCCATGGGACAGCTGTGGAATTATAAATTACACATATAAACTGCTTGATGCTT
GTCACATAGCTGGGGGTTGAAAAAATGATAGCCATTATTTTCTTGGCACTTTTAATGAATTTTTT
ATTATCTCTATTTCTTTCTGCCTATCTCCTCTAATTATGTTTATTACTTATTTTGTTCCTCAGGATG
AGGTCAATTCTCAATATCTGTGCTGTACATAATATACATATATACCAAATATGTGCATATAGTATG
TACATACATACATACTGTGCTAATCTTTTAGTGTTCTCAGCTGATCAAATAGCTACAAATAGATAT
AAGTAATTCGCCACAAGTAATTTATCAACATAAAAAAAATTTACAAAAAAGTTAAGGAATAATTGT
CTCCA

FIG. 12 CONT'D

TGAGCTGCAAAGATCCCTCATTTCACAAGAGTACACCCTAGAGATATTTTAATAGTAAATTTCTC
ACATAGATTTAAAATCACATTTGTTTTGCACATAATTTAGAAAAGATACCTGCTATATAATAAGTA
ATATACTTTTAAGTTTTCTTCAAATATTCTTGGGAAGATGATAATAGGTACTGCTAATTCTATAC
CCAGTTAACATTTTGGAACTAAGGTTGAAAATTGTGACTTAACTATAATTATGCATTAAATCTAC
AACACATCAAAGAATTTTGCATTTTGTACTCCTTACTAAGATCCAGTTTGAGTAGGAAGATAAATT
TTACAGTAATTCTGAATGAGGGAAGTTGGCACAGAGTTTCTAAAAGAGTACCTTCCTTATAGCAA
ATACTAAATAATTGTGCTATATTGAATTTAATTAAATAGAGAATAGTAAAAGGGAGAAAGAAACAT
CCAATGTTTTGAACTTCTAGAGATCTACTCCCAGGGACACATTGTTTTTCTTAGCAAAATCTGTT
TGGAGGTCTGCTCTACTTTCTCAGAGGTCTCCCTTTCATGCTGAAGCTATCTTTTTTCTTGTGG
AACATAAGTAATTAAATACCTTGCAATTATTTACCTAAGAAAGTGTTTCTTCCCGTTTTAAATGC
TCTTACCACCCACATTGGACTCGATTATCAGAATTTTTATCCGGGGCAGCTTCAGGAGCACTTT
GGCACTTCGGGGCTAAACCACAATCTGTTTTTACATGTTTGTGATTATACCCGTTTTGTAGATCA
AGACATTGAAGCTAGTAAAAAAGTCAATTTTTTCAGGGTAACAAAGTAGGTGGTAGAA
CTAGGACAGGGACTCTAATTTCTTACATTATTGCTTTTCTAAATTAAGGGATGCATGGAATTA
TTCTCCATTGCCTTTGCCTTCAAATAATTATCTATTGCACCCAACATCCTATTCTAGAACTCATC
TATGAAGGCTTAACACAGCTGTACCTGGGAGCTCCATTACAGGGCATATATCTCGCTCTCATAA
GCTACTTCCTAAGGAATTCTCTTTAATTATGGGAGCTTTTCCAGACTCTGAAATCTTTTTTCTG
GTAACACAAGTGTGAGGTGTCAATTTATCAGAATGCATCACCCAGTCTTCCCTCCTCAAATGATT
ACTGTAGGCTCCACTCAAGAGCTCATCCCAGTTCAAGACCACCTTCTCCTCCAGAGAAGCAAA
TATATATATACACGTATATATATATATACACGTATATATATATATACACGTATATATATATACAC
GTATATATATATATACACGTATATATATATATACACGTATATATATATATACACGTATATATATATACA
CGTATATATATATACACGTATATATATATATACACGTATATATATATATACACGTATATATATATATAC
ACGTATATATATATACGTGTATATATATATATACATTTTTTTTTTTGAGACGGAGTCTCGCTCTGT
TGCCCAGGCTGGAGTGCAGTGGCGCGATCTCGGCTCACTGCAAGCTCCGCCCCCGGGTTCA
CGCCATTCTCCTTCTCAGCCTCCGGAGTAGCTGGGACTACAGGTGCCCGCCACCTCGCCTGG
CTAATTTTTTGTATCTTTAGTAGAGATGGGGTTTACCGTGTTACCTAGGATGGTCTAGATCTCC
TGACCTCGTGATCCGCCCCGCTCGGCCTCCCAAAGTGCTGGGATTACAGGCGTGAGCCACCG
CGCCTGGCAGAGAAGCAAATATATTGATGGTTGTTACCAATACATGCTCTTGACTAAGAAACCTT
CTTTCTTAATTAATATTGACAACTTTAAGCCGAGTGCCTGACATATATTAGGTACTCAGTTACTCT
TTTTCACTAAAGTTATGAATGATGATTCTAATAAAAGTAACTTATTTGTCTACTAGTTTTATTATG
TTTATTTAATTCATTAGAAAGGCCATGGACATAGTACAAAATTCAAACAATATAAATCATGGAATG
TGAAAAGTAAGTCACATGCCATCCCAGTTCTTCATTTCTTACCTCACAGGTAACAGCTTTTCC
TGTATCTCCCCAGAGATATTCTATGTATATTTTGTTTTAAACCAAGCTATATTTAAACAATTAT
CTTTAATAATAATGTTAATATTGAACTGGTAAAGAAATATGTGTGTATTATCTCACCTCAAGCGT
AAACAATAGAACAAAGAGAGAGCCATTTTGAATTTATGGACAATGAATCTAGAAATAATCTCAA
AAGATTTTGCAGTCAAAAATAGTTCATTAGATACATGAGAACTGTCACTTGGTCTCAGTGTAGA
GCTATTGCCTCAACTCCCTTTATTTTCTAACAAAATCATCTTGCTTATCCCATGAAATACGTGCA
TATTGCCAATCCTACAATGCCGCATCAGAACCAGAACCAACTCTGGAACACTACCTTCTCAAG
TATCTTTCTGTCTCTTTATGGTAATATGTTGAATTAATTCACATCTATTATGACTAGTCTTTGAT
TTGTAGGGTTGCTGAAGTAGTAGCACCCTGCAGGGCTTCTTTAGTTTAAAGAAAGTAATCAG
GTGT

