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(54) **Title**: METHODS AND COMPOSITIONS FOR TYPING MOLECULAR SUBGROUPS OF MEDULLOBLASTOMA

(57) **Abstract**: Immunohistochemical methods and compositions for the typing of molecular subgroups of medulloblastomas are provided. The methods comprise determining a protein expression profile for a sample obtained from a meduUoblastoma by detecting expression of GAB 1, filamin A, or at least two biomarker proteins selected from the group consisting of β-catenin, YAP1, GABl, and filamin A, and typing the meduUoblastoma as a WNT pathway tumor, a SHH pathway tumor, or a non- WNT/non-SHH tumor based on this protein expression profile. Kits for typing a meduUoblastoma according to these three molecular subgroups are provided. The kits comprise at least two antibodies, wherein each of said antibodies specifically binds to a distinct biomarker protein selected from the group consisting of β-catenin, YAP1, GABl, and filamin A, and can optionally comprise one or more of instructions for use, reagents for detecting antibody binding to one or more of said biomarker proteins, and one or more positive control samples.

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METHODS AND COMPOSITIONS FOR TYPING MOLECULAR SUBGROUPS OF MEDULLOBLASTOMA

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FIELD OF THE INVENTION

The present invention relates generally to the field of diagnostic pathology of brain tumors, more particularly to typing of molecular subgroups of medulloblastomas.

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The official copy of the sequence listing is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file named 410091SEQLIST.txt, created on September 26, 2011, and having a size of 175,428 bytes and is filed concurrently with the specification. The sequence listing contained in this ASCII 20 formatted document is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Medulloblastoma, the most common type of primitive neuroectodermal tumor 25 (PNET), is a malignant embryonal tumor of the nervous system, occurring mainly in children and accounting for 25% of all pediatric brain tumors. Most medulloblastoma patients are diagnosed between five and ten years of age (Louis *et al.* (2007) *Acta Neuropathol.* 114(2):97-109), and these tumors occur more often in males than in females. Few medulloblastomas occur under the age of one. These tumors arise in the 30 cerebellum, a region of the brain that continues to develop after birth, during the first two years of human life.

Typically a childhood tumor, medulloblastoma does occur at a very low frequency in adults. Of all medulloblastomas diagnosed in the United States less than ten percent (5%) are found in adults, usually between the ages of 20-44 years. The 35 incidence in adults sharply decreases in frequency after age 45, with very few older adults having this tumor.

A preliminary diagnosis of medulloblastoma is based on clinical symptoms and signs, supplemented by radiological investigations (neuroimaging).

Current treatment regimens for medulloblastoma depend on the age of the patient and the size and/or position of the tumor. In most cases, surgery is performed to remove as much of the tumor as possible with minimal neurological damage. In a minority of patients, the tumor has grown into the brain stem making total removal impossible. Surgery is then followed by radiation therapy to the brain and spinal cord to minimize spreading through the CSF and possible regrowth. Chemotherapy is also given to further deter the spread or regrowth of tumor cells.

The success of current treatments depends, *inter alia*, upon the accurate staging and subtyping of medulloblastomas. Generally, staging is dependent upon the extent of resection, evidence of tumor spread as determined by radiography, and cerebrospinal fluid (CSF) cytology and is graded according to the Chang system (M0-M4). Patients are categorized as low-risk if they undergo gross total tissue resection, with no radiographic evidence of spread, and show no malignant cells on CSF cytology.

Further classification of medulloblastoma depends upon detailed pathologic examination. Histologically, medulloblastomas are divided into five categories according to the WHO classification of tumors of the nervous system based on their morphology, the classic tumor and four variants: desmoplastic/nodular (D/N), medulloblastoma with extensive nodularity (MBEN), large cell medulloblastoma and anaplastic medulloblastoma. The last of these two are sometimes combined into a large cell / anaplastic (LC/A) category, because of their shared aggressive behavior (Kleihues *et al.* (2002) *J. Neuropath. Exp.Neurol.* 61(3):215-225).

Although significant strides have been made in diagnosing and treating these tumors, medulloblastomas remain one of the most challenging pediatric tumors, and improved survival rates have come at a significant cost with significant long-term cognitive and/or neuroendocrine adverse effects among survivors. Presently, identification of pathobiologic/molecular correlates of heterogeneous behavior that could facilitate therapeutic stratification and the application of novel therapies for children with brain tumors, including medulloblastoma, remains a major challenge in the field.

Specifically, cells within medulloblastomas that survive radiation treatment can repopulate the tumor, for example, via activation of the phosphatidylinositol-3 (PI3) kinase pathway. Thus, effective and optimal techniques to identify, type/characterize,

and then target these cells to eliminate the tumor in its entirety while sparing the rest of the brain are still needed. In order for the appropriate therapy to be effectively applied, it is necessary that the type of medulloblastoma present in patients be determined as soon as possible. Therefore, the development of novel ways to type medulloblastomas that may benefit from a distinct therapeutic protocol and, thus improve outcome of patients with these specific medulloblastomas, are also urgently needed.

SUMMARY OF THE INVENTION

In the past year researchers in the field of medulloblastoma have defined four molecular subgroups of the tumor. (See Northcott PA, Korshunov A, Witt H, et al: Medulloblastoma comprises four distinct molecular variants. *J Clin Oncol* 29:1408-14, 2011; Ellison DW: Childhood medulloblastoma: novel approaches to the classification of a heterogeneous disease. *Acta Neuropathol* 120:305-16, 2010; and Ellison DW, Dalton J, Kocak M, et al: Medulloblastoma: clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups. *Acta Neuropathol* 121:381-96, 2011.) Two of these medulloblastoma subgroups are characterized by aberrant upregulation of either the WNT or Sonic Hedghog (SHH) cell signaling pathway. The other two (non-SHH/WNT) subgroups are not obviously characterized by dysfunction of a cell signaling pathways, although one is associated with overexpression of the *MYC* gene. These four molecular subgroups of medulloblastoma are associated with different clinical and histopathological characteristics; e.g., WNT pathway tumors present at a slightly older age among children, are nearly always classic tumors, and have a good outcome with current therapies.

Inventors have tailored therapeutic approaches to medulloblastoma based on a combination of clinical, pathological, and molecular assessments, including the separation of WNT, SHH, and non-SHH/WNT tumors. Northcott PA, Korshunov A, Witt H, et al: Medulloblastoma comprises four distinct molecular variants. *J Clin Oncol* 29:1408-14, 2011; Ellison DW: Childhood medulloblastoma: novel approaches to the classification of a heterogeneous disease. *Acta Neuropathol* 120:305-16, 2010; Ellison DW, Dalton J, Kocak M, et al: Medulloblastoma: clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups. *Acta Neuropathol* 121:381-96, 2011

Immunohistochemical methods and compositions for the typing of molecular subgroups of medulloblastomas are provided. The methods comprise determining a

protein expression profile for a sample obtained from a medulloblastoma by determining presence or absence of expression of at least two biomarker proteins selected from the group consisting of β-catenin, YAP1, GAB1, and filamin A, and typing the medulloblastoma as a WNT pathway tumor, a SHH pathway tumor, or a non-WNT/non-SHH tumor based on this protein expression profile. In some embodiments, expression of at least one of these four biomarker proteins are detected, preferably at least two of these four biomarker proteins is detected, and more preferably where one of the two biomarker proteins is either β-catenin or GAB1. In other embodiments, expression of three of these four biomarker proteins is detected. In yet other embodiments, expression of all four biomarker proteins is detected.

The present invention also provides immunohistochemical methods and compositions for typing a medulloblastoma as a SHH pathway tumor or a non-SHH tumor based on the detection of expression of GAB1 alone within a tumor sample, for typing a medulloblastoma as a non-WNT/non-SHH tumor based on the detection of negative expression of filamin A or YAP1 within a tumor sample, and for typing a medulloblastoma as a WNT pathway tumor based on detection of positive nuclear expression of β-catenin within a tumor sample.

In some embodiments, these immunohistochemical methods comprise evaluating a medulloblastoma tissue sample by first contacting a tissue sample obtained from a medulloblastoma with at least two antibodies, where each of these antibodies specifically binds to a distinct biomarker protein selected from the group consisting of β-catenin, YAP1, GAB1, and filamin A, determining a protein expression profile for the sample based on the detection of binding of these antibodies to their respective biomarker proteins, and typing the medulloblastoma based on this protein expression profile. In some embodiments, the methods utilize a combination of two antibodies, three antibodies, or four antibodies, each of which specifically binds to a distinct biomarker protein selected from the group consisting of β-catenin, YAP1, GAB1, and filamin A.

Kits for typing a medulloblastoma according to these three molecular subgroups are provided. The kits comprise at least two antibodies, wherein each of said antibodies specifically binds to a distinct biomarker protein selected from the group consisting of β-catenin, YAP1, GAB1, and filamin A. In some embodiments, the kits comprise two antibodies, three antibodies, or four antibodies, each of which specifically binds to a distinct biomarker protein selected from the group consisting of β-catenin, YAP1,

GAB1, and filamin A. The kits can optionally comprise one or more of instructions for use, reagents for detecting antibody binding to one or more of said biomarker proteins, and one or more positive control samples.

The medulloblastoma typing methods and kits of the invention permit the rapid
5 and accurate identification of medulloblastomas belonging to these three molecular subgroups. In so doing, the present invention also provides for the identification of subjects with a medulloblastoma that would benefit from known therapies, as well as the selection of subjects for enrollment into clinical trials designed to assess efficacy of new therapies that target the WNT or SHH signaling pathways, or target treatment of
10 medulloblastomas that are not regulated by either of these cell signaling pathways. Accordingly, the present invention encompasses methods and compositions useful for typing medulloblastomas and identifying appropriate and effective therapies for subjects afflicted with this disease.

15 DESCRIPTION OF THE FIGURES

Figure 1 shows a table representing the validation set for the immunohistochemical (IHC) panel to distinguish WNT pathway, SHH pathway, and non-WNT/non-SHH medulloblastomas. Antibodies to four proteins are used: β-catenin, filamin A, GAB1, and YAP1.

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DETAILED DESCRIPTION OF THE INVENTION

Overview

The present invention is directed to immunohistochemical methods and kits for typing medulloblastomas, more particularly for typing these tumors as being associated
25 with one of the following molecular subgroups: (1) medulloblastomas that are associated with activation of the WNT signaling pathway, hereinafter referred to as "WNT pathway tumors"; (2) medulloblastomas that are associated with activation of the sonic hedgehog (SHH) signaling pathway, hereinafter referred to as "SHH pathway tumors"; and (3) medulloblastomas that are not associated with dysregulation of either
30 the WNT signaling pathway or SHH signaling pathway, hereinafter referred to as "non-WNT/non-SHH tumors."

The immunohistochemical methods and kits of the present invention utilize the biomarker proteins, GAB1, filamin A, or a unique combination of at least two biomarker proteins selected from the group consisting of β-catenin, YAP1, GAB1, and

filamin A in order to type medulloblastomas into one of these three molecular subgroups. In this manner, a sample obtained from a medulloblastoma is analyzed to determine a protein expression profile, where analysis comprises detecting expression of at least two of these biomarker proteins. The resulting protein expression profile is predictive of the molecular subgroup to which the medulloblastoma belongs. In some embodiments, expression is detected with the use of at least two antibodies, each of which specifically binds to one of these four biomarker proteins.

The medulloblastoma typing methods and kits of the invention advantageously use surrogate biomarkers of these cell signaling pathways that can readily be detected in fixed tissue samples, including formalin-fixed paraffin wax-embedded (FFPE) tissue samples. The medulloblastoma typing methods and kits provided herein find use in identifying subjects with medulloblastomas that would benefit from standard treatment protocols, 'low-risk' tumor protocols, or which need more aggressive therapeutic intervention for a 'high-risk' tumor. Thus, the present invention also provides means for selecting subjects for clinically proven treatment regimens for medulloblastomas, and for enrolling subjects into clinical trials aimed at testing new treatment regimens for medulloblastomas.

By "medulloblastoma" is intended a malignant primary embryonal tumor that originates in the cerebellum, the part of the brain that controls walking, balance, and fine motor coordination. It is the most common type of embryonal tumor, the latter of which arise from "embryonal" or immature cells at an early stage of their development. These rapidly growing tumors may spread through the cerebrospinal fluid, metastasizing to different locations in the brain and spine. The clinical manifestations and diagnosis of a medulloblastoma are well known to those of skill in the art. Medulloblastomas are known to be molecularly distinct from other histologically similar embryonal tumors such as supratentorial primitive neuroectodermal tumors, central neuroblastomas, ependymoblastomas, medulloepithelioma, and atypical teratoid/rhabdoid tumors.

Recent studies aimed at classifying medulloblastomas on the basis of their gene expression profiles have identified several distinct molecular subgroups, one of which is characterized by activation of the WNT cell signaling pathway (WNT pathway tumors), another of which is characterized by activation of the SHH cell signaling pathway (SHH pathway tumors), along with a third molecular subgroup of tumors, which are not obviously associated with abnormalities of any specific cell signaling

pathway (non-WNT/non-SHH tumors) (see Thompson *et al.* (2006) *J. Clin. Oncol.* 24(12):1924-1930; Northcott PA, Korshunov A, Witt H, *et al*: Medulloblastoma comprises four distinct molecular variants. *J Clin Oncol* 29:1408-14, 201; and Ellison DW: Childhood medulloblastoma: novel approaches to the classification of a heterogeneous disease. *Acta Neuropathol* 120:305-16, 2010).

The non-WNT/non-SHH tumors cluster into two, three, or four further subgroups. These non-WNT/non-SHH tumor subgroups are also more closely related to each other on principal components analysis than they are to WNT pathway tumors or SHH pathway tumors. For purposes of the present invention, those medulloblastomas not regulated by the WNT signaling pathway or the SHH signaling pathway are treated as one molecular subgroup, i.e., non-WNT/non-SHH tumors. The present invention provides methods and compositions for typing medulloblastomas according to these three molecular subgroups. Notably, the typing methods of the present invention can be utilized to type medulloblastomas that cannot be distinguished from other medulloblastomas on the basis of basic clinical or histopathological information (for example, age at diagnosis, sex, and histologic subtype).

In this manner, the present invention provides methods and kits for typing of medulloblastomas that are regulated by activation of the WNT signaling pathway. Such medulloblastomas are referred to herein as "WNT pathway tumors." Activation of the WNT signaling pathway defines a distinct molecular subgroup of medulloblastomas that harbor a characteristic genomic profile, frequently involving monosomy of chromosome 6 and activating mutations in *CTNNB1*, and which are generally independent of tumors containing common characteristic medulloblastoma defects, such as chromosome 17 aberrations (Thompson *et al.* (2006) *J. Clin. Oncol.* 24(12):1924-1930). Upregulation of *CDH1, APC, DKK1, DKK2, DKK4*, WNT inhibitory factor 1 (*WIF*), *LEF1*, *CCDN1*, and *GAD1* may also be present in medulloblastomas that are regulated by the WNT signaling pathway (see, for example, Nikuševa-Martić *et al.* (2007) *Pathology – Research and Practice* 203:779-787; Thompson *et al.* (2006) *J. Clin. Oncol.* 24(12):1924-1930; and Rogers *et al.* (2009) *Brit. J. Cancer*. 100:1292-1302).

In other embodiments, the present invention provides methods and kits for typing of medulloblastomas that are characterized by activation of the SHH signaling pathway. Such medulloblastomas are referred to herein as "SHH pathway tumors." The SHH signaling pathway, which stimulates proliferation of cerebellar granule cells

during cerebellar development, has been implicated in the pathogenesis and etiology of medulloblastoma, particularly those of the desmoplastic/nodular (D/N) histologic subtype. *PTCH1* loss-of-function mutations contribute to approximately 10-20% of sporadic medulloblastoma cases, and mutations in *SMO* and *SUFU* also occur, though
5 these are rare. Upregulation of *GLI1* and *GLI2* (Teglund *et al.* (2010) *Biochim. Biophys. Acta*. 1505:181-208) and *ATOH1*, *PTCH2*, and *SFRP1* have also been shown (Thompson *et al.* (2006) *J. Clin. Oncol.* 24(12):1924-1930).

