Published in final edited form as:

J Neurooncol. 2012 October ; 110(1): 89–98. doi:10.1007/s11060-012-0938-9.

Prospective, high-throughput molecular profiling of human gliomas

Andrew S. Chi,

Department of Neurology, Stephen E. and Catherine Pappas Center for Neuro-Oncology, Massachusetts General Hospital Cancer Center, Yawkey 9E, 55 Fruit Street, Boston, MA 02114, USA

Tracy T. Batchelor,

Department of Neurology, Stephen E. and Catherine Pappas Center for Neuro-Oncology, Massachusetts General Hospital Cancer Center, Yawkey 9E, 55 Fruit Street, Boston, MA 02114, USA

Dora Dias-Santagata,

Translational Research Laboratory, Massachusetts General Hospital Cancer Center, Boston, MA, USA, Department of Pathology, Massachusetts General Hospital Cancer Center, Boston, MA, USA

Darrell Borger,

Translational Research Laboratory, Massachusetts General Hospital Cancer Center, Boston, MA, USA

Charles D. Stiles,

Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA, USA

Daphne L. Wang,

Department of Neurology, Stephen E. and Catherine Pappas Center for Neuro-Oncology, Massachusetts General Hospital Cancer Center, Yawkey 9E, 55 Fruit Street, Boston, MA 02114, USA

William T. Curry,

Department of Neurosurgery, Massachusetts General Hospital, Boston, MA, USA

Patrick Y. Wen,

Center for Neuro-Oncology, Dana-Farber/Brigham and Women's Cancer Center and Department of Neurology, Brigham and Women's Hospital, Boston, MA, USA

Keith L. Ligon,

Center for Molecular Oncologic Pathology, Dana-Farber Cancer Institute and Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Leif Ellisen,

Translational Research Laboratory, Massachusetts General Hospital Cancer Center, Boston, MA, USA

© Springer Science+Business Media, LLC. 2012

Correspondence to: Andrew S. Chi, chi.andrew@mgh.harvard.edu.

Preliminary data of this manuscript was presented at the 2011 American Association of Neurology Annual Meeting in Honolulu, Hawaii.

Conflicts of interest The authors have no conflicts of interest to declare.

Electronic supplementary material The online version of this article (doi:10.1007/s11060-012-0938-9) contains supplementary material, which is available to authorized users.

David N. Louis, and

Department of Pathology, Massachusetts General Hospital Cancer Center, Boston, MA, USA

A. John Iafrate

Translational Research Laboratory, Massachusetts General Hospital Cancer Center, Boston, MA, USA, Department of Pathology, Massachusetts General Hospital Cancer Center, Boston, MA, USA

Andrew S. Chi: chi.andrew@mgh.harvard.edu

Abstract

Gliomas consist of multiple histologic and molecular subtypes with different clinical phenotypes and responsiveness to treatment. However, enrollment criteria for clinical trials still largely do not take into account these underlying molecular differences. We have incorporated a high-throughput tumor genotyping program based on the ABI SNaPshot platform as well as other molecular diagnostic tests into the standard evaluation of glioma patients in order to assess whether prospective molecular profiling would allow rational patient selection onto clinical trials. From 218 gliomas we prospectively collected SNaPshot genotyping data on 68 mutated loci from 15 key cancer genes along with data from clinical assays for gene amplification (*EGFR*, *PDGFRA*, *MET*), 1p/19q co-deletion and *MGMT* promoter methylation. SNaPshot mutations and focal gene amplifications were detected in 38.5 and 47.1 % of glioblastomas, respectively. Genetic alterations in *EGFR*, *IDH1* and *PIK3CA* closely matched frequencies reported in recent studies. In addition, we identified events that are rare in gliomas although are known driver mutations in other cancer types, such as mutations of *AKT1*, *BRAF* and *KRAS*. Patients with genetic alterations that activate signaling pathways were enrolled onto genetically selective clinical trials for malignant glioma as well as for other solid cancers. High-throughput molecular profiling incorporated into the routine clinical evaluation of glioma patients may enable the rational selection of patients for targeted therapy clinical trials and thereby improve the likelihood that such trials succeed.