FIG. 12 CONT'D

CCCTACTGTGTCATGATCTCCACCCTCAGCTGGGTTCTCCAGTCTGGTTTTAAAGAACAAAACA
AAAGGCTTCTCTGTCTGAGTCTTACTCAACCCATCCTCTCTACTCATAAGAGGTATTCCAAACCT
TTACGATTCTCAAACCTCCTAACCGACCATCTTATTTTCACTCTGCAAACAAGCTAACCTCCTCAT
TCATAGAAGGAAGTGCCTCAACTTCCTCCCCGTTCTGACCTTTTCTCCCTCCCAAATCTATGTAT
CTCTTGTGACAAAATCTATAACCACCGCTGTACTTTGAGTTCTATTTCTTCATTATTTTTGAGGGA
CCTCAAGTCCTCAAAAATATCCTATCTTGCCTGTGTACTTAACTTTTCTTTTATTCTTTTCTAACTT
TCCCTTCTCTTCACTTGGCACTTGCCCTTCCAGGTATATGTGTGCTCAGGTCTCCTCCACCTTC
CATCTGCCTCACTTCATGGCATAGGGCCTTGAACATCACAACCAAGCTATGAAAGAGTAGTCA
ACGCAGTGTCCCCACTTCCTTGCCATCCCATTATCCTAGTTTTTCTTTTGGCTCTCTGAGGAGTC
CTTCACAGGCTGGTTTTTCAAGGAATAAGTCTAAATGAATCACTTTTCAAGTTTTCTAAACTTCTATG
CCTTTGCACATCCTCTTACCTCTGCCTAGAATATCTTTCTCCTTCTTTTCCATCTTTAAACTCTCA
CATCATTCTTCAAGACTGGGATCAGCTCTCAGCATCCGGAAGCCTTTGCCTACTAGAGACAAAT
GAGAATGAGTTTGGTCACCTTTTCATTTTCTTGTATCATTCTGTGCTTTATTTTGTCTTTCTAAGA
GCGTTACATGCTTCATTTAATCCCTAAACAACCTGTTTGAGGCAAGTACAGTTATTATCCTAATCAT
GCAAATGAGAAAACAGAGGCCAGACATGTTGAGTAACTTTGATAAAAGTTAAAGAACCAATAA
GTGGAACAGTTGAGGTTTGAACCCTGGCAGTCTGACTGTAGAGATACTATGTTTGACCTACTCC
CCTCTGCCCCCACCCTCATGTCTGCCCTTAGTTTCTGAGCTTGTTGAATGAATGAACAGGTGGTA
GTCTTTTTTTGTTATAAGACTGATCAGAAATTAAGACAGGTTTAAATTTACGTGTAGAATTTTCAA
AACTGCAAAGGCAGTGCAAATCTAAAAAAGAATGGCATTCTCAGGAAAAGAGGAAAAGTAAGTG
TGAGAATAATAATAACAATAACCAACAAACTTTAGTAAATTTAGTAAATGTAGTAAATTTTTACATT
AAAAGCTTTTGGACATACATTATCATATTTTATGGCCACATGAAATATATTATAATCCCATTTTGC
ACATAGGAAATCTGAGACTGGCATAAGGAGCACAGAGATCCAGGACTTTATATTTTCACTTCTCT
AGGATTTTGCACCTCAGGTCGATATGTATGAGTAACTGGGAGTATAATGGGCTCTTTAACAGA
AAAAGTGGAAAGTTTTCCCACTATTATTAATTATTTACATAATATTTTTTTAATTTTATTATTATTT
ATACTTTAAGTTTTAGAGTACATGTGCACAATGTGCAGGTTTGTACATATGTATACATGTGCCA
TGTTGGTGTGCTGCACCCATCAACTCATCTTTAGCATTAGGTATATCTCCTAATGCTATCCCTC
CCCCCTCCCCCTACATAAGATTTATAATGGATAATGGACTTCAATTTCTAGAGCAAAATGGCCC
CACCCAAGGATGCCATAATCCTTCCAGAGCTCTACTGCAAGATATGAGATATACATATCTAAAC
TTGTTCTTGGTATTTCCAAAGCAGTCAACTTTTACACCTGTTTATAATGCATCCAAATGTTGTTTT
TATATGGTTGCATCTCCCATCTTCTTACCAATAGCTATATATATTTTTTACAAGAGCTGAAAGAG
TTCTTGATGTAGGAATCCATGGTAGAGTTTTCAGAGAAATCCCTGAATTCAGTAAAAGTTTTATCT
AGAAATACATGTGCAAGTGAACACATCTTTTTTAAAAAAATCATTACCTACTTTCTTTTTTGAAGA
AGAAGGTATTTATTTCAACAGACTCTTGAAGGAGCCTACTCTTCCCACTCTCCCACCCCCATTAA
GAACCACTGTAGGCCGGGCACGATGGCTCATGCCTGTAATCCCAGCACTTTGGGAGGCTAAGG
TGGGTGGATCACCTGAGGTCAGGAGTTCGAGACAAGCCTAGCCAACATAGTGAAACCCCGTCT
CTACTAATAATACAAAAATTAGCTGGGTATGGCAGCATGTGCCTGTAATCCCAGCTACTCGGGA
GGCTGAGGCAGGAGAATTGCTCGAACCCTGGGAGGCGGAGGTTGCAGTGAACCGAGAGAGATC
GTGCGGTGCCATTTCACTCCAGCCTGGGCAACAGAGCGAACTCCATCTCAAAAAACACACA
AAACAAACAAACAAAAGAAAGAACCATTTGATTAGTGATGGAAATGTGTTCCCTCCCTCCCATC
CTGGCAACCACTTTCTTCTCCTCCATCATAAAATATCTTAACTAACTAAAATAATTTTATTTAT
CGATAGT