The medulloblastoma typing methods and kits described herein can be utilized to type tumors as belonging to the third molecular subgroup referred to herein as "non-
10 WNT/non-SHH tumors." As noted above, this molecular subgroup of medulloblastomas is not obviously associated with abnormalities in either the WNT or SHH signaling pathways.

The methods and kits of the present invention encompass the typing of medulloblastomas as WNT pathway tumors, SHH pathway tumors, or non-WNT/non-
15 SHH tumors that may also be classified based on histology, including general architectural and cytological features such as nodule formation, differentiation along neuronal (neurocytic/ganglionic) and astroctyic lines, and large cell or anaplastic phenotypes. Thus, the methods of the present invention are applicable to medulloblastomas of the classic, desmoplastic/nodular (D/N), large cell (LC), or
20 anaplastic (A) histologic subtypes, as well as medulloblastomas with extensive nodularity (MBENs), medulloblastomas with neuroblastic or neuronal differentiation, and medulloblastomas with glial differentiation. Medulloblastomas of the classic histologic subtype consist of sheets of densely packed, small round cells with a high nuclear:cytoplasmic ratio, as seen with microscopic examination. Medulloblastomas of
25 the desmoplastic/nodular histologic subtype are characterized by scattered islands of neurocytic cells. Internodular desmoplasia is required for the diagnosis of D/N medulloblastomas, including the paucinodular D/N variant, and MBEN. Anaplastic medulloblastomas show marked cytological pleomorphism across most of their area, in association with high mitotic and apoptotic counts. A large-cell medulloblastoma is
30 defined by its groups of uniform large round cells with a single nucleolus, in most cases admixed with groups of anaplastic cells. Medulloblastomas with neuroblastic or neuronal differentiation refer to tumors in which the tumor cells appear similar to abnormal nerve cells; and medulloblastomas with glial differentiation refer to tumors having cells that appear similar to the supportive glial brain cells. MBENs mainly

occur in young children and are clearly associated with a more favorable outcome, whereas patients with LC/A medulloblastomas generally have a poor outcome. The D/N histologic subtype has a more favorable outcome than that of classic medulloblastomas among infants. The typing methods of the present invention advantageously can be used to determine whether a medulloblastoma would best be treated by standard, 'low-risk', or 'high-risk' protocols (i.e., surgery, radiation, chemotherapy, and combinations thereof) or by therapeutics that target the WNT or SHH signaling pathway, irrespective of the histologic subtype of a medulloblastoma.

Furthermore, the medulloblastoma typing methods and kits of the invention are applicable to tumors defined according to the "TNM" classification scheme, a standardized system for medulloblastoma cancer staging that was developed by the American Joint Committee on Cancer (AJCC) and/or according to the Chang classification system of grading medulloblastoma. (See Laurent JP, Chang CH, Cohen ME. A classification system for primitive neuroectodermal tumors (medulloblastoma) of the posterior fossa. *Cance*r. 1985 Oct 1;56(7 Suppl):1807-9). According to TNM classification scheme, a subject diagnosed with a medulloblastoma is assessed for primary tumor size (T), regional lymph node status (N), and the presence/absence of distant metastasis (M) and then classified into stages 0 - IV based on this combination of factors. In this system, primary tumor size is categorized on a scale of 0 - 4 (T0 = no evidence of primary tumor; T1 = <3 cm; T2 = >3 cm; T3 = >3 cm in diameter with definite spread into the brain stem; T4 = >3 cm in diameter with extension up past the aqueduct of Sylvius and/or down past the foramen magnum). Lymph node status is classified as N0 - N3 (N0 = regional lymph nodes are free of metastasis; N1 - N3 = degree of metastasis to lymph nodes and distant sites). Metastasis is categorized (by TNM and Chang classification systems) as M0 = no evidence of metastasis; M1 = tumor cells found in cerebrospinal fluid (by lumbar puncture and cytology study); M2 = tumor beyond primary site but still intracranial; M3 = tumor deposits ("seeds") in spine area that are easily seen on MRI; M4 = tumor spread to areas outside the CNS (outside both brain and spine).

The medulloblastomas to be typed in accordance with the methods and compositions of the invention can be associated with one or more genetic abnormalities. Thus, for example, 33-50% of all pediatric medulloblastomas contain chromosome 17 copy number abnormalities, in particular i17q, an isochromosome on the long arm of chromosome 17. Genetic abnormalities found on chromosomes 1, 6, 7,

8, 9, 10q, 11, and 16 may also be involved. Thus, in some embodiments of the present invention, the medulloblastoma to be typed is associated with genetic abnormalities on one or more of chromosomes 1, 6, 7, 8, 9, 10q, 11, 16, and 17, as well as amplification of the *MYC* or *MYCN* genes.

5

Methods for Typing Medulloblastomas

The present invention thus provides immunohistochemical methods for typing medulloblastomas according to one of the three molecular subgroups previously identified by gene expression profiling (see, for example, Thompson *et al.* (2006) *J.*
10 *Clin. Oncol.* 24(12):1924-1930; and Ellison DW, Dalton J, Kocak M, *et al*: Medulloblastoma: clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups. *Acta Neuropathol* 121:381-96, 2011, herein incorporated by reference in their entirety), *i.e.*, WNT pathway tumors, SHH pathway tumors, and non-WNT/non-SHH tumors. (). These immunohistochemical methods reliably predict
15 whether a medulloblastoma is associated with genomic abnormalities resulting in activation of the WNT signaling pathway, genomic abnormalities resulting in activation of the SHH signaling pathway, or genomic abnormalities that are not associated with either of these two cell signaling pathways.

In accordance with some embodiments of the present invention, the
20 immunohistochemical methods for typing of a medulloblastoma as a WNT pathway tumor, a SHH pathway tumor, or a non-WNT/non-SHH tumor comprise determining a protein expression profile for a sample obtained from a medulloblastoma by detecting expression of at least two biomarker proteins selected from the group consisting of β-catenin, YAP1, GAB1, and filamin A.
25 By "detecting expression" is intended determining the quantity or presence of a biomarker protein. Thus, "detecting expression" encompasses instances where a biomarker protein is determined not to be expressed, not to be detectably expressed, expressed at a low level, expressed at a normal level, or overexpressed. "Detecting expression" also encompasses detecting expression in the nucleus or cytoplasm.
30 Detection of expression of these biomarker proteins within a sample obtained from a medulloblastoma can be achieved using any protein detection means known to those of skill in the art of immunohistochemistry, including those detection methods described herein below. Detecting expression of at least two of these four biomarker proteins

within a medulloblastoma sample yields a unique protein expression profile that is predictive of the molecular subgroup to which the analyzed medulloblastoma belongs. In this manner, the medulloblastoma typing methods of the invention distinguish between WNT pathway tumors, SHH pathway tumors, and non-WNT/non-SHH tumors without having to resort to more complex and costly gene expression profiling. Furthermore, these particular biomarker proteins provide two advantages for immunohistochemical typing of medulloblastomas into these molecular subgroups. First, expression of these particular biomarker proteins is essentially either “on” or “off” within the cells or identified subcellular compartments of the medulloblastoma sample such that their expression can readily be scored as either “positive” or “negative” by anyone of skill in the field with the following qualifications.

With respect to filamin A, positive expression observed in WNT pathway tumor samples is generally weaker than positive expression observed in SHH pathway tumor samples. With respect to β-catenin, WNT pathway tumors generally show nearly all cells with nucleopositive (i.e., positive nuclear expression) immunoreactivity for β-catenin. However, about one-third of WNT pathway tumors show patchy nucleopositive immunoreactivity for β-catenin, but at least 10% of tumor cells are nucleopositive in these samples. There are also very rare non-WNT pathway medulloblastomas with scattered nucleopositive immunoreactivity for β-catenin amounting to less than 1% of tumor cells, which can easily be distinguished from WNT pathway tumor samples. Second, all of these biomarker proteins are readily detectable by immunological techniques even in formalin-fixed paraffin-wax embedded tissue samples.

β-catenin is a critical downstream effector of the WNT/Wg signaling pathway. This protein is encoded by the *CTNNB1* gene (see, for example, Kraus *et al.* (1994) *Genomics* 23(1):272-274; see also, GenBank Accession Nos. NP_001091680 (SEQ ID NO:2, setting forth the 781-aa protein sequence) and NM_001098210 (coding sequence is nucleotides 269-2614; see SEQ ID NO:1, setting forth this coding sequence). It is expressed as both cytoplasmic and nuclear forms. Cytoplasmic β-catenin is inactive and is regulated by a multimeric protein complex consisting of APC, GSK-3β, and AXIN. In its inactive state, β-catenin is phosphorylated by GSK-3β, rendering it degradable by the ubiqitin-proteasome pathway. During activation of the WNT/Wg signaling pathway, the multimeric complex is destabilized, leading to the upregulation

of β-catenin, promoting its translocation to the nucleus. In the nucleus, β-catenin acts as a co-activator of Tcf/Lef transcription factors, which regulate genes involved in cell-cycle progression, apoptosis, and differentiation. Positive nuclear β-catenin expression is therefore a biomarker for activation of the WNT/Wg signaling pathway. β-catenin has been shown to be expressed in the nucleus of medulloblastoma cells, and its positive nuclear expression has further been shown to be a prognostic marker in medulloblastoma (Ellison *et al.* (2005) *J. Clin. Oncol.* 23(31):7951-7957).

YAP1, or Yes-associated protein 1, is a 65 kDa protein encoded by the *YAP1* gene (see, for example, Sudol *et al.* (1995) *J. Biol. Chem.* 270(24):14733-14741; see also GenBank Accession Nos. NP_001123617 (SEQ ID NO:4, setting forth the isoform 1 variant having a 504-aa protein sequence) and NM_001130145 (coding sequence shown in nucleotides 389-1903; see SEQ ID NO:3, setting forth this coding sequence); and GenBank Accession Nos. NP_006097 (SEQ ID NO:6, setting forth the isoform 2 variant having a 450-aa protein sequence) and NM_006106 (coding sequence is nucleotides 389-1741; see SEQ ID NO:5, setting forth this coding sequence)). YAP1 can act both as a co-activator and a co-repressor and is the critical downstream regulatory target in the Hippo signaling pathway that plays a pivotal role in organ size control and tumor suppression by restricting proliferation and promoting apoptosis. The core of this pathway is composed of a kinase cascade wherein MST1/MST2, in complex with its regulatory protein SAV1, phosphorylates and activates LATS1/2 in complex with its regulatory protein MOB1, which in turn phosphorylates and inactivates YAP1 oncoprotein. YAP1 plays a key role in controlling cell proliferation in response to cell contact. Phosphorylation by LATS1/2 inhibits its translocation into the nucleus to regulate cellular genes important for cell proliferation, cell death, and cell migration. Up-regulation of YAP1 has been shown in a subset of human medulloblastomas, in particular, those medulloblastomas regulated by the SHH and WNT pathways. YAP1 is also upregulated in cerebellar granule neuron precursors (CGNPs), which are proposed cells of origin for subtypes of medulloblastomas, possibly through the activation of TEAD1 (Fernandez-L *et al.* (2009) *Genes & Development* 23:2739-2741).

GAB1, or GRB2-associated-binding protein 1, is a member of the IRS1-like multisubstrate docking protein family, and is encoded by the *GAB1* gene (see, for example, Holgado-Madruga *et al.* (1996) *Nature* 379:560-564; see also GenBank Accession Nos. NP_997006 (SEQ ID NO:8, setting forth the "isoform a" variant

having a 724-aa protein sequence) and NM_207123 (coding sequence shown in nucleotides 360-2534; see SEQ ID NO:7, setting forth this coding sequence); and GenBank Accession Nos. NP_002030 (SEQ ID NO:10, setting forth the “isoform b” variant having a 694-aa protein sequence) and NM_002039 (coding sequence shown in nucleotides 360-2444; see SEQ ID NO:9, setting forth this coding sequence)). GAB1 is an important mediator of branching tubulogenesis and plays a central role in cellular growth response, transformation, and apoptosis. Two transcript variants of this gene are known and encode different isoforms. GAB1 is a docking protein that recruits PI3 kinase and other effector proteins in response to the activation of many receptor tyrosine kinases (RTKs). The primary mechanism of EGF-induced stimulation of the PI3 kinase/Akt anti-apoptotic pathway occurs via the docking protein GAB1. The protein GRB2 plays a central role in signaling by RTKs, where its SH2 domain binds to the receptor and its two SH3 domains link to effectors. One target effector is SOS, where GRB2 links RTKs with the Ras signaling pathway. The SH3 domains can also couple to other signaling proteins, including Vav, c-Abl and dynamin. GAB1 is expressed in medulloblastomas and shares amino-acid homology and several structural features with IRS-1, which is a substrate of the EGF and insulin receptors, and can act as a docking protein for several SH2-containing proteins known to be expressed in medulloblastomas. Expression of GAB1 enhances cell growth and transformation (see, for example, Holgrado-Madruga *et al.* (1996) *Nature* 379:560-564).

Filamins are a family of high molecular mass cytoskeletal proteins that organize filamentous actin in networks and stress fibers and are responsible for anchoring various transmembrane proteins to the actin cytoskeleton and providing a scaffold for a wide range of cytoplasmic signaling proteins. Filamin A is a 280-kDa protein that is encoded by the *FLNA* gene (see, for example, Gorlin *et al.* (1993) *Genomics* 17(2):496-498; see also, GenBank Accession Nos. NP_001447 (SEQ ID NO:12, setting forth the “isoform 1” variant having a 2639-aa protein sequence) and NM_001456 (coding sequence shown in nucleotides 250-8169; see SEQ ID NO:11, setting forth this coding sequence); and GenBank Accession Nos. NP_001104026 (SEQ ID NO:14, setting forth the “isoform 2” 2647-aa protein sequence) and NM_001110556 (coding sequence shown in nucleotides 250-8193; see SEQ ID NO:13, setting forth this coding sequence)). Remodeling of the cytoskeleton is central to the modulation of cell shape and migration. Filamin A is a widely expressed protein that regulates reorganization of the actin cytoskeleton by interacting with integrins, transmembrane receptor

complexes, and second messengers. Upregulation or overexpression of filamin A has been observed in various types of cancer. The direct association of filamin A with medulloblastomas has not been previously reported.

Thus, in accordance with these immunohistochemical methods of the present invention, typing of a medulloblastoma as a WNT pathway tumor, a SHH pathway tumor, or a non-WNT/non-SHH tumor comprises determining a protein expression profile for tumor cells in a sample obtained from a medulloblastoma by detecting expression of at least two biomarker proteins selected from the group consisting of β-catenin, YAP1, GAB1, and filamin A.

The unique protein expression profiles displayed by medulloblastomas falling within these three molecular subgroups are summarized in Table 1 below.

Table 1. Immunohistochemical protein expression profiles among tumor cells predictive of WNT pathway medulloblastomas (WNT), SHH pathway medulloblastomas (SHH), and non-WNT/non-SHH medulloblastomas (Other). For YAP1, GAB1, and filamin A, "+" = positive expression, scored by detection in at least 50% of cells in field of view and "–" = negative expression, scored by detection in less than 50% of cells in field of view. For β-catenin expression, "N" denotes nuclear expression, where "+" = positive expression, scored by detection in at least 10% of the nuclei in field of view and "–" = negative expression, scored by detection in less than 10% of the nuclei in field of view; "C" denotes cytoplasmic expression.