Keywords

Tumor genotyping; Molecular profiling; Biomarker; Glioblastoma; Glioma

Introduction

As a result of the success of targeted therapy in several cancer types, recent drug development in oncology has shifted toward therapeutics that selectively target specific molecular alterations [1–6]. These successes were contingent upon the identification of molecular subtypes in histologically identical tumors that were susceptible to specific targeted agents. For example, subsets of advanced non-small cell lung cancers (NSCLC) with either activating mutations in the region encoding the kinase domain of the epidermal growth factor receptor (*EGFR*) gene or activating rearrangements of the anaplastic lymphoma kinase (*ALK*) gene render these tumors sensitive to specific tyrosine kinase inhibitors (TKI) targeting *EGFR* or *ALK*, respectively [2, 4, 7].

Current evidence suggests that histologically identical gliomas represent distinct molecular phenotypes with different prognoses and possibly differential responses to treatment [8–14]. For example, recurrent mutations of codon 132 of the isocitrate dehydrogenase 1 (*IDH1*) gene and the analogous codon (172) of the *IDH2* gene are associated with younger age and longer survival for WHO grade II, grade III (anaplastic) and grade IV (glioblastoma, GBM) gliomas [10, 12, 13].

Although these and other molecular markers have strong prognostic value, genetic markers that have been prospectively validated to predict sensitivity to specific targeted therapies (predictive biomarkers) are currently lacking in gliomas. This contributes to the impression that targeted agents have failed in malignant glioma. However, it is possible that molecular subsets of responsive tumors may be masked in unselected trial populations.

We implemented a high-throughput genetic profiling platform (SNaPshot genotyping) [15] into a comprehensive molecular evaluation of malignant gliomas in order to prospectively detect genomic alterations and rationally direct patient enrollment onto clinical trials of molecularly targeted agents. The SNaPshot genotyping platform is a robust, highly sensitive and specific tumor genotyping assay that enables simultaneous detection of multiple somatic mutations in small quantities of tumor DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tissue in a time frame of 2–3 weeks. This assay detects common mutations in 15 key cancer-related genes, several of which activate signaling pathways that are targeted by drugs currently in clinical development for malignant glioma.

Here, we describe our initial results of prospective, comprehensive molecular profiling of tumor tissues from glioma patients seen at the Dana-Farber/Harvard Cancer Center. We used SNaPshot genotyping in combination with fluorescence in situ hybridization (FISH) for gene amplification/loss and *MGMT* promoter DNA methylation analysis. We aimed to identify molecular markers that may predict sensitivity to targeted agents currently in investigation for malignant glioma.

Methods

Patients and samples

From December 2009 to August 2011 we prospectively profiled 218 gliomas from 212 patients treated at Massachusetts General Hospital (MGH) and Dana-Farber Cancer Institute/Brigham and Women's Hospital (DFCI/BWH) using SNaPshot genotyping. If tissue was sufficient, *EGFR*, *MET*, and platelet-derived growth factor receptor alpha (*PDGFRα*) gene amplification was assessed (details below). If clinically indicated, methylation-specific PCR (MS-PCR) to determine *MGMT* promoter methylation status and FISH for 1p and 19q deletion status was performed. The time from test requisition to report finalization of all molecular tests was approximately 2–3 weeks.

Testing was performed as part of routine medical care in CLIA-certified clinical laboratories. All results were obtained from the patient medical record and collected in an IRB-approved patient database. A counseling session with background information about SNaPshot genotyping was conveyed to patients undergoing testing along with the information that established treatment algorithms based on results of genetic profiling are lacking. Patients then signed an informed consent form. Patients in this cohort enrolled on recurrent GBM clinical trials were previously treated with radiation and temozolomide as described [16].

SNaPshot genotyping assay

SNaPshot genotyping assays were performed in the MGH Translational Research Laboratory (TRL). This assay is based on the SNaPshot assay from Applied Biosystems and was developed in the TRL for clinical tumor genotyping [15]. It can sensitively and specifically detect low-level somatic mutations in DNA extracted from FFPE tumor samples. The MGH SNaPshot assay consists of a multiplexed PCR step followed by a single-base extension reaction that generates allele-specific fluorescently labeled probes. Mutations in 68 commonly mutated loci from 15 genes (*AKT1*, *APC*, *BRAF*, *CTNNB1*,

EGFR, HER2, IDH1, KIT, KRAS, MEK1, NOTCH1, NRAS, PIK3CA, PTEN, TP53) are detected in nine multiplexed reactions (Table 1).