G

FIG. 12 CONT'D

GTGGGTAGAGGGTCTGCAGCGGGAGCAGGGGATGGCGGGCGACTCTGGAGGACGAAGTTTG
CAGGGGAATTGGAATCAGGTAGCGCTTCGATTCTCCGGAAAAAGGGGAGGCTTCCTGGGGAG
TTTTCAGAAGGGGTTTGTAAATCACAGACCTCCTCCTGGCGACGCCCTGGGGGCTTGGGAAGCC
AAGGAAGAGGAATGAGGAGCCACGCGCGTACAGATCTCTCGAATGCTGAGAAGATCTGAAGG
GGGGAACATATTTGTATTAGATGGAAGTATGCTCTTTATCAGATACAAAATTTACGAACGTTTTGG
GATAAAAAGGGAGTCTTAAAGAAATGTAAGATGTGCTGGGACTACTTAGCCTCCAATTCACAGA
TACCTGGATGGAGCTTATCTTTCTTACTAGGAGGGATTATCAGTGGAATCTGTGGTGTATGTT
GGAATAAATATCGAATATAAATTTTGATCGAAATTATTCAGAAGCGGCCGGGCGCGGTGCCTCA
CGCCTTGTAATCCCTTCACCTTTGGGAGATCAAGGCGGGGGGAATCACCTGAGGTCGGGAGTTC
GAGACCAGCCTGGCCAACAGGTGAAACCTCGCCTCTACTAAAAATACAAAAAGTAGCCGGGGG
TGGTGGCAGGCGCCTGTAATCCCAGCTACTCGGGAGGTTGAGGCAGGAGAATCGCTTGAACC
CGGGAGGCTGAGGTTGTAGTGAACAGCGAGATGGAGCCACTTCACTCCAGCCTGGGTGACAG
AGTGAGACTTTGTGAAAGAAAGAAAGAGAGAAAGAGAGAGAGAAAAATTATTCAGAAGCAACT
ACATATTGTGTTTATTTTAACTGAGTAGGGCAAATAAATATATGTTTGCTGTAGGAACCTTAGGAA
ATAATGAGCCACATTCATGTGATCATTCCAGAGGTAATATGTAGTTACCATTTTGGGAATATCTG
CTAACATTTTGTCTTTTTACTATCTTTAGCTTACTTGATATAGTTTATTTGTGATAAGAGTTTTCA
ATTCCTCATTTTTGAACAGAGGTGTTTCTCCTCTCCCTACTCCTGTTTTGTGAGGGAGTTAGGGG
AGGATTTAAAGTAATTAATACATGGGTAACCTTAGCATCTCTAAAATTTTGCCAACAGCTTGAAC
CCGGGAGTTTTGGCTTTGTAGTCCTACAATATCTTAGAAGAGACCTTATTTGTTTAAAAACAAAAA
GGAAAAAGAAAAGTGGATAGTTTTGACAATTTTTAATGGAGACGGGAGAAGAACATGTAGAAAA
GGGGAAATGATGTTGGCTTAGAATCCTAACTACATTGGTGTTTAATATAGGAACATTTATTTATAT
AACATTTTAAAGTACTAAATTCATATTAGTATATTATCAAATGGATATATTATCAAATGGGTTTAAG
CATCCTACACATTTTAATTCAATTGATTCATTTTCTTTTTGCTTTGGATTTCTATCATGATTTAAAT
ATTTACATATGGGTACTTTTTAGATTTTTCTACTATGAAATATAAGAAAAACCTTTAAGGCTAG
TTTTATGACCAAGACGAAGGACTTCATTGAATACACAAAACAATAAATACTGCAACATTTTGTCT
TTTCTTTTTGTAGCTGCAATTTGGTTTGCTTATACTTTCTCTTTGTCTCTTTGAAAACCTGAGTCAG
TTTCACTTTCTCAGGACAGGATTTAATAACCATAATATAATTTAGTATAATTCCTTGATTTAGGCA
AATTATGCAATTTGTGTTTAGTATGAAATGTACCTAAAAATAAGTAACTCCTCTTTAACACCACCA
TCCTCAAATAATATAACAAATAACAGTTATCCTAAAATAAATTGTCTACTTCCACCATGCAGCAC
TCAAATTTTAAGGTTGCTATGACTGCAGACAGTATTTTAAATTCCTCTCTGGAAATGGCTTTGTT
TCCAAGATGATTTAGGAACCAAAGAGGTGACCATCTCTTGTTAATGAACTCTCAAATCATAAAC
CTGGGAAGTGTTTTAGTTTCTACTGCTGCTGTTACAAATTATCACAAATGTGTTAGCTAAACA
AACACAAAATTATTATTTTACAGTTCTAGAGATCAGAAGTCAAAAATGGGTCCACAAGGTTTCAT
TCCTTTTGAAACTCTAAGGGGCAATCTGTTTCTTGCTTTTCCAGCTTCTAGTGACCATCAAA
TTCCTTGGCTCATGGTCTCTGTATTTTCTCTGTGGCCTGTGCTTCCATTCTTGATCTTCTCTCT
GACTGTGACCCTCTAATAAAAAACACTTGGGGTTATGTTGGGCCCACCCTGAAAATTCTGGATAA
TCTCCCTCAAGACCATTAATTAATCACATCTGCAAAGCCTCTTTTGCCACATAAGTTAATGTATT
AAAAGTTTTTGAGGATTAGGACATAGACATTGGGGGTGGGGGGGCATTATTCAGCCTACCACA
GGAAGGAATTTTAGGGTTAATTAAGTAGCCTTCTTATTTTATACTTGAAGAAATTGAAGTTTTGG
AATTGGAGAGCATTATGCTAAATGAAATAAGCCAAACACAGAAAGACAAATATCACATGTTCTCA
CTTATCTGT