Molecular Subgroup	β-catenin		YAP1	GAB1	Filamin A
	N	C			
WNT	+	+	+	-	+
SHH	-	+	+	+	+
Other	-	+	-	-	-

As evidenced by Table 1, in those embodiments of the invention where the protein expression profile is based on the detection of expression of two biomarker proteins, one of the biomarker proteins is either β-catenin or GAB1, as detection of expression of either of these biomarker profiles in combination with detection of expression of one of the other three biomarker proteins yields a protein expression profile that distinguishes between WNT pathway tumors, SHH pathway tumors, and non-WNT/non-SHH tumors.

Also, as can be seen from Table 1, β-catenin expression is generally positive in the cytoplasm of cells of medulloblastomas, regardless of these three molecular subgroups. In contrast, positive expression of β-catenin within the nuclei of cells of a medulloblastoma is predictive of a WNT pathway tumor.

5 Where the protein expression profile of a medulloblastoma sample is determined by detecting expression of β-catenin and at least one of YAP1, GAB1, or filamin A, a protein expression profile characterized by positive nuclear expression of β-catenin is predictive of a WNT pathway medulloblastoma. If, however, the protein expression profile of a medulloblastoma sample is characterized by negative nuclear

10 expression of β-catenin, detecting expression of any one of YAP1, GAB1, filamin A, or a combination thereof within the medulloblastoma sample allows for the determination of a unique protein expression profile that distinguishes between SHH pathway tumors and non-WNT/non-SHH tumors. In this manner, medulloblastomas regulated by activation of the SHH signaling pathway exhibit a protein expression profile that is

15 characterized not only by negative nuclear expression of β-catenin, but also positive expression of any one or more of YAP1, GAB1, or filamin A. In contrast, medulloblastomas that have no association with the WNT or SHH signaling pathways exhibit a protein expression profile among tumor cells that is characterized by negative nuclear expression of β-catenin and negative expression of YAP1, GAB1, and filamin

20 A.

Alternatively, where the protein expression profile of a medulloblastoma sample is determined by detecting expression of GAB1 and expression of at least one of β-catenin, YAP1, or filamin A, a protein expression profile characterized by positive expression of GAB1 is predictive of a SHH pathway medulloblastoma. If, however,

25 the protein expression profile of a medulloblastoma sample is characterized by negative expression of GAB1, detecting positive nuclear expression of β-catenin is predictive of a WNT pathway medulloblastoma. Where the protein expression profile is characterized by negative expression of GAB1 and negative nuclear expression of β-catenin, the medulloblastoma is typed as a non-WNT/non-SHH tumor.

30 In like manner, where the protein expression profile of a medulloblastoma sample is determined by detecting expression of YAP1 and at least one of β-catenin, GAB1, or filamin A, a protein expression profile characterized by negative expression of YAP1 is predictive of a non-WNT/non-SHH pathway tumor. If, however, the protein expression profile is characterized by positive YAP1 expression, typing of the

analyzed medulloblastoma as a SHH pathway or WNT pathway tumor is readily achieved by the detection of expression of β-catenin or GAB1. In such embodiments, a protein expression profile characterized by positive YAP1 expression and positive GAB1 expression and/or negative nuclear β-catenin expression is predictive of a SHH pathway medulloblastoma. In like manner, a protein expression profile characterized by positive YAP1 expression and negative GAB1 expression and/or positive nuclear β-catenin expression is predictive of a WNT pathway medulloblastoma.

In yet other embodiments, where the protein expression profile of a medulloblastoma sample is determined by detecting expression of filamin A and at least one of β-catenin, GAB1, or YAP1, a protein expression profile characterized by negative expression of filamin A is predictive of a non-WNT/non-SHH tumor. If, however, the protein expression profile is characterized by positive filamin A expression, typing of the analyzed medulloblastoma as a SHH pathway or WNT pathway tumor is readily achieved by the detection of expression of GAB-1 or β-catenin (nuclear). In such embodiments, a protein expression profile characterized by positive filamin A expression and positive GAB1 expression and/or negative nuclear β-catenin expression is predictive of a SHH pathway medulloblastoma. In like manner, a protein expression profile characterized by positive filamin A expression and negative GAB1 expression and/or positive nuclear β-catenin expression is predictive of a WNT pathway medulloblastoma.

Thus, in some embodiments, the medulloblastoma typing methods of the present invention comprise determining a protein expression profile among tumor cells for a sample obtained from a medulloblastoma of interest by detecting expression of two of these four biomarker proteins, wherein one of the biomarker proteins is β-catenin, and the second biomarker protein is either YAP1, GAB1, or filamin A. In other embodiments, the medulloblastoma typing methods of the invention comprise determining a protein expression profile for a sample obtained from a medulloblastoma of interest by detecting expression of two of these four biomarker proteins, wherein one of the biomarker proteins is GAB1, and the second biomarker protein is either β-catenin, YAP1, or filamin A. In yet other embodiments, the medulloblastoma typing methods of the present invention comprise determining a protein expression profile for a sample obtained from a medulloblastoma of interest by detecting expression of two of these four biomarker proteins, wherein one of the biomarker proteins is YAP1, and the second biomarker protein is either β-catenin or GAB1. In still other embodiments, the

medulloblastoma typing methods of the present invention comprise determining a protein expression profile for a sample obtained from a medulloblastoma of interest by detecting expression of two of these four biomarker proteins, wherein one of the biomarker proteins is filamin A, and the second biomarker protein is either β-catenin or GAB1. In some preferred embodiments, the medulloblastoma typing methods of the present invention comprise determining a protein expression profile for a sample obtained from a medulloblastoma of interest by detecting expression of β-catenin and YAP1.

In some embodiments, the medulloblastoma typing methods of the present invention comprise determining a protein expression profile for a sample obtained from a medulloblastoma of interest by detecting expression of three of these four biomarker proteins, wherein one of the biomarker proteins is β-catenin, and the other two biomarker proteins are selected from YAP1, GAB1, and filamin A. In other embodiments, the medulloblastoma typing methods of the present invention comprise determining a protein expression profile for a sample obtained from a medulloblastoma of interest by detecting expression of three of these four biomarker proteins, wherein one of the biomarker proteins is GAB1, and the other two biomarker proteins are selected from β-catenin YAP1, and filamin A. In yet other embodiments, the medulloblastoma typing methods of the present invention comprise determining a protein expression profile for a sample obtained from a medulloblastoma of interest by detecting expression of three of these four biomarker proteins, wherein one of the biomarker proteins is YAP1, and the other two biomarker proteins are selected from β-catenin, GAB1, and filamin A. In still other embodiments, the medulloblastoma typing methods of the present invention comprise determining a protein expression profile for a sample obtained from a medulloblastoma of interest by detecting expression of three of these four biomarker proteins, wherein one of the biomarker proteins is filamin A, and the other two biomarker proteins are selected from β-catenin, GAB1, and YAP1. In some preferred embodiments, the medulloblastoma typing methods of the present invention comprise determining a protein expression profile for a sample obtained from a medulloblastoma of interest by detecting expression of β-catenin, GAB1, and YAP1.

In yet other embodiments, the medulloblastoma typing methods of the present invention comprise determining a protein expression profile among tumor cells for a sample obtained from a medulloblastoma of interest by detecting expression of β-catenin, GAB1, YAP1, and filamin A.

The present invention is in part based upon the discovery that GAB1 and filamin A are differentially expressed in these three molecular subgroups of medulloblastomas. In this manner, GAB1 shows positive expression in the cytoplasm of cells of medulloblastomas of the SHH pathway tumor subgroup, yet is not expressed within cells of medulloblastomas of the WNT pathway subgroup or the non-WNT/non-SHH tumor subgroup. Filamin A shows positive expression in the cytoplasm of cells of medulloblastomas of the WNT pathway and SHH pathway tumor subgroups, yet is not expressed within cells of medulloblastomas of the non-WNT/non-SHH tumor subgroup. Accordingly, the present invention also provides methods of typing medulloblastomas based on positive or negative expression of either of these biomarker proteins.

In this manner, in some embodiments, the present invention provides an immunohistochemical method for typing a medulloblastoma as a SHH pathway tumor or a non-SHH tumor. By "non-SHH tumor" is intended the tumor is not a SHH pathway tumor, and thus falls within one of the other two molecular subgroups described herein, i.e., is either a WNT pathway tumor or a non-WNT/non-SHH tumor. This method comprises determining a protein expression profile for a sample obtained from the medulloblastoma by detecting expression of GAB1 and typing the medulloblastoma as a SHH pathway tumor or a non-SHH tumor based on this protein expression profile. In this manner, where the protein expression profile is characterized by positive expression of GAB1, the medulloblastoma is typed as a SHH pathway tumor. In contrast, where the protein expression profile is characterized by negative expression of GAB1, the medulloblastoma is typed as a non-SHH tumor. In the latter case, detection of expression of an additional biomarker protein disclosed herein (for example β-catenin, YAP1, or filamin A) facilitates typing of the medulloblastoma tumor as either a WNT pathway tumor or a non-WNT/non-SHH tumor.

In other embodiments, the present invention provides an immunohistochemical method for typing a medulloblastoma as a non-WNT/non-SHH tumor. This method comprises determining a protein expression profile for a sample obtained from the medulloblastoma by detecting expression of filamin A and typing the medulloblastoma as a non-WNT/non-SHH tumor pathway tumor or a non-SHH pathway tumor based on this protein expression profile. In this manner, where the protein expression profile is characterized by negative expression of filamin A, the medulloblastoma is typed as a non-WNT/non-SHH tumor. In contrast, where the protein expression profile is

characterized by positive expression of filamin A, the medulloblastoma is typed as falling outside the non-WNT/non-SHH tumor subgroup. In the latter case, detection of expression of an additional biomarker protein disclosed herein (for example β-catenin or GAB1) facilitates typing of the medulloblastoma tumor as either a WNT pathway tumor or a SHH pathway tumor.

In both of these embodiments, detection of expression of GAB1 or filamin A can be achieved using any protein detection method known to those of skill in the art. In some embodiments, expression is detected using an antibody that specifically binds GAB1 or an antibody that specifically binds filamin A, in the manner described herein below.

Expression of these biomarker proteins can be detected by any protein detection method known to those of skill in the art. In some embodiments, the medulloblastoma typing methods of the invention rely on the use of a unique combination of at least two, three, or four antibodies, each of which specifically binds to a distinct biomarker protein selected from the group consisting of β-catenin, YAP1, GAB1, and filamin A, to determine a protein expression profile within a medulloblastoma tissue sample. By "distinct" biomarker protein is intended the antibody binds specifically to that biomarker protein, and not to any of the other biomarker proteins to be detected. Thus, for example, β-catenin is a distinct biomarker protein for an antibody that specifically binds β-catenin. In like manner, YAP1 is a distinct biomarker protein for an antibody that specifically binds YAP1. Similarly, GAB1 is a distinct biomarker protein for an antibody that specifically binds GAB1. Likewise, filamin A is a distinct biomarker protein for an antibody that specifically binds filamin A. The distinct biomarker protein to which an antibody selectively binds is referred to herein as the respective "binding partner" for that antibody. Thus, for example, β-catenin is the binding partner for an antibody that specifically binds β-catenin. In like manner, YAP1 is the binding partner for an antibody that specifically binds YAP1. Similarly, GAB1 is the binding partner for an antibody that specifically binds GAB1. Likewise, filamin A is the binding partner for an antibody that specifically binds filamin A. Antibodies that specifically bind to these respective biomarker proteins are well known to those of skill in the art and include commercially available antibodies, as well as antibodies that can be prepared using standard procedures well known to those of skill in the art, and further described elsewhere herein.

In some embodiments, the medulloblastoma typing methods of the invention comprise contacting a tissue sample obtained from a medulloblastoma with a combination of at least two antibodies, each of which specifically binds to one of these four distinct biomarker proteins, determining a protein expression profile for the tissue sample based on detection of binding of at least two of these antibodies to their respective biomarker proteins, and typing the medulloblastoma as a WNT pathway tumor, a SHH pathway tumor, or a non-WNT/non-SHH tumor based on this protein expression profile. Detection of binding of a particular antibody to its distinct biomarker protein (i.e., its binding partner) is indicative of positive expression of that biomarker protein. In like manner, the lack of detection of binding of a particular antibody to its distinct biomarker protein is indicative of negative expression of that biomarker protein. Any antibody binding detection methods known to those of skill in the art can be utilized, including those described elsewhere herein.

In certain embodiments, the medulloblastoma typing methods of the invention comprise contacting a tissue sample obtained from a medulloblastoma with two antibodies selected from the group consisting of an antibody that specifically binds β-catenin, an antibody that specifically binds YAP1, an antibody that specifically binds GAB1, and an antibody that specifically binds filamin A; determining a protein expression profile for this tissue sample based on detection of binding of these two antibodies to their respective binding partners; and typing the medulloblastoma as a WNT pathway tumor, a SHH pathway tumor, or a non-WNT/non-SHH tumor based on the resulting protein expression profile, as outlined herein above and in Table 1. In specific embodiments, the tissue sample is contacted with an antibody that specifically binds β-catenin (i.e., a β-catenin antibody), and an antibody that specifically binds either GAB1 (i.e., a GAB1 antibody), YAP1 (i.e., a YAP1 antibody), or filamin A (i.e., a filamin A antibody). In some preferred embodiments, the tissue sample is contacted with an antibody that specifically binds β-catenin and an antibody that specifically binds YAP1.

In such embodiments, positive detection of binding of the β-catenin antibody to its binding partner within the nuclei of cells of the tissue sample indicates positive nuclear expression of β-catenin, and thus yields a protein expression profile that is predictive of a WNT pathway medulloblastoma. Alternatively, where nuclear expression of β-catenin is found to be negative (i.e., binding of the β-catenin antibody to its binding partner is not detected), positive detection of binding of the GAB1,

YAP1, or filamin A antibody to its respective binding partner (i.e., GAB1, YAP1, or filamin A) within tumor cells of the tissue sample indicates positive expression of GAB1, YAP1, or filamin A, thus yielding a protein expression profile that is predictive of a SHH pathway medulloblastoma. In contrast, negative detection of binding of the β-catenin antibody to its binding partner in nuclei of the tumor cells of the tissue sample in combination with negative detection of binding of the GAB1, YAP1, or filamin A antibody to its respective binding partner indicates negative nuclear expression of β-catenin and negative expression of the other biomarker protein (i.e., YAP1, GAB1, or filamin A), thus yielding a protein expression profile that is predictive of a non-WNT/non-SHH medulloblastoma.

In other specific embodiments, the tissue sample obtained from the medulloblastoma is contacted with an antibody that specifically binds GAB1 (i.e., a GAB1 antibody), and an antibody that specifically binds either β-catenin (i.e., a β-catenin antibody), YAP1 (i.e., a YAP1 antibody), or filamin A (i.e., a filamin A antibody). In such embodiments, positive detection of binding of the GAB1 antibody to its binding partner in tumor cells of the tissue sample indicates positive expression of GAB1, thus yielding a protein expression profile that is predictive of a SHH pathway medulloblastoma. Alternatively, where expression of GAB1 is found to be negative (i.e., binding of the GAB1 antibody to GAB1 is not detected), positive detection of binding of the β-catenin antibody to its binding partner within the nuclei of tumor cells of the tissue sample indicates positive nuclear expression of β-catenin, and thus yields a protein expression profile that is predictive of a WNT pathway medulloblastoma. In yet other embodiments, negative detection of binding of the GAB1 antibody to its binding partner in tumor cells of the tissue sample and negative detection of binding of the β-catenin antibody to its binding partner within the nuclei of the cells of the tissue sample, or negative detection of binding of the filamin A antibody to its binding partner in cells of the tissue sample, is indicative of negative expression of GAB1 in combination with negative nuclear expression of β-catenin expression or negative expression of filamin A, thus yielding a protein expression profile that is predictive of a non-WNT/non-SHH medulloblastoma.