Cytogenetics and copy number detection

To determine gene amplification and chromosomal deletion status, 5 μ m sections of FFPE tumor material were prepared and regions containing a majority of tumor cells were selected. Three dual-color FISH assays were performed to assay the *EGFR*, *MET* and *PDGFRA* genes. BAC probes CTD-2113A18 (chromosome 7p *EGFR* locus) and CTB-13N12 (chromosome 7q *MET* locus) were used with a centromere 7 copy number control (CEP7; Abbott) and RP11-58C6 (chromosome 4q *PDGFRA* locus) was used with a centromere 4 copy number control (CEP4; Abbott). Signal quantitation was used to generate gene/copy number control ratios. A ratio of >2.0 was considered amplified. Focal amplification was considered amplified and was specified in the diagnosis. *EGFR* amplification status at DFCI/BWH was determined by chromogenic in situ hybridization (CISH) with the SPOT-Light *EGFR* amplification probe (#84-1300) and CISH kit (#84-9246) (Invitrogen). Samples with greater than ten *EGFR* probe signals per nucleus in $>50\%$ of the tumor cells were considered high level amplification. For data analysis, tumors positive by FISH or CISH were considered amplified. For clinical trial enrollment, FISH was used to confirm CISH results.

1p and 19q status was determined at MGH using two separate dual-color FISH assays using the Vysis 1p36/1q25 and 19q13/19p13 FISH Probe Kit (Abbott). Signal quantitation was used to generate 1p/1q and 19q/19p ratios. A ratio of ≥ 0.75 was considered loss and <0.75 as maintained. At DFCI/BWH, tumors were tested using disaggregated whole nuclei preparations from 50 μ m FFPE sections and using a molecular probe assay for 1p/19q FISH (Abbott #04N60-020) using similar cutoffs for determination of loss.

MGMT promoter methylation status

To determine *MGMT* promoter methylation status, 5 μ m sections of a FFPE tumor specimen were prepared and regions containing a majority of tumor cells were selected. Following manual microdissection, DNA was extracted and subjected to bisulfite treatment. MS-PCR was performed as described [17]. *MGMT* promoter methylation status was not used as criteria for enrollment in the clinical trials reported herein.

Statistical analysis

Associations between the occurrences of genetic alterations were analyzed using Fisher's exact test. All reported *P* values are two-sided.

Results

Molecular profiling

A SNaPshot mutation was detected in 84/218 tumor specimens (38.5 %) (Fig. 1; Table 2, Supplementary Table). Of the genes interrogated by SNaPshot, the most frequently mutated was *IDH1*, which was found in 47/218 specimens (21.6 %). *IDH1* mutation was found significantly more frequently in secondary (5/8, 62.5 %) than primary GBM (12/156, 7.7 %) ($p < 0.0004$), and more frequently in WHO grade II and III gliomas (30/54, 55.6 %) than glioblastomas (17/164, 10.4 %) ($p < 1 \times 10^{-10}$). In the grade II and grade III glioma subset, ten of 11 oligodendrogliomas (90.9 %) had an *IDH1* mutation, whereas nine of 15 (60 %) oligoastrocytomas and 11 of 28 (39.3 %) astrocytomas harbored an *IDH1* mutation, indicating an association between *IDH1* mutation and oligodendrogliomas versus tumors with an astrocytic component ($p = 0.0147$).

In gliomas of all types, *IDH1* mutation was significantly associated with 1p/19q co-deletion and inversely associated with *EGFR* amplification. In tumors with 1p/19q co-deletion, *IDH1* mutation was detected in 15 of 17 (88.2 %) cases whereas only 13 of 53 (24.5 %) tumors without 1p/19q co-deletion had *IDH1* mutation ($p < 1 \times 10^{-5}$). Conversely, only 1 of 47 *EGFR*-amplified tumors (2.1 %) had *IDH1* mutation whereas 11 of 66 (16.7 %) tumors without *EGFR* amplification were *IDH1*-mutated ($p = 0.014$). Interestingly, *IDH1* mutation was concurrent with other oncogene alterations in several GBMs, including *KRAS* mutation, *EGFR* amplification, *PDGFRA* amplification, and one tumor with concurrent *PIK3CA* and *PTEN* mutations.