FIG. 12 CONT'D

GAAATATAAAACAATTACATTCTTAGCAGTAAAGAGTAGAATGGTGGTTACTAGAGCTGGGGGG
TGGGAGGAATGGGGAGATGGTAATCAAGATATAAAGCCTCAGTTAAGATGGGAGGAATAAGTTT
GATTGTTTTTTTTGAGATGTGTTTCATAGCATGATGAATATAGCTAAATAGTAAATCCCAAATGCT
CTCATTGACAAAAATGTCAAATATTTGAGATGATGGATAGGTTACTTAGCTTGACTTAATAATTC
CCCATTGTGTTCAAAGATCATAACTTCATATTGTACCACATAAATATATACAACGTACTATCCCA
ATATATAATTTTAAACTAATATAATGAAAAAGAAATTGAAGTTCAACATTCCCAGAAGCTAAGTG
TAACTTAAAAGTTTTGTGAGAATTTGTTTTAACAAACAAACAAGTTTTCTTTTTTAACAATTACCA
CATTCTGCGCTTGATATACAGCAGTGAACAAAAAAAAAAAAAAAAATCTCCAGGCCTAACATAA
TTTCAGGAAGAAATTTTCAGTAGTTGTATCTCAGGGGAAATACAGGAAGTTAGCCTGGAGTAAAA
GTCAGTCTGTCCCTGCCCTTTGCTATTTTGCCCGTGCCCTCACAGTGCTCTCTGCCTGTGACGA
CAGCTCCGCAGAAGTTCGGAGGATATAATGGAATTCATTGTGTACTGAAGAATGGATAGAGAAC
TCAAGAAGGAAATTGGAAGCTGGAAGCAAATGTAGGGGTAATTAGACACCTGGGGCTTGTGTG
GGGGTCTGCTTGCGGTGAGGGGGCTCTACACAAGCTTCCTTTCCGTCATGCCGGCCCCCAC
CCTGGCTCTGACCATTCTGTTCTCTCTGGCAGGTCATGATGATGGGCAGCGCCCGAGTGGCGG
AGCTGCTGCTGCTCCACGGCGCGGAGCCCAACTGCGCCGACCCCGCCACTCTCACCCGACCC
GTGCACGACGCTGCCCGGGAGGGCTTCCTGGACACGCTGGTGGTGTGACCCGGGCGCGGGG
CGCGGCTGGACGTGCGCGATGCCTGGGGCCGTCTGCCCGTGGACCTGGCTGAGGAGCTGGG
CCATCGCGATGTGCGACGGTACCTGCGCGCGGCTGCGGGGGGCACCAGAGGCAGTAACCAT
GCCCCGATAGATGCCGCGGAAGGTCCCTCAGGTGAGGACTGATGATCTGAGAATTTGTACCCT
GAGAGCTTCCAAAGCTCAGAGCATTCAATTTCCAGCACAGAAAGTTCAGCCCGGGAGACCAGT
CTCCGGTCTTGCCCTCAGCTCACGCGCCAATCGGTGGGACGGCCTGAGTCTCCCTATCGCCCTG
CCCCGCCAGGGCGGCAAATGGGAAATAATCCCGAAATGGACTTGCGCACGTGAAAGCCCATT
TGATACATTATACTTCCCAAAGCATACCACCACCCAAACACCTACCCTCTGCTAGTTCAAGGCCTA
GACTGCGGAGCAATGAAGACTCAAGAGGCTAGAGGTCTAGTGCCCCCTCTTCCTCCAACTAG
GGCCAGTTGCATCCACTTACCAGGTCTGTTTCCTCATTTGCATACCAAGCTGGCTGGACCAACC
TCAGGATTTCCAAACCCAATTGTGCGTGGCATCATCTGGAGATCTCTCGATCTCGGCTCTTCTG
CACAACCTCAACTAATCTGACCCTCCTCAGCTAATCTGACCCTCCGCTTTATGCGGTAGAGTTTTCT
CAGAGCTGCCCCAGGGGGTTCTGGGGACATCAGGACCAAGACTTCGCTGACCCTGGCAGTCT
GTGCACCGGAGTTGGCTCCTTTCCCTCTTAACTTGTGCAAGAGATCGCTGAGCGATGAAGGT
AGAATTATGGTCCTCCTTGCCCTTGCCCTTTCTTTTTGTGATCTCAAAGCATCCTCCCTCCGCCC
CCATTCCATGGCCCCAGTTCCCTACTCCCACAGCTGTCTGCTGAAACTGCCAACATTACTCAAT
TGTTTTCTGGGGGGAGGAACATTTTTTTTTGAAACAAAATAGATATATGAAACAGTACACGGGAAT
TAACACGAATATTTAAGGTAAAACATGACCTTGAAGATTATGAAATCCATCTTATTTTGGCCCAG
AACGGGGGCATTGGGCTCCTTGGGCCATAGGGGAGCTGGGGAGGACAGGGTGAAGAGTTAG
CTCTAAGCCCTCTGCTTGGAGATGCTGTAAATACAGAACGCAAAATCACCTTCGAAGTTAAAGA
CGCGAAGTTCTTCTTTACTCGGCCCTCCTCCCCTCCCCCCCCGCCAATCCCTCCAGTTACAGC
TAGCATCCAGGTCCCGGGAGGTGAAGAAGGAGACTTCGGCTCCAGTTACAGCTAGCATCCGG
GTCCCGATTTAGAAGGAGCTGCCAATTACAGCGCGGTTCCAGGGCTGAGCAAAAAGCCTGAGG
AGCCAAGTGGGAGAGGGAGTAAACTACTGAATTGGGCCACAAGCAAATGAATAAACTGAACG
ACTCTTAACCAAACCTAATATATTTAATCCAAACACACAAGTCTTTCATTTCTTCCCTCCTCCCT
CCTT