In yet other specific embodiments, the tissue sample of the medulloblastoma is contacted with an antibody that specifically binds YAP1 (i.e., a YAP1 antibody), and an antibody that specifically binds either β-catenin (i.e., a β-catenin antibody) or an antibody that specifically binds GAB1 (i.e., a GAB1 antibody). In such embodiments,

negative detection of binding of the YAP1 antibody to its binding partner in cells of the tissue sample is indicative of negative expression of YAP1, thus yielding a protein expression profile that is predictive of a non-WNT/non-SHH medulloblastoma. Alternatively, where YAP1 expression is found to be positive (i.e., binding of the YAP1 antibody to YAP1 is detected in cells of the tissue sample), positive detection of binding of the GAB1 antibody to its binding partner is indicative of positive GAB1 expression, thus yielding a protein expression profile that is predictive of a SHH pathway medulloblastoma. In yet other embodiments, where YAP1 expression is found to be positive, negative detection of binding of β-catenin antibody to its binding partner within the nuclei of cells of the sample tissue is indicative of negative nuclear β-catenin expression, thus yielding a protein expression profile that is also predictive of a SHH pathway medulloblastoma. In still other embodiments, where YAP1 expression is found to be positive, positive detection of binding of β-catenin antibody to its binding partner within the nuclei of cells of the sample tissue is indicative of positive nuclear β-catenin expression, thus yielding a protein expression profile that is predictive of a WNT pathway medulloblastoma.

In still other specific embodiments, the tissue sample is contacted with an antibody that specifically binds filamin A (i.e., a filamin A antibody), and an antibody that specifically binds β-catenin (i.e., a β-catenin antibody) or an antibody that specifically binds GAB1 (i.e., a GAB1 antibody). In such embodiments, negative detection of binding of the filamin A antibody to its binding partner in cells of the tissue sample is indicative of negative filamin A expression, thus yielding a protein expression profile that is predictive of a non-WNT/non-SHH medulloblastoma. Alternatively, where filamin A expression is found to be positive (i.e., binding of the filamin A antibody to filamin A is detected), positive detection of binding of the GAB1 antibody to its binding partner in cells of the tissue sample is indicative of positive expression of GAB1, thus yielding a protein expression profile that is predictive of a SHH pathway medulloblastoma. In yet other embodiments, where filamin A expression is found to be positive, negative detection of binding of the β-catenin antibody to its binding partner in nuclei of cells of the tissue sample is indicative of negative nuclear expression of β-catenin, thus yielding a protein expression profile that is also predictive of a SHH pathway medulloblastoma. In still other embodiments, where filamin A expression is found to be positive, positive detection of binding of the β-catenin antibody to its binding partner in nuclei of cells of the tissue sample is

indicative of positive nuclear expression of β-catenin, thus yielding a protein expression profile that is predictive of a WNT pathway medulloblastoma.

In other embodiments, the tissue sample is contacted with three antibodies selected from the group consisting of an antibody that specifically binds β-catenin, an antibody that specifically binds YAP1, an antibody that specifically binds GAB1, and an antibody that specifically binds filamin A; a protein expression profile for this tissue sample is determined based on detection of binding of these three antibodies to their respective binding partners; and the medulloblastoma is typed as a WNT pathway tumor, a SHH pathway tumor, or a non-WNT/non-SHH tumor based on the resulting protein expression profile, as outlined herein above and in Table 1. In specific embodiments, the tissue sample is contacted with an antibody that specifically binds β-catenin, and two antibodies selected from the group consisting of an antibody that specifically binds YAP1, an antibody that specifically binds GAB1, and an antibody that specifically binds filamin A. In other specific embodiments, the tissue sample is contacted with an antibody that specifically binds GAB1, and two antibodies selected from the group consisting of an antibody that specifically binds β-catenin, an antibody that specifically binds YAP1, and an antibody that specifically binds filamin A. In yet other specific embodiments, the tissue sample is contacted with an antibody that specifically binds YAP1, and two antibodies selected from the group consisting of an antibody that specifically binds β-catenin, an antibody that specifically binds GAB1, and an antibody that specifically binds filamin A. In still other specific embodiments, the tissue sample is contacted with an antibody that specifically binds filamin A, and two antibodies selected from the group consisting of an antibody that specifically binds β-catenin, an antibody that specifically binds GAB1, and an antibody that specifically binds YAP1. In some preferred embodiments, the tissue sample is contacted with an antibody that specifically binds β-catenin, an antibody that specifically binds YAP1, and an antibody that specifically binds GAB1.

In yet other embodiments, the tissue sample is contacted with four antibodies selected from the group consisting of an antibody that specifically binds β-catenin, an antibody that specifically binds YAP1, an antibody that specifically binds GAB1, and an antibody that specifically binds filamin A; a protein expression profile for this tissue sample is determined based on detection of binding of these four antibodies to their respective binding partners; and the medulloblastoma is typed as a WNT pathway

tumor, a SHH pathway tumor, or a non-WNT/non-SHH tumor based on the resulting protein expression profile, as outlined herein above and in Table 1.

Thus, the immunohistochemical typing methods of the invention require the detection of expression of GAB1, filamin A, or at least two biomarker proteins selected from the group consisting of β-catenin, GAB1, YAP1, and filamin A, in a sample obtained from a medulloblastoma. It is recognized that detection of more than one biomarker protein in a body sample may be used to more accurately type the medulloblastoma to one of these three molecular subgroups. Therefore, in some embodiments, two or more biomarker proteins are used, more preferably, two or more complementary biomarker proteins. By “complementary” is intended that detection of the combination of biomarker proteins in a medulloblastoma sample results in the accurate determination of molecular subgroup in a greater percentage of cases than would be identified if only one of the biomarker proteins was used. Thus, in some cases, a more accurate determination of the molecular subgroup of medulloblastoma can be made by using at least two biomarker proteins. Accordingly, where at least two biomarker proteins are used, at least two antibodies directed to distinct biomarker proteins will be used to practice the immunohistochemical methods disclosed herein. These antibodies may be contacted with the medulloblastoma sample simultaneously or successively.

When a combination of two or more biomarker proteins is used, the biomarkers will typically be substantially statistically independent of one another. By “statistically independent” biomarker proteins is intended that the molecular subgroups identified thereby are independent such that one biomarker protein does not provide substantially repetitive information with regard to the complementary biomarker protein. This may ensure, for instance, that a second biomarker protein is not used in conjunction with a first biomarker protein when the two are not substantially statistically independent. The dependence of the two biomarker proteins may indicate that they are duplicative and that the addition of a second biomarker adds no additional value to the predictive power of a given pair of biomarker proteins. In order to optimize the predictive power of a given panel of biomarker proteins it is also desirable to reduce the amount of signal “noise” by minimizing the use of biomarker proteins that provide duplicative predictive information when compared to another biomarker protein in the panel. Where independent predictive biomarker proteins are used to practice the present methods, the predictive value is increased by detecting the expression of two, three, or four of these

biomarker proteins. In such cases, any combination of independent biomarkers can be used.

One of skill in the art will also recognize that a panel of biomarker proteins can be used to type a medulloblastoma as a WNT pathway tumor, a SHH pathway tumor,
5 or a non-WNT/non-SHH tumor in accordance with the methods of the invention. In some embodiments, a panel comprising at least two biomarker proteins selected from the group consisting of β-catenin, filamin A, GAB1, and YAP1, is utilized. One particular panel of biomarker proteins may comprise, for example, all or a subset of said proteins. In certain aspects of the invention, a panel comprises at least two
10 statistically independent biomarker proteins. In particular embodiments, the immunohistochemical methods for typing medulloblastoma comprise collecting a tissue sample from a medulloblastoma, contacting the sample with at least two antibodies, each specific for a different biomarker protein selected from the group consisting of β-catenin, filamin A, GAB1, and YAP1, detecting antibody binding, and determining if
15 the biomarker proteins are expressed. That is, samples are incubated with the antibodies for a time sufficient to permit the formation of antibody-biomarker protein complexes, and antibody binding is detected, for example, by a labeled secondary antibody.

20 *Biomarker Proteins*

It is recognized that the β-catenin, YAP1, GAB1, and filamin A biomarker proteins to be detected within the medulloblastoma sample may be the full-length polypeptides, or may be detectable fragments or naturally occurring variants thereof. By "fragment" is intended a portion of the amino acid sequence and hence protein
25 encoded thereby. A fragment of a biomarker polypeptide will generally consist of at least 25, 30, 50, 100, 150, 200, or 250 contiguous amino acids, or up to the total number of amino acids present in a full-length biomarker protein of the invention. "Variant" is intended to mean substantially similar sequences. Generally, variants of a particular biomarker of the invention will have at least about 40%, 45%, 50%, 55%,
30 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to that biomarker as determined by sequence alignment programs known in the art. The protein and corresponding coding sequence for each of these markers is known in the art, and are further identified in the Sequence Listing provided concurrently herewith.

As used herein, "sequence identity" or "identity" in the context of two polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions that are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity." Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

Unless otherwise stated, sequence identity/similarity values provided herein refer to the value obtained using GAP Version 10 using the following parameters: % identity and % similarity for an amino acid sequence using GAP Weight of 8 and Length Weight of 2, and the BLOSUM62 scoring matrix; or any equivalent program thereof. By "equivalent program" is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical amino acid

residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by GAP Version 10.

Tumor Samples

5 The methods and kits of the present invention find use in typing medulloblastomas from any subject in need thereof. The subject may be a patient undergoing treatment for a recurring incidence of medulloblastoma, or a patient initially diagnosed with a medulloblastoma, and may be a patient for whom eligibility for enrollment into a clinical trial is to be determined. Tumor samples may be obtained
10 from a subject by removing a tissue sample (i.e., biopsy). Methods for collecting biopsy samples from medulloblastomas are well known in the art. In some embodiments, a sample is obtained, for example, from resected tissue. Where a medulloblastoma tissue sample must be stored prior to assaying for the presence or absence of two or more of the β-catenin, GAB1, YAP1, and filamin A biomarker
15 proteins, the sample may be frozen for later preparation or immediately placed in a fixative solution. Tissue samples of the invention may be processed into serial sections using a microtome instrument or a cryostat. Techniques for producing thin sections of a sample by means of a microtome or cryostat are well known in the art (U.S. Patent Application Publication No. 20100118133; Wang *et al.* (2008) *Biotech. Histochem.*
20 83(3):179-189, and Vollmer *et al.* (1989) *J. Steroid Biochem.* 33:41-47). Tumor tissue samples may be transferred to a glass slide for viewing under magnification. Fixative and staining solutions may be applied to the tissues for preserving the specimen and for facilitating examination. In preferred embodiments, the sample is a formalin-fixed, paraffin wax-embedded (FFPE) medulloblastoma tissue sample.

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Immunohistochemical Detection of Biomarker Proteins

In accordance with the immunohistochemical methods of the invention, a protein expression profile is determined for a sample obtained from a medulloblastoma by detecting expression of GAB1, detecting expression of filamin A, or detecting
30 expression of at least two biomarker proteins selected from the group consisting of β-catenin, YAP1, GAB1, and filamin A. The medulloblastoma is then typed as a WNT pathway tumor, a SHH pathway tumor, or a non-WNT/non-SHH tumor based on this protein expression profile, in the manner set forth above. As noted above, any means for detecting expression of a particular biomarker protein of interest is contemplated.

In specific embodiments, expression of a biomarker protein in the medulloblastoma sample is detected by means of a binding protein capable of interacting specifically with that biomarker protein, or a naturally occurring variant or fragment thereof. Preferably, labeled antibodies, binding portions thereof, or other binding partners may be used. The word "label" when used herein refers to a detectable compound or composition that is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition that is detectable.

In particular embodiments, the immunohistochemical typing method comprises contacting a tissue sample obtained from the medulloblastoma with at least two antibodies selected from the group consisting of an antibody that specifically binds β-catenin, an antibody that specifically binds YAP1, an antibody that specifically binds GAB1, and an antibody that specifically binds filamin A; determining a protein expression profile for the tissue sample based on detection of binding of the at least two antibodies to their respective biomarker proteins; and typing the medulloblastoma as a WNT pathway tumor, a SHH pathway tumor, or a non-WNT/non-SHH tumor based on this protein expression profile. By "contacting" in the context of an antibody and a tissue sample is intended the medulloblastoma tissue sample is exposed to the antibody for a sufficient time to allow the formation of a complex between the antibody and its binding partner (i.e., its respective biomarker protein) if that binding partner is present within the cells of the tissue sample.

One of skill in the art will recognize that the immunohistochemical methods described herein below may be performed manually or in an automated fashion using, for example, the Autostainer Universal Staining System (DAKO). One protocol for antibody staining (i.e., immunohistochemistry) of medulloblastoma tissue samples is provided in Examples 2 and 4 below.

In one embodiment, the medulloblastoma tissue sample is collected following tissue resection, as is well known in the art. The sample may be freshly frozen for later preparation or immediately placed in a fixative solution. In this manner, the tissue sample may be fixed by treatment with a reagent such as formalin, paraformaldehyde, gluteraldehyde, methanol, or the like, and embedded in paraffin. Methods for preparing slides for immunohistochemical analysis from fresh-frozen and formalin-fixed paraffin-embedded tissue samples are well known in the art and can be used with the

immunohistochemical typing methods of the present invention.

In some embodiments of the immunohistochemical methods of the invention, a medulloblastoma tissue sample may need to be modified in order to make one or more of the biomarker proteins (i.e., respective antigens), where present, accessible to antibody binding. For example, formalin fixation of tissue samples results in extensive cross-linking of proteins that can lead to the masking or destruction of antigen sites and, subsequently poor antibody staining. As used herein, "antigen retrieval" or "antigen unmasking" refers to methods for increasing antigen accessibility or recovering antigenicity in, for example, formalin-fixed, paraffin-embedded tissue samples. Any method for making antigens more accessible for antibody binding may be used in the practice of the invention, including those antigen retrieval methods known in the art. See, for example, Hanausek and Walaszek, eds. (1998) *Tumor Marker Protocols* (Humana Press, Inc., Totowa, New Jersey); Meera *et al.* (1995) *Eur. J. Morphol.* 33(4):337-358; and Shi *et al.*, eds. (2000) *Antigen Retrieval Techniques: Immunohistochemistry and Molecular Morphology* (Eaton Publishing, Natick, MA), all of which are herein incorporated by reference in their entirety.

Antigen retrieval methods include but are not limited to treatment with proteolytic enzymes (e.g., trypsin, chymoptrypsin, pepsin, pronase, etc.) or antigen retrieval solutions. Antigen retrieval solutions of interest include, for example, citrate buffer or tris buffer. In some embodiments, antigen retrieval comprises applying the antigen retrieval solution to a formalin-fixed tissue sample and then heating the sample in an oven (e.g., 60°C), steamer (e.g., 95°C), or pressure cooker (e.g., 120°C) at specified temperatures for defined time periods. In other aspects of the invention, antigen retrieval may be performed at room temperature. Incubation times will vary with the particular antigen retrieval solution selected and with the incubation temperature. For example, an antigen retrieval solution may be applied to a sample for as little as 5, 10, 20, or 30 minutes or up to overnight. The design of assays to determine the appropriate antigen retrieval solution and optimal incubation times and temperatures is standard and well within the routine capabilities of those of ordinary skill in the art.

Following antigen retrieval, the medulloblastoma tissue sample is blocked using an appropriate blocking agent, e.g., hydrogen peroxide. An antibody directed to a biomarker of interest is then incubated with the sample for a time sufficient to permit antigen-antibody binding.

As noted above, the immunohistochemical typing methods of the present invention detect expression of at least two biomarker proteins selected from the group consisting of β-catenin, GAB1, YAP1, and filamin A. Therefore, in particular embodiments, at least two antibodies, each of which is directed to a distinct biomarker protein, are used to type the medulloblastoma as a WNT pathway tumor, a SHH pathway tumor, or a non-WNT/non-SHH tumor. Each individual antibody may be contacted with a separate tissue section obtained from a single medulloblastoma sample, and the resulting data pooled. Alternatively, these antibodies may be contacted with a single tissue sample section sequentially as individual antibody reagents or simultaneously as an antibody cocktail.