Point mutations in the *PIK3CA* gene were observed in 9/164 (5.5 %) of GBMs. Four tumors had mutation of R88, which is located in the P85-binding domain, and five had mutations in “hot-spot” regions (four H1047 kinase domain mutations and one helical domain Q546 mutation). One *PIK3CA* mutant anaplastic oligodendroglioma also harbored *IDH1* and *PTEN* point mutations and two *PIK3CA* mutant GBMs had concurrent *EGFR* amplification.

Amplification of the *EGFR* gene was detected in 45 of 108 (41.7 %) malignant gliomas and 42 of 102 (41.2 %) of GBMs (Fig. 2). *EGFR* amplification was occasionally concurrent with other genetic alterations, including two tumors with *PIK3CA* mutation, three with both *EGFR* and *MET* amplification and one with *IDH1* mutation. None of the *EGFR*-amplified tumors harbored 1p/19q co-deletion (0/9).

Our population included several oncogene mutations that are rare in malignant gliomas. Three GBMs had *KRAS* mutation (1.8 %), one *CTNNB1* mutation (0.6 %), one *BRAF* V600E catalytic domain mutation (0.6 %), one *AKT1* mutation, and one with an *EGFR* active site mutation (G719D) known to sensitize to *EGFR* inhibitors in NSCLC. Four GBMs displayed *MET* gene amplification (4/102, 3.9 %) (Fig. 2) and two had *PDGFRA* amplification.

In this dataset, there were seven matched sets of tumors that were profiled at diagnosis and at progression. Two tumors with *IDH1* mutation and 1p/19q co-deletion retained these mutations at re-resection. One was a grade II oligodendroglioma that transformed into an anaplastic oligodendroglioma, and the other was an anaplastic oligodendroglioma that had similar histology at recurrence. Four matched GBM tumors had identical molecular profiles at diagnosis and at recurrence. Two had only *EGFR* amplification, one recurrent GBM retained the *PIK3CA* and *TP53* point mutations detected at diagnosis, and one had no genetic alterations detected.

Two GBMs had a different molecular profile at recurrence. One *EGFR*-amplified tumor remained *EGFR*-amplified at recurrence however also developed a new *PTEN* point mutation in the recurrent tumor. Another GBM had both *EGFR* and *MET* amplification at diagnosis, however at recurrence both alterations were lost. Interestingly, a small area of this original tumor had oligodendroglial histology without either *EGFR* or *MET* amplification, and at recurrence the majority of the tumor contained this oligodendroglial population.

Discussion

Clinical application of molecular profiling

Prospective molecular profiling enabled rational selection of clinical trials for several patients. Recently, seven patients with *EGFR* amplification in their tumors at diagnosis were enrolled at recurrence onto a genetically-selective (*EGFR*-amplified) phase II trial of PF-00299804, an irreversible *EGFR* TKI (NCT01112527). Efficacy results are pending, however one patient who progressed within 2 months required urgent surgery for malignant

cerebral edema. The tissue was molecularly profiled and intriguingly, the recurrent tumor harbored a *PTEN* point mutation that was not present in the original tumor at diagnosis. Another patient had a minor response and is currently stable after 14 months.

One GBM patient had *MET* gene amplification in her tumor at diagnosis, which occurs infrequently in GBM (5 % [18]). At recurrence she enrolled onto a phase I study of crizotinib, a dual MET and ALK tyrosine kinase inhibitor, for advanced solid tumor patients with *MET* gene amplification or *ALK* gene rearrangement/amplification in their tumors (A8081001). Within 2 weeks she had significant improvement in short term memory and orientation and she had a sustained 40 % reduction in the area of contrast enhancement on MRI [19]. She ultimately progressed after 6 months.

One GBM patient who harbored both *EGFR* and *MET* amplification in his tumor at diagnosis was a potential candidate for one of two genetically-selective clinical trials; PF-00299804 for *EGFR*-amplified recurrent GBM and crizotinib for advanced solid cancers with *MET* or *ALK* genetic alterations. At recurrence, this patient required surgical resection due to rapid, symptomatic growth of the tumor. Surprisingly, both *EGFR* and *MET* amplification were lost in the recurrent tumor, and he therefore was not enrolled onto either trial.