FIG. 12 CONT'D

CTCTTACTCCCCAACACCCCCTCTTCAAGCACAAATTAATTATATGGTTAGATTCTACTGCGTGAT
CAGCCCTGTTCTAGGTGGTGGGCACGCCAAGGTGAATGAGACCAAACAAGAGTCTTGCCCTCA
TGGGGTTTACATTTGGAGACAGAGTCGATCTGTTGCCAACCTGGAGTGCAGTGGCGCGATCA
CAGCTCACTGCAGCCTCAAACCTCCCTGGCTCAAGGGGTTCTCCACCTGAGCCTCCCGACTAG
CTGGGACCACAGGTGCACGCCACGACGCCTGGGTTTGTGTTGTTTGTGTTAATAGAGACGAAGGT
CTCACCATGTTATCTGGGCTCAAGCGATCATCCCCCTCCTCCTCTAAAGTACTGGGATTACA
GTCCCAAGCTATCTTGCCCGACCTGGGAAACAGACGTAAAGGAAGATAACAATCTATTTTCAGA
GAGCGAGTTTATAAAACCAATGCAATGGGTAAATATGAAGTGTGAATAGGAGGAGAAGCTAAAG
AGTGGTCGGAGAATCTAATGCAAGCTACGGGAGAAAGAACTCAAGTGCAAATGCTGCCTCAG
GAATAAACGTAAAAAGAGACTTTCAAGTGCAAATGCTCCCTCAGGAATAAAATAATCTTGAGACT
CTCAAGTGTAATGCTGCCTCGGGAGAACCGAACGGCGAGCTGGAGCCCATACGCAACGAGA
TTAGAGAGGAAGGCAGAAAGCCAGAGCACATGAATAAATGAGCATCCATTTTGTTCAGAAATGA
TCGGAAACCATTTGTGGGTTTGTAGAAGCAGGCATGCGTAGGGAAGCTACGGGATTCCGCCGA
GGAGCGCCAGAGCCTGAGGCGCCCTTTGGTTATCGCAAGCTGGCTGGCTCACTCCGCACCAG
GTGCAAAAGATGCCTGGGGATGCGGGAAGGGAAAGGCCACATCTTCACGCCTTCGCGCCTGG
CATTGTGAGCAACCACTGAGACTCATTATATAACACTCGTTTTCTTCTTGCAACCCTGCGGGCC
GCGCGGTGCGGCTTTCTCTGCCCTCCGCCGGGTGGACCTGGAGCGCTTGAGCGGTGCGCGC
GCCTGGAGCAGCCAGGCGGGCAGTGGACTAGCTGCTGGACCAGGGAGGTGTGGGAGAGCGG
TGGCGGCGGGTACATGCACGTGAAGCCATTGCGAGAAGTTTATCCATAAGTATTTCAATGCCG
GTAGGGACGGCAAGAGAGGAGGGCGGGATGTGCCACACATCTTGACCTCAGGTTTCTAACG
CCTGTTTTCTTTCTGCCCTCTGCAGACATCCCCGATTGAAAGAACCAGAGAGGCTCTGAGAAAC
CTCGGGAAACTTAGATCATCAGTCACCGAAGGTCCTACAGGGCCACAAGTCCCCCGCCACAA
CCCACCCCGCTTTCTAGTATTTTCAATTTAGAAAATAGAGCTTTTAAAAATGTCCTGCCTTTTAACG
TAGATATATGCCTTCCCCCACTACCGTAAATGTCCATTTATATCATTTTTTATATATTCTTATAAAA
ATGTAAAAAAGAAAAACACCGCTTCTGCCTTTTCACTGTGTTGGAGTTTTCTGGAGTGAGCACTC
ACGCCCTAAGCGCACATTCATGTGGGCATTTCTTGCGAGCCTCGCAGCCTCCGGAAGCTGTGG
ACTTCATGACAAGCATTTTGTGAACTAGGGAAGCTCAGGGGGGTTACTGGCTTCTCTTGAGTCA
CACTGCTAGCAAATGGCAGAACCAAAGCTCAAATAAAAATAAAATAATTTTCATTCATTCACTC