Techniques for detecting antibody binding to a biomarker protein of interest are well known in the art. In this manner, antibody binding to a biomarker protein of interest may be detected through the use of chemical reagents that generate a detectable signal that corresponds to the level of antibody binding and, accordingly, to the level of biomarker protein expression. For example, antibody binding can be detected through the use of a secondary antibody that is conjugated to a labeled polymer. Examples of labeled polymers include but are not limited to polymer-enzyme conjugates. The enzymes in these complexes are typically used to catalyze the deposition of a chromogen at the antigen-antibody binding site, thereby resulting in cell staining that corresponds to expression level of the biomarker protein of interest. Enzymes of particular interest include horseradish peroxidase (HRP) and alkaline phosphatase (AP). Commercial antibody detection systems, such as, for example the DAKO Envision™+ system and Biocare Medical's MACH 3™ system, may be used to practice the present invention.

Detection of antibody binding to a biomarker protein of interest can be facilitated by coupling the antibody to a detectable substance or "label." Examples of detectable substances or labels include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an

example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, Texas Red, AlexaFluor conjugates, and aequorin; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S, or ^{3}H. In some embodiments, antibody binding to a biomarker protein of interest is detected through
5 the use of an HRP-labeled polymer that is conjugated to a secondary antibody.

In particular embodiments where the antibodies are contacted with the medulloblastoma tissue in a cocktail, the antibodies are chosen so they do not cross react with each other. For example, each antibody used may be produced in a different host, such that there are no cross reactivity interactions among the secondary antibodies
10 being used.

Following the contacting of the tissue sample with the antibodies of interest, the mounted tissue sections are stained for antibody binding using, for example, the chromogen 3,3-diaminobenzidine (DAB) ,and then counterstained with hematoxylin and, optionally, a bluing agent such as ammonium hydroxide. In some aspects of the
15 invention, slides are reviewed microscopically by a pathologist to assess cell staining (i.e., biomarker expression) and to determine the protein expression profile for GAB1, filamin A, or at least two biomarkers selected from the group consisting of β-catenin, GAB1, YAP1, and filamin A. Alternatively, the stained tissue sections may be reviewed via automated microscopy or by personnel with the assistance of computer
20 software that facilitates the identification of positive staining cells.

The antibodies used to practice the invention are selected to have specificity for the biomarker proteins of interest. Methods for making antibodies and for selecting appropriate antibodies are known in the art. See, for example, Celis, ed. (2006) *Cell Biology & Laboratory Handbook*, 3rd edition (Elsevier Academic Press, Burlington,
25 MA), which is herein incorporated in its entirety by reference. In some embodiments, commercial antibodies directed to specific biomarker proteins may be used to practice the invention. The antibodies of the invention may be selected on the basis of desirable staining of histological samples. That is, in preferred embodiments the antibodies are selected with the end sample type (e.g., formalin-fixed, paraffin-embedded
30 medulloblastoma tissue samples) in mind and for binding specificity.

One of skill in the art will recognize that optimization of staining reagents and conditions, for example, antibody titer and detection chemistry parameters, is needed to maximize the signal to noise ratio for a particular antibody. Antibody concentrations that maximize specific binding to the biomarkers of the invention and minimize non-

specific binding (or "background") will be determined. In particular embodiments, appropriate antibody titers are determined by initially testing various antibody dilutions on formalin-fixed, paraffin-embedded normal and medulloblastoma tissue samples. The design of assays to optimize antibody titer and detection conditions is standard and well within the routine capabilities of those of ordinary skill in the art. Some antibodies require additional optimization to reduce background staining and/or to increase specificity and sensitivity of staining.

Furthermore, one of skill in the art will recognize that the concentration of a particular antibody used to practice the methods of the invention will vary depending on such factors as time for binding, level of specificity of the antibody for the biomarker protein, and method of body sample preparation. Moreover, when multiple antibodies are used in a single sample, the required concentration may be affected by the order in which the antibodies are applied to the sample, i.e., simultaneously as a cocktail or sequentially as individual antibody reagents. Furthermore, the detection chemistry used to visualize antibody binding to a biomarker of interest must also be optimized to produce the desired signal to noise ratio. Techniques for optimizing staining reagents and conditions for immunohistochemistry are well known in the art.

In order to score biomarker protein expression, the medulloblastoma tissue sample to be examined may be compared with tissue samples from corresponding regions (i.e., cerebellum) obtained from a healthy person, a non-medulloblastoma tissue sample, or another type of brain tumor sample, including those of neuroectodermal origin. That is, the "normal" level of expression is the level of expression of the biomarker protein in, for example, a cerebellar sample from a human subject or patient not afflicted with medulloblastoma. Such a sample can be present in standardized form. In some embodiments, determination of biomarker protein expression requires no comparison between the tumor sample and a corresponding tissue sample that originates from a healthy person. In this manner, detection of expression of at least two biomarker proteins, or at least three biomarker proteins selected from the group consisting of β-catenin, GAB1, YAP1, and filamin A, or detection of expression of all four of these biomarker proteins yields a protein expression profile that is indicative of a molecular group of medulloblastoma, i.e., a WNT pathway tumor, a SHH pathway tumor, or a non-WNT/non-SHH tumor, and may preclude the need for comparison to a corresponding tissue sample that originates from a healthy person. Control samples may also be positive controls or negative controls. By "positive controls" is intended

samples and tissues known to show a positive expression profile for one or more of the biomarker proteins of the invention, such as tissue and microarray samples from other tissues and cancerous tissues including but not limited to medulloblastoma, colon, placenta, tonsil, and appendix, or an internal positive control such as intrinsic vascular elements.

Also encompassed in the immunohistochemical methods of the invention is a method for validating or verifying the accurate performance of the typing method, wherein the method comprises processing the biological sample, detecting the expression of the biomarkers of interest, and comparing the expression of the biomarker or biomarkers of interest in the sample to control samples. In some embodiments of the invention, the biological and control tissue samples are affixed to the same microscope slide, where both the tissue sample and respective control sample are subjected to the same condition of reagents, same time, and temperature. In other embodiments, the control sample is fixed, for example by formalin, paraformaldehyde, gluteraldehyde, methanol, or the like and embedded in paraffin, affixed onto the microscope slide with its corresponding biological sample, and subjected to the same conditions as the biological sample.

One of skill in the art will appreciate that any or all steps in the immunohistochemical typing methods of the invention could be implemented by personnel or, alternatively, performed in an automated fashion. Thus, the steps of medulloblastoma sample preparation, sample staining, and detection of biomarker expression may be automated. Moreover, in some embodiments, the immunohistochemical methods of the invention are used in conjunction with computerized imaging equipment and software to facilitate the identification of positive-staining cells by a pathologist.

Production of Antibodies for Use in the Medulloblastoma Typing Methods of the Invention

The immunohistochemical methods of the invention contemplate the use of antibodies that specifically bind to β-catenin, GAB1, YAP1, or filamin A in order to detect expression of one or more of these biomarker proteins. By "specifically binds" is intended the antibody binds to an epitope of the particular biomarker protein of interest, and does not bind to another biomarker protein of interest. By "epitope" is

intended the part of an antigenic molecule to which an antibody is produced and to which the antibody will bind.

The terms "antibody" and "antibodies" broadly encompass naturally occurring forms of antibodies and recombinant antibodies such as single-chain antibodies, chimeric and humanized antibodies and multi-specific antibodies as well as fragments and derivatives of all of the foregoing, which fragments and derivatives have at least an antigenic binding site. Thus, the term "antibody" is used in the broadest sense and covers fully assembled antibodies, antibody fragments that can bind antigen (e.g., Fab', $F'(ab)_2$, Fv, single chain antibodies, diabodies), and recombinant peptides comprising the foregoing. As previously noted, any antibody intended for use in the methods and kits of the present invention may be labeled with a detectable substance to facilitate biomarker protein detection in the medulloblastoma sample.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen-binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')2, and Fv fragments; diabodies; linear antibodies (Zapata *et al.* (1995) *Protein Eng.* 8(10):1057-1062); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize 35 readily. Pepsin treatment yields an F(ab')2 fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment that contains a complete antigen recognition and binding site. In a two-chain Fv species, this region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv species, one heavy- and one light-chain variable domain can be covalently linked by flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable

domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (C_H1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy-chain C_H1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')2 antibody fragments originally were produced as pairs of Fab' fragments that have hinge cysteines between them.

Monoclonal antibodies can be prepared using the method of Kohler *et al.* (1975) *Nature* 256:495-496, or a modification thereof. Typically, a mouse is immunized with a solution containing an antigen. Immunization can be performed by mixing or emulsifying the antigen-containing solution in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally. Any method of immunization known in the art may be used to obtain the monoclonal antibodies of the invention. After immunization of the animal, the spleen (and optionally, several large lymph nodes) are removed and dissociated into single cells. The spleen cells may be screened by applying a cell suspension to a plate or well coated with the antigen of interest. The B cells expressing membrane bound immunoglobulin specific for the antigen bind to the plate and are not rinsed away. Resulting B cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium. The resulting cells are plated by serial dilution and are assayed for the production of antibodies that specifically bind the antigen of interest (and that do not bind to unrelated antigens). The selected monoclonal antibody (mAb)-secreting hybridomas are then cultured either *in vitro* (e.g., in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

As an alternative to the use of hybridomas, antibody can be produced in a cell line such as a CHO cell line, as disclosed in U.S. Patent Nos. 5,545,403; 5,545,405; and 5,998,144; incorporated herein by reference. Briefly the cell line is transfected with vectors capable of expressing a light chain and a heavy chain, respectively. By transfecting the two proteins on separate vectors, chimeric antibodies can be produced. Another advantage is the correct glycosylation of the antibody. A monoclonal antibody can also be identified and isolated by screening a recombinant combinatorial

immunoglobulin library (e.g., an antibody phage display library) with a biomarker protein to thereby isolate immunoglobulin library members that bind the biomarker protein. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP϶ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent No. 5,223,409; PCT Publication Nos. WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; 93/01288; WO 92/01047; 92/09690; and 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J.* 12:725-734.

Polyclonal antibodies can be prepared by immunizing a suitable subject (e.g., rabbit, goat, mouse, or other mammal) with a biomarker protein immunogen. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized biomarker protein. At an appropriate time after immunization, e.g., when the antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497, the human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol. Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985) in *Monoclonal Antibodies and Cancer Therapy*, ed. Reisfeld and Sell (Alan R. Liss, Inc., New York, NY), pp. 77-96) or trioma techniques. The technology for producing hybridomas is well known (*see* generally Coligan *et al.*, eds. (1994) *Current Protocols in Immunology* (John Wiley & Sons, Inc., New York, NY); Galfre *et al.* (1977) *Nature* 266:55052; Kenneth (1980) in *Monoclonal Antibodies: A New Dimension In Biological Analyses* (Plenum Publishing Corp., NY; and Lerner (1981) *Yale J. Biol. Med.* 54:387-402).

In another embodiment, antibodies for use in the methods of the invention can be obtained commercially from any source. Exemplary antibodies include, but are not limited to, β-catenin antibody (#610154 and others, available from BD Biosciences, USA, as well as catalog #760-4242, available from Ventana, USA); GAB1 antibody (#ab27439 and others, available from Abcam, USA); filamin A antibody (#10R-F113A

and others, available from Fitzgerald, USA), and YAP1 antibody (#sc-101199 and others, available from Santa Cruz Biotechnology, USA).

Further Subtyping of Medulloblastoma Samples

5 The results of the immunohistochemical methods described above can be combined with assessment of clinical information, conventional diagnostic methods, and expression of other molecular markers (for example, but not limited to, Ki67, p27 etc.) known in the art to be involved in the etiology of medulloblastoma, should such combined information be deemed beneficial for determination of a treatment plan for 10 the subject whose medulloblastoma has been analyzed and typed as a WNT pathway tumor, a SHH pathway tumor, or a non-WNT/non-SHH tumor in accordance with the immunohistochemical typing methods of the present invention.

Thus, the immunohistochemical methods of the present invention can be further combined with the standard histological preparations (hematoxylin & eosin) to assess 15 general architectural and cytological features, including nodule formation, differentiation along neuronal (neurocytic / ganglionic) and astrocytic lines, and large cell or anaplastic phenotypes. In other embodiments of the invention, reticulin preparations are used to evaluate desmoplasia. Thus, for example, detection of internodular desmoplasia is required for histologic typing of D/N medulloblastoma, 20 including the paucinodular D/N variant and MBEN.

In like manner, any biomarker whose expression is indicative of a particular molecular subgroup of medulloblastoma can be used in conjunction with the methods of the present invention. Such biomarkers include genes and proteins that are, for example, involved in cell proliferation, cell cycle control, or the generalized 25 mechanisms of cancer motility and invasion. Biomarkers of potential interest can include, but are not limited to, for example, Ki67 and p27 (see for example de Haas *et al.* (2008) *Clin. Cancer Res.* 14(13):4154-4160; and Thompson *et al.* (2006) *J. Clin. Oncol.* 24(12):1924-1931; Kool *et al.* (2008) PLoS. 3(8):e3088; all of which are herein incorporated by reference in their entirety).

30 Expression of these additional biomarker proteins can be detected using any of the standard detection methods known in the art, and include detection at the protein or nucleic acid level. Such methods are well known in the art and include but are not limited to western blots, northern blots, ELISA, immunoprecipitation, immunofluorescence, flow cytometry, immunohistochemistry, nucleic acid

hybridization techniques, for example, in situ hybridization (ISH), nucleic acid reverse transcription methods, nucleic acid amplification, and gene sequencing methods. Thus, for example, where detection is at the level of the protein, detection can utilize antibodies that are directed against these additional biomarker proteins. These antibodies can be used in various methods such as Western blot, ELISA, multiplexing technologies, immunoprecipitation, or immunohistochemistry techniques.

In some embodiments, it may be desirable to further determine the genetic abnormalities that contribute to the particular molecular subgroup into which the medulloblastoma has been typed. For example, DNA ISH can be used to determine the status of specific loci on chromosomes. Sample cells and tissues are usually treated to fix the target transcripts in place and to increase access of the probe. The probe hybridizes to the target sequence at elevated temperature, and then the excess probe is washed away. The probe that was labeled with either radio-, fluorescent- or antigen-labeled bases is localized and quantitated in the tissue using autoradiography, fluorescence microscopy or immunohistochemistry, respectively. ISH can also use two or more probes, labeled with radioactivity or the other non-radioactive labels, to simultaneously detect two or more transcripts. For example, the genetic abnormalities may be detected using fluorescence in situ hybridization (FISH).

As used herein, a "probe" is an isolated polynucleotide to which is attached a conventional detectable label or reporter molecule, e.g., a radioactive isotope, ligand, chemiluminescent agent, enzyme, etc. Such a probe is complementary to a strand of a target polynucleotide, which in specific embodiments of the invention comprise a polynucleotide comprising regions of loci known to harbor chromosomal copy number abnormalities (CNAs) in medulloblastomas. Particular embodiments include, but are not restricted to, chromosome 6 (including SGK1), chromosome 17 (including *HIC1*), chromosome 9, *PTCH1*, *MYC*, and *MYCN*. Deoxyribonucleic acid probes can include those generated by PCR using specific primers to the regions listed above, olignucleotide probes synthesized *in vitro*, or DNA obtained from bacterial artificial chromosome or fosmid libraries. Probes include not only deoxyribonucleic or ribonucleic acids but also polyamides and other probe materials that can specifically detect the presence of the target DNA sequence. For nucleic acid probes, examples of detection reagents include, but are not limited to radiolabeled probes, enzymatic labeled probes (horse radish peroxidase, alkaline phosphatase), affinity labeled probes (biotin, avidin, or steptavidin), and fluorescent labeled probes (6-FAM, VIC, TAMRA, MGB).