We identified many newly diagnosed malignant glioma patients who may be candidates for genetically-selective targeted inhibitor clinical trials open at our institution at the time of recurrence, including *EGFR*-amplified patients (PF-00299804 trial), *MET*-amplified patients (crizotinib trial) and patients with *PIK3CA* or *PTEN* point mutations [BKM120 for recurrent GBM patients with activated PI3K pathway (NCT01339052)]. Patients in whom we detected alterations such as *PDGFRA* amplification are potential candidates for genetically un-selective clinical trials investigating agents such as cediranib, sunitinib and IMC-3G3.

Molecular associations

Our institution has developed a practical yet robust platform to genotype tumors in a short time frame. This has allowed us to incorporate molecular profiling into the standard clinical evaluation of glioma patients and has enabled more rational enrollment into early-stage clinical trials.

In general, the results from our molecular profiling were consistent with previously reported prevalences. For example, the frequencies of *EGFR* amplification (41.2 %) and *MGMT* promoter methylation (53.5 %) in GBMs were similar to prior reports [11, 20–22]. *IDH1* mutations were associated with secondary versus primary GBM, grade II and grade III gliomas versus GBMs, oligodendrogliomas versus astrocytic gliomas (in grade II and grade III gliomas) and 1p/19q co-deletion, whereas they were inversely associated with *EGFR* amplification [12, 13].

Our observed frequencies of uncommon genetic alterations were also consistent with prior reports, including *MET* amplification (3.9 vs. 5 % [18]), *PIK3CA* hot-spot mutation (5.5 vs. 6.6–15 % [18, 23]) and *KRAS* hot-spot mutation (1.8 vs. 0–1.1 % [18, 24, 25]). We detected slightly lower mutation frequencies in other genes, such as *BRAF V600E* mutation in GBM (0.6 vs. 3.2–6 % [24, 26]). These differences may be due to our limited sample size, however it is noteworthy that our data was obtained prospectively during routine patient care using clinical assays.

Prospective molecular profiling also revealed several potential clinical associations for further exploration, including potential predictive biomarkers, links between mutations and responses to therapy, and selection criteria for targeted therapies. We identified one genetic

marker (*MET* amplification) that warrants further investigation in GBM. In the case of the *EGFR*-amplified GBM patient who required urgent surgery after rapid progression on an *EGFR* inhibitor, a *PTEN* point mutation had developed during the interval since diagnosis. Although speculative, the *PTEN* mutation could have had a role in her tumor's intrinsic resistance to *EGFR* inhibition. Another patient's recurrent tumor surprisingly lost amplification of two oncogenes just prior to enrollment onto genetically-selective clinical trials. Although this patient avoided treatment with therapies that he may have been resistant to and would have likely experienced toxicity from, this case raises a concern about the validity of using tumor tissue from initial diagnosis for enrollment onto targeted therapy trials at the time of recurrence.

Comprehensive molecular profiling may provide additional benefit if a resection is performed at tumor recurrence since the mutational status of tumors may change after therapy [27]. Tissue can be analyzed to confirm the retention of previously observed alterations and to assess whether new alterations have developed. Although tumor genetic heterogeneity remains a challenge [28], determining the fraction or abundance of specific alterations in situ with methods such as FISH would assist the formulation of combination and sequential targeted strategies.

By applying a comprehensive assay that interrogates commonly mutated oncogenes across multiple cancer types, we identified oncogene mutations that are infrequent in GBM (e.g. *EGFR* G719D, *BRAF*, *KRAS*, and *AKT1*) although are currently being targeted in solid cancer phase I trials. Although CSF penetration of many solid cancer phase I drugs is either limited or unknown, these cases open opportunities to explore rare mutations as predictive biomarkers in GBM. Two *BRAF* mutant glioma patients may be eligible for one of several *BRAF*-mutant selective phase I clinical trials. Three patients with *KRAS* mutation may be eligible for one of several trials testing MEK inhibitor and PI3K inhibitor combinations, a formula which has preclinical activity in *KRAS* mutant lung cancers [29].

A limitation of targeted sequencing platforms is that mutations at non-interrogated loci can be missed, resulting in false negative testing. For tumor suppressors, our SNaPshot panel interrogates loci that are mutated with at least 3 % frequency, however this design still covers less than 30 % of the *TP53* and 10 % of the *PTEN* point mutations that have been reported in GBM [30]. Our observed point mutation frequencies for *TP53* (12.2 %) and *PTEN* (2.4 %) in GBM cases were much lower than previous reports (60–90 % for *TP53* [31–33] and 14–32 % for *PTEN* [18, 34–36]), reflecting this limitation. However, our SNaPshot panel is intentionally weighted to interrogate oncogenes, which have activating mutations at only a few hot-spots. Oncogenes are better suited for targeted sequencing than tumor suppressors, where mutations tend to occur throughout the gene. Our panel also favors oncogenes that are being targeted either in clinical trials or with FDA-approved targeted therapies.