p16INK4a (SEQ ID NO: 8)

MEPAAGSSMEPSADWLATAAARGRVEEVRLLEAGALPNAPNSYGRRIQVMMMGSARVAELLLL
HGAEPNCADPATLTRPVHDAAREGFLDTLVVLRHAGARLDVRDAWGRLPVDLAEELGHRDVARYL
RAAAGGTRGSNHARIDAAEGPSDIPD

p14ARF (SEQ ID NO: 9)

MVRRFLVTLRIRACGPPRVRFVHIPRLTGEWAAPGAPAAVALVLMLLRSQRLGQQPLPRRPGH
DDGQRPSGGAAAAPRRGAQLRRPRHSHPTRARRCPGGLPGHAGGAAPGRGAAGRARCLGPSAR
GPG

FIG. 12 CONT'D

MDM2 cds (SEQ ID NO: 10)

ATGGTGAGGAGCAGGCAAATGTGCAATACCAACATGTCTGTACCTACTGATGGTGCTGTAACCA
CCTCACAGATTCCAGCTTCGGAACAAGAGACCCTGGTTAGACCAAAGCCATTGCTTTTTGAAGTT
ATTAAAGTCTGTTGGTGACAAAAAGACACTTATACTATGAAAGAGGTTCTTTTTTATCTTGGCC
AGTATATTATGACTAAACGATTATATGATGAGAAGCAACAACATATTGTATATTGTTCAAATGATC
TTCTAGGAGATTTGTTTGGCGTGCCAAGCTTCTCTGTGAAAGAGCACAGGAAAATATATACCAT
GATCTACAGGAACTTGGTAGTAGTCAATCAGCAGGAATCATCGGACTCAGGTACATCTGTGAGT
GAGAACAGGTGTCACCTTGAAGGTGGGAGTGATCAAAAGGACCTTGTACAAGAGCTTCAGGAA
GAGAAACCTTCATCTTCACATTTGGTTTCTAGACCATCTACCTCATCTAGAAGGAGAGCAATTAG
TGAGACAGAAGAAAATTCAGATGAATTATCTGGTGAACGACAAAGAAAACGCCACAAATCTGAT
AGTATTTCCCTTTCTTTGATGAAAGCCTGGCTCTGTGTGTAATAAGGGAGATATGTTGTGAAAG
AAGCAGTAGCAGTGAATCTACAGGGACGCCATCGAATCCGGATCTTGATGCTGGTGTAAGTGA
ACATTCAGGTGATTGGTTGGATCAGGATTCAGTTTCAGATCAGTTTAGTGTAGAATTTGAAGTTG
AATCTCTCGACTCAGAAGATTATAGCCTTAGTGAAGAAGGACAAGAACTCTCAGATGAAGATGA
TGAGGTATATCAAGTTACTGTGTATCAGGCAGGGGAGAGTGATACAGATTCATTTGAAGAAGAT
CCTGAAATTTCTTAGCTGACTATTGGAAATGCACTTCATGCAATGAAATGAATCCCCCCTTCC
ATCACATTGCAACAGATGTTGGGCCCTTCGTGAGAATTGGCTTCCTGAAGATAAAGGGAAAGAT
AAAGGGGAAATCTCTGAGAAAGCCAACTGGAAAACCTCAACACAAGCTGAAGAGGGCTTTGAT
GTTCTGATTGTAAAAAACTATAGTGAATGATTCCAGAGAGTCATGTGTTGAGGAAAATGATGA
TAAAATTACACAAGCTTCACAATCACAAGAAAGTGAAGACTATTCTCAGCCATCAACTTCTAGTA
GCATTATTTATAGCAGCCAAGAAGATGTGAAAGAGTTTGAAGGGGAAGAAACCCAAGACAAAGA
AGAGAGTGTGGAATCTAGTTTGCCCTTAATGCCATTGAACCTTGTGTGATTTGTCAAGGTCGA
CCTAAAAATGGTTGCATTGTCCATGGCAAAACAGGACATCTTATGGCCTGCTTTACATGTGCAA
AGAAGCTAAAGAAAAGGAATAAGCCCTGCCCAGTATGTAGACAACCAATTCAAATGATTGTGCT
AACTTATTTCCCTAG