One skilled in the art will readily recognize that the nucleic acid probes described in the present invention can readily be incorporated into one of the established kit formats described herein below.

As used herein, "primers" are isolated polynucleotides that are annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a polymerase, e.g., a DNA polymerase. Primer pairs refer to their use for amplification of a target polynucleotide, e.g., by the polymerase chain reaction (PCR) or other conventional nucleic-acid amplification methods. "PCR" or "polymerase chain reaction" is a technique used for the amplification of specific DNA segments (see, U.S. Pat. Nos. 4,683,195 and 4,800,159; herein incorporated by reference).

Probes and primers are of sufficient nucleotide length to bind to the target DNA sequence and specifically detect and/or identify a polynucleotide comprising the region of interest with the genetic abnormality. It is recognized that the hybridization conditions or reaction conditions can be determined by the operator to achieve this result. This length may be of any length that is of sufficient length to be useful in a detection method of choice. Generally, 8, 11, 14, 16, 18, 20, 22, 24, 26, 28, 30, 40, 50, 75, 100, 200, 300, 400, 500, 600, 700 nucleotides or more, or between about 11-20, 20-30, 30-40, 40-50, 50-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, or more nucleotides in length are used. Such probes and primers can hybridize specifically to a target sequence under high stringency hybridization conditions. Probes and primers according to embodiments of the present invention may have complete DNA sequence identity of contiguous nucleotides with the target sequence, although probes differing from the target DNA sequence and that retain the ability to specifically detect and/or identify a target DNA sequence may be designed by conventional methods. Accordingly, probes and primers can share about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater sequence identity or complementarity to the target polynucleotide. Probes can be used as primers, but are generally designed to bind to the target DNA or RNA and are not used in an amplification process.

Methods for preparing and using probes and primers are described, for example, in *Molecular Cloning: A Laboratory Manual,* 2.sup.nd ed, vol. 1-3, ed. Sambrook *et al.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1989 (hereinafter, "Sambrook *et al.*, 1989"); *Current Protocols in Molecular Biology*, ed. Ausubel *et al.*,

Greene Publishing and Wiley-Interscience, New York, 1992 (with periodic updates) (hereinafter, "Ausubel *et al.*, 1992"); and Innis *et al.,* PCR Protocols: A Guide to Methods and Applications, Academic Press: San Diego, 1990. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended
5 for that purpose such as the PCR primer analysis tool in Vector NTI version 10 (Informax Inc., Bethesda Md.); PrimerSelect (DNASTAR Inc., Madison, Wis.); and Primer (Version 0.5.COPYRGT., 1991, Whitehead Institute for Biomedical Research, Cambridge, Mass.). Additionally, the sequence can be visually scanned and primers manually identified using guidelines known to one of skill in the art.

10 In specific embodiments, probes for detecting a genetic abnormality in medulloblastomas are labeled with appropriate fluorescent or other markers and then used in hybridizations. Example 6 provided herein sets forth one protocol that can be used with the present invention, which effectively detects the genetic abnormalities, but one of skill in the art will recognize that many variations of these assay can be used
15 equally well. Specific protocols are well known in the art and can be readily adapted for the present invention. Guidance regarding methodology may be obtained from many references including: *In situ Hybridization: Medical Applications* (eds. G. R. Coulton and J. de Belleroche), Kluwer Academic Publishers, Boston (1992); *In situ Hybridization: Neurobiology; Advances in Methodology* (eds. J. H. Eberwine, K. L.
20 Valentino, and J. D. Barchas), Oxford University Press Inc., England (1994); *In situ Hybridization: A Practical Approach* (ed. D. G. Wilkinson), Oxford University Press Inc., England (1992)); Kuo *et al.* (1991) *Am. J. Hum. Genet.* 42:112-119; Klinger *et al.* (1992) *Am. J. Hum. Genet.* 51:55-65; and Ward *et al.* (1993) *Am. J. Hum. Genet.* 52:854-865). There are also kits that are commercially available and that provide
25 protocols for performing FISH assays (available from e.g., Oncor, Inc., Gaithersburg, MD). Patents providing guidance on methodology include U.S. 5,225,326; 5,545,524; 6,121,489 and 6,573,043. All of these references are hereby incorporated by reference in their entirety and may be used along with similar references in the art and with the information provided in the Examples section herein to establish procedural steps
30 convenient for a particular laboratory.

Thus, the methods disclosed herein can also be combined with other prognostic methods or analyses, including, but not limited to, tumor size, expression levels of Ki67, p53, $p27^{Kip1}$, synaptophysin, MYC, MYCN, GFAP, CCNB1, LDHB, TrkC, AXIN1 family members, Akt, Pin1, Notch2, and/or NeuN, and molecular cytogenetic

data identifying CNAs, for example, in chromosome 6 (including *SGK1*), chromosome 9 (including *PTCH1* deletions) and chromosome 17 (including *HIC1*), and the like.

Assessing the Outcome of Medulloblastoma Patients

5 The accurate assessment of disease risk remains a major goal in children with medulloblastoma. Although no specific biochemical test exists for assessing the presence of medulloblastoma, histologically identical medulloblastomas are composed of distinct subgroups with different prognoses. For example, the expression of Erb2 is indicative of a poor outcome, while expression of TrkC or neurotrophin-3 receptor is 10 indicative of a good outcome. Additionally, gene mutations impairing the WNT signaling transduction pathway have been found in approximately 15% of human sporadic medulloblastomas, and 25% of cases associated with a favorable disease outcome (Clifford *et al.* (2006) *Cell Cycle* 5(22):2666-2670). Combined factors, among others, that contribute to a favorable outcome in medulloblastoma patients 15 include age <3 years, complete resection of the tissue, WNT/Wg pathway activation, nuclear β-catenin expression, and monosomy of chromosome 6. Combined factors, among others, that contribute to poor or bad outcomes in medulloblastoma patients include age <3 years, residual disease > 1.5 cm^2 after resection, metastasis, chromosome 17p loss, as well as expression of Erb B2 receptor, C-myc, and OTX (20 Mueller and Chang (2009) *Neurotherapeutics* 6(3):570-586).

The immunohistochemical typing methods of the present invention offer insight into the prognosis and potential stratification of medulloblastoma patients by rapidly categorizing their medulloblastomas as being WNT pathway tumors, SHH pathway tumors, or non-WNT/non-SHH tumors. In this manner, medulloblastomas typed as 25 WNT pathway tumors are indicative of a good outcome following standard therapy or treatment with a WNT signaling pathway inhibitor, particularly when combined with other factors that contribute to a favorable outcome. As used herein, "indicative of a good outcome" refers to an increased likelihood that the patient will remain cancer free following treatment. In some embodiments, "indicative of a good outcome" refers to 30 an increased likelihood that the patient will remain cancer free for at least five, more particularly at least ten years. In one embodiment, Patients having a medulloblastoma typed as a WNT pathway tumor in accordance with the immunohistochemical typing methods of the present invention advantageously can be treated using less aggressive therapeutic regimens.

Alternatively, medulloblastomas typed as SHH pathway tumors or non-WNT/non-SHH tumors have a worse outcome, relative to medulloblastomas of the WNT pathway tumor subgroup, particularly when combined with other clinical, pathologic, and molecular factors that contribute to an unfavorable outcome. By "indicative of a poor outcome" is intended an increased likelihood of relapse or recurrence of the underlying cancer or tumor, metastasis, or death following treatment. For example, "indicative of a poor outcome" may refer to an increased likelihood or relapse or recurrence of the underlying cancer or tumor, metastasis, or death within five years, more particularly ten years post-treatment. Patients having a medulloblastoma typed as a SHH pathway tumor in accordance with the immunohistochemical typing methods of the present invention advantageously can be treated with specific therapeutic regimens that inhibit the SHH signaling pathway (for example, SHH signaling pathway inhibitors). This is also true for WNT-pathway tumors and specific WNT-pathway antagonists. Patients having a medulloblastoma typed as a non-WNT/non-SHH tumor in accordance with the immunohistochemical typing methods of the present invention advantageously can be identified as needing standard treatment protocols (i.e., surgery, radiation, and chemotherapy) using more aggressive approaches based on clinical or pathological presentation (for example, histologic subtype or molecular cytogenetic data).

The immunohistochemical typing methods of the present invention thus provide new means for quickly identifying beneficial/optimal treatment regimens for medulloblastoma patients that allow more appropriate allocation to standard treatment regimens, the potential application of targeted therapies, and the possibility of reducing long-term adverse events. Accordingly, the invention can lead to an improvement in a patient's overall survival as well as their event-free survival. As used herein, the term "overall survival" refers to the chances of staying alive for a group of individuals suffering from medulloblastoma. It denotes the percentage of individuals in the group who are likely to be alive after a particular duration of time. At a basic level, the overall survival is representative of cure rates. Five-year rates are reported for many cancers because those who survive five years are quite likely to be cured of their disease. In some slow growing and low grade malignancies where late relapses are common, the ten-year overall survival is more representative of cure rates. As used herein, the term "event-free survival" refers to the possibility of having a particular

group of defined events, such as metastases, recurrence, or death, after a treatment that is designed to delay or prevent that group of events.

Determining Eligibility for Entry into Clinical Trials and Selecting Treatment Regimens

In medulloblastoma patients, the standard front-line therapy includes adjuvant, platinum-based chemotherapy. However, cisplatin- and carboplatin-associated toxicity produces serious adverse effects. Therefore, there is a need in the art to find novel therapies for treating medulloblastoma patients. Many small molecule inhibitors of the SHH or WNT signaling pathway with the potential to treat a subset of medulloblastoma patients are being considered for, or are being tested in, clinical trials. These include, but are not limited to, the SHH signaling pathway inhibitors GDC-0449, LDE225, LEQ506, and those disclosed in U.S. Patent No. 7,498,304 and International Patent Application Publication No. WO 2009/132023 A2, which describe SHH signaling pathway inhibitors. See also, for example, U.S. Patent Application Publication Nos. 20100203113 and 20100137394, describing WNT signaling pathway inhibitors. The methods of the present invention provide a means to identify patients with medulloblastomas within molecular subgroups that can benefit from experimental treatments with such signaling pathway inhibitors. In a further embodiment, the present invention identifies a patient that can benefit from a clinical trial for treating medulloblastomas with a WNT pathway or SHH pathway inhibitor.

The methods of the invention can therefore be used to assist in selecting appropriate courses of treatment and to identify patients that would benefit from more or less aggressive therapy, or new avenues of therapy. In particular embodiments, the immunohistochemical techniques of the present invention are used to identify a medulloblastoma as being regulated by the WNT or SHH signaling pathway, and thus a tumor that could favorably respond to existing therapeutic agents, or experimental therapeutic agents, that are inhibitors of the WNT or SHH signaling pathway, respectively. In this manner, the immunohistochemical typing methods of the invention provide a means to identify subjects with a medulloblastoma of the WNT pathway tumor subgroup or the SHH pathway tumor subgroup that renders the subject eligible for enrollment into appropriate clinical trials. In other embodiments, the immunohistochemical typing methods of the invention provide a means to identify subjects with a medulloblastoma of the non-WNT/non-SHH tumor subgroup, who

would not be responsive to existing therapeutic agents, or experimental therapeutic agents, that are inhibitors the WNT or SHH signaling pathway. Rather, in the latter case, these patients would be identified as needing treatment regimens suitable for treatment of this molecular subgroup of medulloblastomas.

5 The immunohistochemical typing methods disclosed herein also find use in identifying a patient with a medulloblastoma that would benefit from a selected treatment. By “identifying a patient with a medulloblastoma that would benefit from a selected treatment” is intended assessing the likelihood that a patient will experience a positive or negative outcome with a particular treatment and potentially reducing the 10 long-term adverse events by optimization of current therapies.. Thus, the immunohistochemical typing methods of the inventions provide a means to identify possible treatments that could result in a positive or a negative outcome. As used herein, “result in a positive treatment outcome” refers to an increased likelihood that the patient will experience beneficial results from the selected treatment (e.g., complete 15 or partial remission, reduced tumor size, increased survival, etc.). By “result in a negative treatment outcome” is intended an increased likelihood that the patient will not benefit from the selected treatment with respect to the progression of the medulloblastoma.

In certain embodiments of the present invention, typing a medulloblastoma as a 20 WNT pathway tumor identifies a patient with a medulloblastoma that will have a positive outcome to standard therapy (i.e., surgery, radiation or chemotherapy) and/or to therapy with a WNT signaling pathway inhibitor. In other embodiments, typing a medulloblastoma as a SHH pathway tumor identifies a patient with a medulloblastoma that will have a positive outcome to therapy with a SHH signaling pathway inhibitor. 25 In yet other embodiments, typing a medulloblastoma as a non-WNT/non-SHH tumor identifies a patient with a medulloblastoma that will have a negative treatment outcome with a WNT or SHH signaling pathway inhibitor, and for which standard therapy with a more aggressive treatment regimen based on clinical or pathological presentation is warranted to achieve a positive treatment outcome.

30

Kits

Kits for practicing the methods of the invention are further provided. By “kit” is intended any manufacture (e.g., a package or a container) comprising at least one reagent, e.g. an antibody, for specifically detecting the expression of a biomarker of the

invention. The kit may be promoted, distributed, or sold as a unit for performing the methods of the present invention. Additionally, the kits may contain a package insert describing the kit and methods for its use.

In particular embodiments, kits for practicing the immunohistochemical methods of the invention are provided. Such kits are compatible with both manual and automated immunohistochemistry techniques for subtyping medulloblastoma (e.g., cell staining) as described herein. In some embodiments, these kits comprise at least two antibodies selected from the group consisting of an antibody that specifically binds β-catenin, an antibody that specifically binds YAP1, an antibody that specifically binds GAB1, and an antibody that specifically binds filamin A. In some of these embodiments, the kits comprise an antibody that specifically binds β-catenin and an antibody that specifically binds GAB1, YAP1, or filamin A. In other embodiments, these kits comprise three antibodies selected from the group consisting of an antibody that specifically binds β-catenin, an antibody that specifically binds YAP1, antibody that specifically binds GAB1, and an antibody that specifically binds filamin A. In some of these embodiments, the kits comprise an antibody that specifically binds β-catenin, an antibody that specifically binds GAB1, and an antibody that specifically binds YAP1. In yet other embodiments, these kits comprise an antibody that specifically binds β-catenin, an antibody that specifically binds YAP1, an antibody that specifically binds GAB1, and an antibody that specifically binds filamin A.

Each antibody may be provided in the kit as an individual reagent or, alternatively, as an antibody cocktail comprising two or more of the antibodies directed to these different biomarker proteins of interest. Furthermore, any or all of the kit reagents may be provided within containers that protect them from the external environment, such as in sealed containers or contains that protect the reagents from light. Positive and/or negative controls may be included in the kits to validate the activity and correct usage of reagents employed in accordance with the invention. Controls may include samples, such as tissue sections, cells fixed on glass slides, etc., known to be either positive or negative for the presence of the biomarker protein of interest. The design and use of controls is standard and well within the routine capabilities of those of ordinary skill in the art. Chemicals for the detection of antibody binding to the biomarker, a counterstain, and a bluing agent to facilitate identification of positive staining cells are optionally provided.

Alternatively, the immunochemistry kits of the present invention are used in conjunction with commercial antibody binding detection systems, such as, for example the DAKO Envision™+ system (for example, DAKO EnVision™+ System, HRP) and Biocare Medical's MACH 3™ system (for example, MACH3 detection kits labeled
5 with either HRP or alkaline phosphatase (AP). Any chemicals that detect antigen-antibody binding may be used in the practice of the invention. In some embodiments, the detection chemicals comprise a labeled polymer conjugated to a secondary antibody. For example, a secondary antibody that is conjugated to an enzyme that catalyzes the deposition of a chromogen at the antigen-antibody binding site may be
10 provided. Such enzymes and techniques for using them in the detection of antibody binding are well known in the art. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or
15 acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include
20 luciferase, luciferin, Texas Red, AlexaFluor conjugates, and aequorin; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S, or ^{3}H.