MDM2 protein (SEQ ID NO: 11)

MCNTNMSVPTDGAVTTSQIPASEQETLVRPKPLLLKLLKSVGAQKDTYTMKEVLFYLGQYIMTKRLY
DEKQQHIVYCSNDLLGDLFGVPSFSVKEHRKIYTMIIYRNLVVVNQQESSDSGTSVSENRCHEGGS
DQKDLVQELQEEKPSSSHLVSRPSTSSRRRAISETEENSDELSEGERQKRHKSDSISLSFDESLALC
VIREICCERSSSESTGTPSNPDLDAVSEHSGDWLDQDSVSDQFSVEFEVESLDSYSLSEEGQ
ELSDDEDDEVYQVTYQAGESDTSFEEDPEISLADYWKCTSCNEMNPPLPSHCNRCWALRENWLP
EDKGKDKGEISEKAKLENSTQAEEGFDVPDCKKTIVNDSRESCVEENDDKITQASQSQESDYDYSQP
STSSSIYSSQEDVKEFEREETQDKEESVSSLPLNAIEPCVICQGRPKNGCIVHGKTGHLMACFTCA
KKLKKRNKPCPVCRQPIQMIVLTYFP

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/32748

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68 (2018.01)

CPC - C12Q 1/6886, C12Q 2600/12, C12Q 2600/106, C12Q 2600/118, C12Q 2600/118, C12Q 2600/158

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)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	US 2017/0081730 A1 (THE JOHNS HOPKINS UNIVERSITY et al.) 23 March 2017 (23.03.2017) (hereinafter 'JHU') para [0015], [0030], [0039], [0047], [0049], [0159], [0161], Fig. 6, Table 3	2, 4/2, 38 ---- 1, 3, 4/1, 16, 17, 20, 27, 28, 31
Y	MAHER et al. "Marked Genomic Differences Characterize Primary and Secondary Glioblastoma Subtypes and Identify Two Distinct Molecular and Clinical Secondary Glioblastoma Entities" Cancer Res, 1 December 2006, Vol 66, No 23, pg 11502-11513; abstract, pg 11504, Fig. 1, pg 11505, col 1, para 1	1, 3, 4/1, 16, 17, 20, 27, 28, 31



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

02 September 2018

Date of mailing of the international search report

14 SEP 2018

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/32748

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.: 5
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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-4, 6-26, 38, drawn to a genetic panel to diagnose glioma. The diagnosis of glioma will be searched to encompass a genetic panel for identifying W1 glioma. It is believed that claims 1-4, 16, 17, 20, 27, 28, 31, 38 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass W1 glioma. Additional genetic panels for identifying glioma subtypes will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected glioma subtypes. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a genetic panel for identifying M1 glioma (Claims 1-2, 11, 16, 23, 38).

- Please see extra sheet for continuation -

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 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.:
1-4, 16, 17, 20, 27, 28, 31, 38 limited to W1 glioma

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/US 18/32748

Continuation of:

Box No. III. Observations where unity of invention is lacking

Group II+: Claims 27-37, 39-54, drawn to a method of diagnosing whether a subject having a specific glioma subtypes. Group II+ will be searched upon payment of additional fees. The method may be searched, for example, to the extent that the glioma subtype encompasses W1 subtype for an additional fee and election as such. It is believed that claims 27, 28, 31, 39, 52 read on this exemplary invention. Additional methods for identifying glioma subtypes will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected glioma subtypes. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. Another exemplary election would be a method for identifying glioma subtype M1 (Claims 27, 34, 39, 40, 42, 46, 48, 53).

Group III: claims 55-57, drawn to a kit comprising primers, probes and stains.

The inventions listed as Groups I+, II+ and III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Groups I+ include the special technical feature of a genetic panel for diagnosing glioma, not required by Groups II+ and III.

Groups II+ include the special technical feature of a method of contacting a glioma sample with primers for detecting gene mutations, not required by Groups I+ and III.

Group III includes the special technical feature of a kit comprising reagents, not required by Groups I+ and II+.

The inventions of Groups I+ and II+ each include the special technical feature of a specific glioma subtype, recited therein, not required by any of the other inventions of Groups I+ and II+.

Common Technical Features

The inventions of Groups I+ and II+ share the technical feature of define molecular subtypes of glioma.

The inventions of Groups I+, II+ and III share the technical feature of IDH1/2, chromosomes 1, 14, 19, CDK4/MDM2, and CDKN2A.

However, these shared technical features do not represent a contribution over prior art in view of US 2017/0081730 A1 to The Johns Hopkins University et al. (hereinafter 'JHU') and the article "Marked Genomic Differences Characterize Primary and Secondary Glioblastoma Subtypes and Identify Two Distinct Molecular and Clinical Secondary Glioblastoma Entities" by Maher et al. (hereinafter 'Maher') (Cancer Res 2006; 66: (23). December 1, 2006, pg 11502-11513).

JHU teaches (instant claim 2) a genetic panel to diagnose glioma (para [0015], a method of detecting or diagnosing glioblastoma multiforme (GBM) or minimal residual disease of GBM or molecular relapse of GBM in a human.) wherein the genetic panel assesses: (i) chromosome copy number alterations (CNAs) (para [0159], The mutational data obtained from sequencing and analysis of copy number alterations were integrated in order to identify GBM candidate cancer genes (CAN-genes) that would be most likely to be drivers and therefore worthy of further investigation.) and (ii) genetic amplifications associated with cell cycle progression (para [0030], FIG. 6. Model of malignant glioma development. For each tumor type common genetic alterations (IDH1/IDH2 mutation, TP53 mutation, 1p 19q loss, and CDKN2A loss) are indicated....In general, tumors on the right acquire IDH alterations, while those on the left do not.) in biopsied cells (para [0039], When testing for a somatic mutation in a brain tissue suspected of being cancerous.).

JHU teaches (instant claim 1) a genetic panel to diagnose glioma (para [0015], a method of detecting or diagnosing glioblastoma multiforme (GBM) or minimal residual disease of GBM or molecular relapse of GBM in a human.), wherein the genetic panel assesses and defines molecular subtypes of glioma by the following CNAs: 1p19q presence or codeletion (para [0030], FIG. 6. Model of malignant glioma development. For each tumor type common genetic alterations (IDH1/IDH2 mutation, TP53 mutation, 1p 19q loss, and CDKN2A loss) are indicated....In general, tumors on the right acquire IDH alterations, while those on the left do not.); and genetic sequences associated with IDH 1/2 status (para [0030], Fig. 6, IDH-dependent pathway vs. IDH-independent pathway. [0049], Our study revealed IDH1 mutation to be a novel and significantly more specific marker for secondary GBM.); and CDK4 amplification (par [0161], Analysis of additional gene members within pathways affected by these genes identified alterations of critical genes in the TP53 pathway (TP53, MDM2, MDM4), the RB 1 pathway (RB1, CDK4, CDKN2A), and the PI3K/PTEN pathway (PIK3CA, PIK3R1, PTEN, IRS1); Table 3, CDK4 amplification) and/or CDKN2A deletion (para [0030], CDKN2A loss); and/or CDK4 and MDM2 coamplification (Table 3, for example, sample Br16X, CDK4 and MDM2 coamplification.). JHU does not specifically teach incorporating chromosomes gain and loss into diagnosis.

Maher teaches that chromosomes gain and loss can be used to define glioma subgroups (abstract, Clinically annotated primary and secondary glioblastoma samples were subjected to high-resolution copy number analysis using oligonucleotide-based array comparative genomic hybridization. Unsupervised classification using genomic non-negative matrix factorization methods identified three distinct genomic subclasses.) by the following CNAs: chromosome 19 gain (pg 11505, col 1, para 1, Gain of ch19, in addition to ch7 gain and ch10 loss, was significantly overrepresented in primary compared with secondary glioblastomas (P < 0.01, FDR 10%).). Maher further teaches that the occurrence of chromosome 1 gain is often observed in primary GBM (pg 11504, Fig. 1); while the occurrence of chromosome 14 loss is often observed in secondary GBM (pg 11504, Fig. 1). Given that chromosome gain and loss can be used to define subtypes of glioma, as taught by Maher, it would have been obvious to one of ordinary skill in the art to have incorporated the copy number alterations of chromosomes 1, 19 and 14 to the method of diagnosing of glioma of JHU, and thus, to have provided more definitive characterization of glioma subtypes.

- see extra sheet for continuation -

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/32748

Continuation of:

Box No. III. Observations where unity of invention is lacking

JHU further teaches (instant claim 27) a method of diagnosing whether a subject having a specific subtype of glioma (para [0015]), the method comprising:

(a) obtaining a glioma sample from the subject (para [0039]);

(b) contacting the glioma sample with a IDH1/2 mutant or IDH1/2 wildtype primer and detecting binding or lack thereof indicative of IDH1/2 mutant or wildtype (para [0047], Polynucleotides can also be used as primers or probes. [0030], Fig. 6.; [0049], Our study revealed IDH1 mutation to be a novel and significantly more specific marker for secondary GBM.).

if the IDH1/2 is IDH1/2 wildtype (para [0030], Fig. 6, IDH-independent pathway.). JHU does not specifically teach contacting the sample with a primer and detecting binding or lack thereof between chromosome 1 and the primer indicative of presence or absence of chromosome 1 gain.

Maier teaches that chromosomes gain and loss can be used to define glioma subgroups (abstract, Clinically annotated primary and secondary glioblastoma samples were subjected to high-resolution copy number analysis using oligonucleotide-based array comparative genomic hybridization. Unsupervised classification using genomic non-negative matrix factorization methods identified three distinct genomic subclasses.). Maier further teaches that the occurrence of chromosome 1 gain is often observed in primary GBM (pg 11504, Fig. 1). Thus, it would have been obvious to one of ordinary skill in the art to applied the chromosome 1 gain to the method of JHU, and thus to have reached the claim limitation wherein primary GBM is characterized as IDH-independent and chromosome 1 gain.

JHU further teaches contacting the sample with a primer and detecting binding or lack thereof between CDK4/MDM2 and the primer indicative of presence or absence of CDK4/M DM2 coamplification (Table 3, for example, sample Br16X, CDK4 and MDM2 coamplification.). Thus, it would have been obvious to one of ordinary skill in the art to have determined whether the glioma subtype having IDH-dependent pathway and chromosome 1 gain, can be further characterized exhibiting CDK4/MDM2 coamplification, and thus to have identified the glioma sample as a W1 glioma.

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I+, II+ and III therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.