In one embodiment, the kit comprises a secondary antibody that is conjugated to an HRP-labeled polymer. Chromogens compatible with the conjugated enzyme (e.g., DAB in the case of an HRP-labeled secondary antibody) and solutions, such as
25 hydrogen peroxide, for blocking non-specific staining may be further provided. The kits of the present invention may also comprise a counterstain, such as, for example, hematoxylin or hematoxilyn and eosin. A bluing agent (e.g., ammonium hydroxide) may be further provided in the kit to facilitate detection of positive staining cells.

Exemplary Embodiments:

30 One embodiment of the invention includes an immunohistochemical method for typing a medulloblastoma as a WNT pathway tumor, a sonic hedgehog (SHH) pathway tumor, or a non-WNT/non-SHH tumor, said method comprising determining a protein expression profile for a sample obtained from said medulloblastoma by detecting

expression of at least two biomarker proteins selected from the group consisting of β-catenin, YAP1, GAB1, and filamin A, and typing said medulloblastoma as a WNT pathway tumor, a SHH pathway tumor, or a non-WNT/non-SHH tumor based on said protein expression profile.

5 In another embodiment, the immunohistochemical method further comprises a) contacting a tissue sample obtained from said medulloblastoma with at least two antibodies selected from the group consisting of an antibody that specifically binds β-catenin, an antibody that specifically binds YAP1, an antibody that specifically binds GAB1, and an antibody that specifically binds filamin A; b) determining a protein
10 expression profile for said tissue sample based on detection of binding of said at least two antibodies to said biomarker proteins; and c) typing said medulloblastoma as a WNT pathway tumor, a SHH pathway tumor, or a non-WNT/non-SHH tumor based on said protein expression profile.

In yet another embodiment the immunohistochemical method includes at least
15 one antibody that specifically binds β-catenin, wherein said protein expression profile is characterized by positive nuclear expression of β-catenin, wherein said medulloblastoma is typed as a WNT pathway tumor.

In yet another embodiment of the invention, the immunohistochemical method includes at least one antibody that specifically binds β-catenin, wherein said protein
20 expression profile is characterized by negative nuclear expression of β-catenin. The method can further comprise, evaluation of a protein expression profile characterized by positive and/or negative expression of one or more additional biomarker proteins selected from the group consisting of filamin A, GAB1, and YAP1, wherein positive expression of one or more biomarker proteins in a medulloblastoma is typed as a SHH
25 pathway tumor. Alternatively, wherein said protein expression profile is characterized by negative expression of one or more additional biomarker proteins selected from the group consisting of filamin A, GAB2, and YAP1, said medulloblastoma is typed as a non-WNT/non-SHH tumor.

In yet another embodiment of the invention, the immunohistochemical method
30 includes at least one antibody that specifically binds GAB1, wherein a protein expression profile is characterized by positive expression of GAB1, and the medulloblastoma is typed as a SHH pathway tumor. Alternatively, where the protein expression profile is characterized by negative expression of GAB1, and said protein expression profile is characterized by positive nuclear expression of β-catenin, the

medulloblastoma is typed as a WNT pathway tumor. In yet another embodiment, the protein expression profile is characterized by negative nuclear expression of β-catenin, the medulloblastoma is typed as a non-WNT/non-SHH pathway-regulated tumor.

In embodiments of the invention a sample is contacted with at least three antibodies, wherein said antibodies are selected from the group consisting of an antibody that specifically binds to β-catenin, an antibody that specifically binds to YAP1, an antibody that specifically binds to GAB1, or an antibody that specifically binds to filamin A.

In yet other embodiments, a sample is contacted with an antibody that specifically binds to β-catenin, an antibody that specifically binds to YAP1, an antibody that specifically binds to GAB1, and an antibody that specifically binds to filamin A. It is contemplated that in any of the aforementioned embodiments a sample can be contacted with said antibodies sequentially, or alternatively a sample can be contacted with said antibodies simultaneously

One embodiment of the invention is an immunohistochemical method for typing a medulloblastoma as a SHH pathway tumor or a non-SHH tumor, the method comprising determining a protein expression profile for a sample obtained from a medulloblastoma by detecting expression of GAB1 and typing said medulloblastoma as a SHH pathway tumor or a non-SHH tumor based on said protein expression profile. In one embodiment, the protein expression profile is characterized by positive expression of GAB1, wherein said medulloblastoma is typed as a SHH pathway tumor. Alternatively, the protein expression profile is characterized by negative expression of GAB1, wherein said medulloblastoma is typed as a non-SHH tumor.

In another embodiment of the invention, an immunohistochemical method for typing a medulloblastoma as a non-WNT/non-SHH tumor is disclosed; the method comprising determining a protein expression profile for a sample obtained from said medulloblastoma by detecting expression of filamin A, and typing said medulloblastoma as a non-WNT/non-SHH tumor based on said protein expression profile. In one embodiment, the said protein expression profile is characterized by negative expression of filamin A, and said medulloblastoma is typed as a non-WNT/non-SHH tumor.

An embodiment of the invention also includes a method of identifying a subject with a medulloblastoma that would benefit from treatment with a WNT signaling pathway inhibitor or a sonic hedgehog (SHH) pathway signaling inhibitor, said method

comprising typing said medulloblastoma as a WNT pathway tumor according to the aforementioned methods, or typing said medulloblastoma as a SHH pathway tumor according to the methods above thereby identifying said subject. The embodiment of the can further comprise treating said subject with said WNT signaling pathway
5 inhibitor when said medulloblastoma is typed as a WNT pathway tumor or with a SHH signaling pathway inhibitor when said medulloblastoma is typed as a SHH pathway tumor.

In yet another embodiment a method for selecting a therapy for a subject with a medulloblastoma is disclosed; the method comprising typing a sample obtained from a
10 medulloblastoma according to the method of any one of the aforementioned embodiments and selecting a therapy based on the typing.

In one embodiment a method for determining whether a subject is eligible for entry into a clinical trial for treating medulloblastomas regulated by the WNT signaling pathway is included, comprising typing a medulloblastoma in said subject according to
15 the method of any one of embodiments discussed herein, where said subject is eligible if said medulloblastoma is typed as a WNT pathway tumor. Alternatively, a method for determining whether a subject is eligible for entry into a clinical trial for treating medulloblastomas regulated by the SHH signaling pathway is disclosed, comprising typing a medulloblastoma in said subject according to the method of any one of
20 aforementioned embodiments discussed herein. Accordingly, the subject is eligible if said medulloblastoma is typed as a SHH pathway tumor.

Another embodiment of the invention is a method for determining whether a subject is eligible for entry into a clinical trial for treating medulloblastomas that are non-WNT/non-SHH tumors, comprising typing a medulloblastoma in said subject
25 according to the method of any one of the aforementioned embodiments disclosed herein. Accordingly, the subject is eligible if said medulloblastoma is typed as a non-WNT/non-SHH tumor.

One embodiment of the invention comprises a kit for typing a medulloblastoma as a WNT pathway tumor, a sonic hedgehog (SHH) pathway tumor, or a non-
30 WNT/non-SHH tumor, the kit comprising at least two antibodies selected from the group consisting of an antibody that specifically binds β-catenin, an antibody that specifically binds YAP1, an antibody that specifically binds GAB1, or an antibody that specifically binds filamin A. In a preferred embodiment, the kit can comprise at least three of said antibodies, for example, an antibody that specifically binds β-catenin, an

antibody that specifically binds YAP1, an antibody that specifically binds GAB1, or an antibody that specifically binds filamin A. Optionally, the kit of any one of embodiments can further comprise reagents for the detection of antibody binding to said β-catenin, YAP1, GAB1, filamin A, or any combination thereof. The kit of any one of the embodiments can further comprise at least one positive control sample. It is also contemplated that the kit of any one of embodiments can further comprise instructions for use.

The article "a" and "an" are used herein to refer to one or more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one or more element.

Throughout the specification the word "comprising," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Example 1

Study tumor cohorts

The main study materials consisted of FFPE tissues from 235 medulloblastomas, representing primary surgical resections from children treated on the SIOP / UKCCSG CNS9102 (PNET3) and CNS9204 trials and from other infants (aged <3 years) and adults (aged >16 years) treated at Washington University, St. Louis, the Children's Hospital of Los Angeles, and Emory University, Atlanta. The cohort's demographics match those of other previously reported medulloblastoma patient populations. (See Ellison DW, Dalton J, Kocak M, *et al*: Medulloblastoma: clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups. *Acta Neuropathol* 121:381-96, 2011; herein incorporated by reference in its entirety).

A separate series of pediatric medulloblastomas (n=26) was used to validate the immunohistochemical assay, the total being limited by availability of FFPE tissue. Gene expression data (Affymetrix U133Av2) were available for the cohort (n=46) from which this validation set was derived.

Example 2

Histology and Immunohistochemistry

Standard histological preparations (hematoxylin & eosin) were used to assess general architectural and cytological features, including nodule formation, differentiation along neuronal (neurocytic / ganglionic) and astrocytic lines, and large cell or anaplastic phenotypes.

Reticulin preparations were used to evaluate desmoplasia. Internodular desmoplasia was required for a diagnosis of D/N medulloblastoma, including the paucinodular D/N variant, and MBEN. The paucinodular D/N medulloblastoma displays scattered small nodules amid widespread desmoplasia. Intranodular cells in this variant uncommonly demonstrate the differentiated neurocytic phenotype of the conventional D/N tumor, but do express neuronal proteins and show low Ki-67 immunolabeling. The MBEN is defined by its large irregularly shaped nodules, pronounced internodular neurocytic differentiation, and sparse internodular desmoplastic regions.

As defined by the WHO classification of CNS tumors and restated in criteria adopted for COG trials in North America, the anaplastic medulloblastoma shows marked cytological pleomorphism across most of its area, in association with high mitotic and apoptotic counts. The large cell medulloblastoma is defined by its groups of uniform large round cells with a single nucleolus, in most cases admixed with groups of anaplastic cells. Large cell and anaplastic tumors were combined in study datasets as LC/A tumors.

Immunohistochemistry was undertaken according to established protocols with antibodies to GFAP (DAKO #M0761; 1:250), synaptophysin (Leica Microsystems #NCL-L-Synap-299; 1:400), NEU-N (Chemicon #MAB377; 1:10,000), $p27^{Kip1}$ (DAKO #M7203; 1:50), and Ki-67 (DAKO #M7240; 1:200).

SHH pathway tumors, WNT pathway tumors, and non-SHH/non-WNT tumors were disclosed by immunohistochemistry using a combination of four antibodies: β-catenin (BD #610154; 1:800; antigen retrieval - citrate buffer 20mins Bond), GAB1 (Abcam #ab27439; 1:50; antigen retrieval - citrate buffer 20mins Bond), filamin A (Fitzgerald #10R-F113A; 1:100; antigen retrieval - TRIS buffer 30mins Benchmark XT), and YAP1 (Santa Cruz #sc-101199; 1:50; antigen retrieval - citrate buffer 20mins Bond). Positive control tissues for these antibodies were: β-catenin - tissue micro-array

containing samples of normal colon and colonic carcinoma; GAB1 - tonsil; filamin A - appendix; YAP1 - placenta.

Example 3

Frequencies and phenotypes among pathological variants - defining the tumor cohort

Classic medulloblastomas dominated the study cohort, accounting for 72% of all tumors. Most classic tumors (86%) appeared as sheets of uniform small cells with a high nuclear:cytoplasmic ratio and round hyperchromatic nuclei, while the remainder (14%) had a dominant spindle-cell morphology. Focal neuronal differentiation was evident in some classic tumors, manifesting either as nodules of uniform neurocytic cells without surrounding reticulin-positive desmoplasia (non-desmoplastic nodular 'biphasic' phenotype; 7%), or as dense clusters of tiny round cells (4%), or as foci of neuropil-like matrix with an irregular border, variable area, and scattered ganglion or neurocytic cells (ganglioneuroblastoma phenotype; n=1). In these tumors, foci of neuronal differentiation demonstrated: (i) the expected moderate to strong immunoreactivities for synaptophysin and NEU-N, (ii) up-regulation of p27, and (iii) a reduced growth fraction, as assessed by Ki-67 immunolabeling. Small foci of tumor cells with cytological features suggesting astrocytic differentiation were present in only three tumors. Among childhood medulloblastomas, immunoreactivity for GFAP was generally present in reactive astrocytes, rarely in tumor cells, but GFAP-positive tumor cells were readily found in adult cases, occurring in classic and D/N, but not LC/A, tumors.

Desmoplastic medulloblastomas, which contributed 17% of all tumors, were classified as conventional D/N medulloblastoma (67%), paucinodular D/N medulloblastoma (13%), and MBEN (20%). Intranodular cells showed the expected neuronal immunophenotype and low growth fraction described above for non-desmoplastic nodular tumors. Foci of internodular cells in a few D/N tumors showed marked cytological pleomorphism amounting to anaplasia, and this cytology was occasionally associated with invasion by such cells of peripheral areas within nodules.

Anaplastic and large cell tumors contributed 10% and 1%, respectively, of the total cohort. Several non-desmoplastic tumors among infants consisted of a monomorphic population of round cells with one or more prominent nucleoli and abundant mitotic activity. Their cytological features were distinct from the

conventional classic medulloblastoma, bearing similarities to the large cell phenotype, but without cytomegaly. Tumors with this phenotype made up approximately one third of non-desmoplastic tumors from children less than 3 years old.

5 ***Example 4***

Medulloblastomas with SHH or WNT signaling pathway aberrations

Immunohistochemical assay

Gene expression data separating medulloblastomas (n=46) into five molecular subgroups, including two characterized by aberrant SHH or WNT pathway activation, 10 were used to choose potential surrogate markers of SHH pathway activation. A subset of these tumors (n=26), for which FFPE material was available, was then used to validate a novel assay that divided tumors into three molecular categories: WNT pathway tumors, SHH pathway tumors, and non-SHH/non-WNT tumors, using four antibodies. GAB1 and filamin A were chosen as potential SHH pathway tumor 15 markers for use alongside β-catenin, an established marker of WNT pathway medulloblastomas, and YAP1, which is a marker of WNT and SHH pathway tumors. Results of the validation of this assay are shown in Figure 1. The validation set represents the majority of the series used to originally produce gene expression subgroups (Thompson *et al.* (2006) *J. Clin. Oncol.* 24(12):1924-1931). This was the 20 first study to identify molecular subgroups of medulloblastoma. Since then, consensus among researchers in the field proposes four main molecular subgroups: WHT, SHH, group 3 (Thompson group A), and group 4 (Thompson group C). The anti-GAB1 antibody identified only tumors with a SHH pathway profile or *PTCH1* mutation, but the anti-filamin A antibody identified WNT and SHH pathway tumors, but not non-25 SHH/non-WNT tumors (Figure 1).

Example 6

Medulloblastoma molecular subgroups - histopathological associations

SHH pathway medulloblastomas

30 Combined immunoreactivities for GAB1, filamin A, and YAP1, indicating a SHH pathway tumor profile, were found in 31% of medulloblastomas, including all desmoplastic tumors. Desmoplastic medulloblastomas constituted 54% of SHH pathway tumors, classic and LC/A tumors contributing 29% and 17% respectively. While non-desmoplastic tumors generally showed uniform immunoreactivities for

GAB1, YAP1, and filamin A, in the three types of desmoplastic tumor they displayed stronger staining in internodular regions. One exceptional classic tumor with focal anaplasia showed regional variation for filamin A, YAP1, and GAB1 immunoreactivities, which tended to align with the anaplastic phenotype.

WNT pathway medulloblastomas

Widespread intermediate or strong cytoplasmic β-catenin immunoreactivity was a feature of most medulloblastomas; few showed only patchy weak cytoplasmic staining for this antigen. WNT pathway medulloblastomas were identified by nuclear, as well as cytoplasmic, β-catenin immunoreactivity. In many cases, nuclear and cytoplasmic β-catenin staining combined to blanket almost all tumor cells, but strong nuclear β-catenin immunoreactivity was also seen in cell clusters alongside weak or negligible nuclear positivity. WNT pathway tumors defined by nuclear β-catenin immunoreactivity also expressed filamin A. Typically, this was patchy, weak to moderate immunostaining and less intense than that seen in SHH pathway tumors. Nuclear immunoreactivity for YAP1 was also a feature of WNT pathway tumors. WNT pathway tumors contributed 14% of all medulloblastomas in this series.

Nearly all WNT pathway medulloblastomas were classic tumors. LC/A tumors were also included, but rare (6%), and desmoplastic medulloblastomas were not represented among WNT pathway tumors. Uniform β-catenin immunoreactivity across the cells of a classic medulloblastoma was the usual phenotype. However, one large cell medulloblastoma showed a biphasic pattern for nuclear β-catenin immunoreactivity. The large cell phenotype was mainly associated with moderately strong cytoplasmic β-catenin immunoreactivity, but lacked β-catenin nucleopositivity, while clusters of cells elsewhere showed a WNT pathway phenotype.

Non-SHH/WNT medulloblastomas

Medulloblastomas falling outside the SHH and WNT pathway categories (55%) displayed cytoplasmic, but not nuclear immunoreactivity for β-catenin (i.e., negative expression within the nuclei). Tumor cells within these non-WNT/non-SHH tumors were immunonegative for GAB1, YAP1, and filamin A, but intrinsic vascular elements were positive, providing an internal control for the method. This subgroup of medulloblastomas was dominated by classic tumors (92%), including 100% of the non-

desmoplastic nodular tumors and all of those that contained small clusters of densely packed neurocytic cells. LC/A tumors made up the remainder.

Example 7

Medulloblastoma molecular subgroups

Cytogenetic associations

Molecular cytogenetic data were generated using iFISH and probes to loci known to harbor CNAs in medulloblastoma: chromosome 6 (including *SGK1*), chromosome 17 (including *HIC1*), *PTCH1*, *MYC*, and *MYCN*. Monosomy 6 was detected in 27 tumors (13%). Most of these were WNT pathway (92%) or classic (96%) tumors. WNT pathway tumors showed very few CNAs at other targeted loci. In contrast, nearly all (97%) *PTCH1* deletions, manifesting as either monosomy 9, heterozygous deletion, or relative imbalance in the setting of hyperploidy, were present in SHH pathway medulloblastomas. The one exception was classified as a non-WNT/non-SHH tumor. Almost two thirds (64%) of *PTCH1* deletions were found in desmoplastic tumors. A much higher proportion of medulloblastomas without chromosome 17 CNAs was evident among SHH and WNT pathway tumors versus non-WNT/non-SHH tumors: SHH 76%, WNT 71%, non-SHH/non-WNT 17%. Most cases (71%) of *MYC* or *MYCN* amplification also occurred in the non-SHH/non-WNT tumor subgroup.

Clinical associations

The three molecular subgroups of medulloblastoma demonstrated distinct clinical associations. SHH pathway tumors dominated medulloblastomas from infants and adults, in part reflecting the association between these age groups and desmoplastic tumors. In contrast, WNT pathway tumors nearly all presented between the ages of 6 and 12 years. The ratio of male:female patients also varied among molecular subgroups, exceeding 2:1 in the non-WNT/non-SHH tumor subgroup.

Clinical data on metastatic disease and outcome were available for children aged 3-16 years and entered into the SIOP PNET3 trial. The frequency of metastatic disease at presentation was higher in the non-WNT/non-SHH tumor subgroup than in the other two. Survival analyses revealed that WNT pathway medulloblastomas had significantly better progression-free and overall survivals than SHH pathway or non-WNT/non-SHH tumors. No significant difference in outcome was shown for LC/A

tumors belonging to the SHH-pathway or non-WNT/non-SHH categories of medulloblastoma, and the trend towards a better outcome for non-LC/A SHH pathway tumors relates to the presence of desmoplastic tumors in this category.

5 All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

10 Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

15

THAT WHICH IS CLAIMED

1. An immunohistochemical method for typing a medulloblastoma as a WNT pathway tumor, a sonic hedgehog (SHH) pathway tumor, or a non-WNT/non-SHH tumor, said method comprising determining a protein expression profile for a sample obtained from said medulloblastoma by detecting expression of β-catenin, YAP1, GAB1, or filamin A, and typing said medulloblastoma as a WNT pathway tumor, a SHH pathway tumor, or a non-WNT/non-SHH tumor based on said protein expression profile.

2. The method of Claim 1, comprising:

a) contacting a tissue sample obtained from said medulloblastoma with at least two antibodies specific to biomarker proteins selected from the group consisting of an antibody that specifically binds β-catenin, an antibody that specifically binds YAP1, an antibody that specifically binds GAB1, or an antibody that specifically binds filamin A;

b) determining a protein expression profile for said tissue sample based on detection of binding of said at least two antibodies to said biomarker proteins; and

c) typing said medulloblastoma as a WNT pathway tumor, a SHH pathway tumor, or a non-WNT/non-SHH tumor based on said protein expression profile.

3. The method of Claim 2, wherein at least one of said antibodies specifically binds β-catenin.

4. The method of Claim 3, wherein said protein expression profile is characterized by positive nuclear expression of β-catenin, wherein said medulloblastoma is typed as a WNT pathway tumor.

5. The method of Claim 3, wherein said protein expression profile is characterized by negative nuclear expression of β-catenin.

6. The method of Claim 5, wherein said protein expression profile is characterized by positive expression of one or more additional biomarker proteins selected from the group consisting of filamin A, GAB1, and YAP1, wherein said medulloblastoma is typed as a SHH pathway tumor.

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7. The method of Claim 5, wherein said protein expression profile is characterized by negative expression of one or more additional biomarker proteins selected from the group consisting of filamin A, GAB2, and YAP1, wherein said medulloblastoma is typed as a non-WNT/non-SHH tumor.

10

8. The method of Claim 2, wherein at least one of said antibodies specifically binds GAB1.

9. The method of Claim 8, wherein said protein expression profile is
15 characterized by positive expression of GAB1, wherein said medulloblastoma is typed as a SHH pathway tumor.

10. The method of Claim 8, wherein said protein expression profile is characterized by negative expression of GAB1.

20

11. The method of Claim 10, wherein said protein expression profile is characterized by positive nuclear expression of β-catenin, wherein said medulloblastoma is typed as a WNT pathway tumor.

25 12. The method of Claim 10, wherein said protein expression profile is characterized by negative nuclear expression of β-catenin, wherein said medulloblastoma is typed as a non-WNT/non-SHH pathway-regulated tumor.

13. The method of any one of Claims 2-12, wherein said sample is contacted
30 with at least three antibodies, wherein said antibodies are selected from the group consisting of an antibody that specifically binds to β-catenin, an antibody that specifically binds to YAP1, an antibody that specifically binds to GAB1, and an antibody that specifically binds to filamin A.

14. The method of any one of Claims 2-12, wherein said sample is contacted with an antibody that specifically binds to β-catenin, an antibody that specifically binds to YAP1, an antibody that specifically binds to GAB1, and an antibody that specifically binds to filamin A.

5

15. The method of any of Claims 2-14, wherein said sample is contacted with said antibodies sequentially.

16. The method of any one of Claims 2-14, wherein said sample is contacted
10 with said antibodies simultaneously.

17. An immunohistochemical method for typing a medulloblastoma as a SHH pathway tumor or a non-SHH tumor, said method comprising determining a protein expression profile for a sample obtained from said medulloblastoma by
15 detecting expression of GAB1 and typing said medulloblastoma as a SHH pathway tumor or a non-SHH tumor based on said protein expression profile.

18. The method of Claim 17, wherein said protein expression profile is characterized by positive expression of GAB1, wherein said medulloblastoma is
20 typed as a SHH pathway tumor.

19. The method of Claim 17, wherein said protein expression profile is characterized by negative expression of GAB1, wherein said medulloblastoma is typed as a non-SHH tumor.

25

20. An immunohistochemical method for typing a medulloblastoma as a non-WNT/non-SHH tumor, said method comprising determining a protein expression profile for a sample obtained from said medulloblastoma by detecting expression of filamin A, and typing said medulloblastoma as a non-WNT/non-SHH tumor based on
30 said protein expression profile.

21. The method of Claim 20, wherein said protein expression profile is characterized by negative expression of filamin A, wherein said medulloblastoma is typed as a non-WNT/non-SHH tumor.

22. A method of identifying a subject with a medulloblastoma that would benefit from treatment with a WNT signaling pathway inhibitor or a sonic hedgehog (SHH) pathway signaling inhibitor, said method comprising typing said medulloblastoma as a WNT pathway tumor according to the method of any one of Claims 4, 11, and 13-16, or typing said medulloblastoma as a SHH pathway tumor according to the method of any one of Claims 6, 9, 13-16, and 18, thereby identifying said subject.

23. The method of Claim 22, further comprising treating said subject with said WNT signaling pathway inhibitor when said medulloblastoma is typed as a WNT pathway tumor.

24. The method of Claim 22, further comprising treating said subject with said SHH signaling pathway inhibitor when said medulloblastoma is typed as a SHH pathway tumor.

25. A method for selecting a therapy for a subject with a medulloblastoma, said method comprising typing a sample obtained from said medulloblastoma according to the method of any one of Claims 1-22, and selecting a therapy based on said typing.

26. A method for determining whether a subject is eligible for entry into a clinical trial for treating medulloblastomas regulated by the WNT signaling pathway, comprising typing a medulloblastoma in said subject according to the method of any one of Claims 1-16, wherein said subject is eligible if said medulloblastoma is typed as a WNT pathway tumor.

27. A method for determining whether a subject is eligible for entry into a clinical trial for treating medulloblastomas regulated by the SHH signaling pathway, comprising typing a medulloblastoma in said subject according to the method of any one of Claims 1-18, wherein said subject is eligible if said medulloblastoma is typed as a SHH pathway tumor.

28. A method for determining whether a subject is eligible for entry into a clinical trial for treating medulloblastomas that are non-WNT/non-SHH tumors, comprising typing a medulloblastoma in said subject according to the method of any one of Claims 1-16, 20, and 21, wherein said subject is eligible if said
5 medulloblastoma is typed as a non-WNT/non-SHH tumor.

29. A kit for typing a medulloblastoma as a WNT pathway tumor, a sonic hedgehog (SHH) pathway tumor, or a non-WNT/non-SHH tumor, said kit comprising at least two antibodies selected from the group consisting of an antibody that
10 specifically binds β-catenin, an antibody that specifically binds YAP1, an antibody that specifically binds GAB1, and an antibody that specifically binds filamin A.

30. The kit of Claim 29, comprising at least three of said antibodies.

15 31. The kit of Claim 29, comprising an antibody that specifically binds β-catenin, an antibody that specifically binds YAP1, an antibody that specifically binds GAB1, and an antibody that specifically binds filamin A.

32. The kit of any one of Claims 29-31, wherein said kit further comprises
20 reagents for the detection of antibody binding to said β-catenin, YAP1, GAB1, filamin A, or any combination thereof.

33. The kit of any one of Claims 29-32, wherein said kit further comprises at least one positive control sample.
25

34. The kit of any one of Claims 29-33, wherein said kit further comprises instructions for use.

FIG 1

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#U113 FILE	CTNNB1 mutation	PTCH1 mutation	Pathological variant	Molecular Subgroup	β-catenin IHC	Filamin A IHC	GAB1 IHC	YAP1 IHC
103			classic	A	C-intermediate	negative	negative	negative
118			anaplastic	A	C-intermediate	negative	negative	negative
121			anaplastic	A	C-strong	negative	negative	negative
80			anaplastic	A	C-intermediate/focal	negative	negative	negative
98			classic	A	C-strong	negative	negative	negative
131			anaplastic	A	C-strong focal	negative	negative	negative
140			classic-GNB	A	C-strong	negative	negative	negative
125	X		classic	B	N-strong	C-weak/focal	negative	N & C- strong
139	X		classic	B	N-strong	C-intermediate/ focal	negative	N & C- strong
84	X		classic	B	N-strong	C-intermediate/ focal	negative	N & C- strong
143	X		classic	B	N-strong	C-intermediate/ focal	negative	N & C- strong
92			classic	C	C-strong	negative	negative	negative
79			classic	C	C-intermediate/focal	negative	negative	negative
129			classic	C	C-intermediate	negative	negative	negative
130			classic	C	C-intermediate	negative	negative	negative
81			classic	C	C-strong	negative	negative	negative
96			classic	C	C-intermediate	negative	negative	negative
89			classic	C	C-intermediate	negative	negative	negative
90		X	anaplastic	C'	C-intermediate	C-intermediate	C-strong	N & C- strong
102			anaplastic	D	C-intermediate	C-intermediate	C-intermediate	N & C- strong
126		X	D/N	D	C-intermediate (nodules)	C-intermediate (nodules)	C-intermediate (nodules)	N & C- strong (nodules)
127		X	D/N	D	C-strong (nodules)	C-strong (nodules)	C-intermediate (nodules)	N & C- strong (nodules)
109			D/N	D	C-strong (nodules)	C-strong (nodules)	C-intermediate (nodules)	N & C- strong (nodules)
114			classic	E	C-strong	negative	negative	negative
153			anaplastic	E	C-intermediate	negative	negative	negative

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/054197

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/50 G01N33/574
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

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

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

EPO-Internal, WPI Data, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ELLISON DAVID W ET AL: "beta-Catenin status predicts a favorable outcome in childhood medulloblastoma: the United Kingdom Children's Cancer Study Group Brain Tumour Committee", JOURNAL OF CLINICAL ONCOLOGY, AMERICAN SOCIETY OF CLINICAL ONCOLOGY, US, vol. 23, no. 31, 1 November 2005 (2005-11-01), pages 7951-7957, XP009154567, ISSN: 0732-183X	1,25,26
Y	the whole document	29,32-34

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[X] 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

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 December 2011	09/01/2012

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2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FATTET SARAH ET AL: "Beta-catenin status in paediatric medulloblastomas: correlation of immunohistochemical expression with mutational status, genetic profiles, and clinical characteristics", JOURNAL OF PATHOLOGY, JOHN WILEY & SONS LTD, GB, vol. 218, no. 1, 1 May 2009 (2009-05-01), pages 86-94, XP009154562, ISSN: 0022-3417 [retrieved on 2009-01-06]	1,25,26
Y	the whole document	29,32-34

X	FERNANDEZ-L AFRICA ET AL: "YAP1 is amplified and up-regulated in hedgehog-associated medulloblastomas and mediates Sonic hedgehog-driven neural precursor proliferation", GENES AND DEVELOPMENT, COLD SPRING HARBOR LABORATORY PRESS, PLAINVIEW, NY, US, vol. 23, no. 23, 1 December 2009 (2009-12-01), pages 2729-2741, XP009154564, ISSN: 0890-9369	1,25,27
Y	the whole document	29,32,34

A	DAVID W ELLISON: "Childhood medulloblastoma: novel approaches to the classification of a heterogeneous disease", ACTA NEUROPATHOLOGICA, SPRINGER, BERLIN, DE, vol. 120, no. 3, 23 July 2010 (2010-07-23) , pages 305-316, XP019847041, ISSN: 1432-0533 the whole document	1-34

X,P	DAVID W ELLISON ET AL: "Medulloblastoma: clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups", ACTA NEUROPATHOLOGICA, SPRINGER, BERLIN, DE, vol. 121, no. 3, 26 January 2011 (2011-01-26), pages 381-396, XP019882166, ISSN: 1432-0533, DOI: 10.1007/S00401-011-0800-8 the whole document	1-34