Sanger sequencing or next-generation sequencing methods would provide more comprehensive genetic information than our targeted sequencing panel. However, tissue quantity, cost, and time are significant limiting factors in the clinical setting. Although our method has limitations in scale and modest expense, newer approaches such as next-generation sequencing present additional challenges such as bioinformatics and technical expertise that currently limit their implementation in clinical practice. Our focused genotyping panel does not include some mutations commonly found in gliomas (e.g. non-kinase domain *EGFR* mutations) and includes others that are rare in glioma, however it is practical, moderately comprehensive, robust and ultimately feasible for application in a cancer center. We report examples where our profiling approach may have had clinical consequence and it provides targeted therapy clinical trials in GBM the opportunity for

improved outcomes. Ultimately our approach may increase the likelihood of replicating the successes already observed with targeted therapy in other cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Andrew S. Chi is supported by a Joan Ambriz American Brain Tumor Association Basic Research Fellowship and an Early Career research award from the Ben and Catherine Ivy Foundation. Ethical Standards: All results were obtained from the medical record of patients and collected in an IRB-approved patient database. Molecular testing was performed as part of routine medical care in CLIA-certified clinical laboratories. All molecular tests comply with the current laws of the USA.

References

1. Druker B, Talpaz M, Resta D, Peng B, Buchdunger E, Ford J, Lydon N, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers C. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med*. 2001; 344:1031–1037. [PubMed: 11287972]
2. Lynch T, Bell D, Sordella R, Gurubhagavatula S, Okimoto R, Brannigan B, Harris P, Haserlat S, Supko J, Haluska F, Louis D, Christiani D, Settleman J, Haber D. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2004; 350:2129–2139. [PubMed: 15118073]
3. Bollag G, Hirth P, Tsai J, Zhang J, Ibrahim PN, Cho H, Spevak W, Zhang C, Zhang Y, Habets G, Burton EA, Wong B, Tsang G, West BL, Powell B, Shellooe R, Marimuthu A, Nguyen H, Zhang KY, Artis DR, Schlessinger J, Su F, Higgins B, Iyer R, D'Andrea K, Koehler A, Stumm M, Lin PS, Lee RJ, Grippo J, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, Chapman PB, Flaherty KT, Xu X, Nathanson KL, Nolop K. Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. *Nature*. 2010; 467:596–599. [PubMed: 20823850]
4. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, Ou SH, Dezube BJ, Janne PA, Costa DB, Varella-Garcia M, Kim WH, Lynch TJ, Fidias P, Stubbs H, Engelman JA, Sequist LV, Tan W, Gandhi L, Mino-Kenudson M, Wei GC, Shreeve SM, Ratain MJ, Settleman J, Christensen JG, Haber DA, Wilner K, Salgia R, Shapiro GI, Clark JW, Iafrate AJ. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med*. 2010; 363:1693–1703. [PubMed: 20979469]
5. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, O'Dwyer PJ, Lee RJ, Grippo JF, Nolop K, Chapman PB. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med*. 2010; 363:809–819. [PubMed: 20818844]
6. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus a monoclonal antibody against *HER2* for metastatic breast cancer that overexpresses *HER2*. *N Engl J Med*. 2001; 344:783–792. [PubMed: 11248153]
7. Paez J, Janne P, Lee J, Tracy S, Greulich H, Gabriel S, Herman P, Kaye F, Lindeman N, Boggon T, Naoki K, Sasaki H, Fujii Y, Eck M, Sellers W, Johnson B, Meyerson M. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 2004; 304:1497–1500. [PubMed: 15118125]
8. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, Alexe G, Lawrence M, O'Kelly M, Tamayo P, Weir BA, Gabriel S, Winckler W, Gupta S, Jakkula L, Feiler HS, Hodgson JG, James CD, Sarkaria JN, Brennan C, Kahn A, Spellman PT, Wilson RK, Speed TP, Gray JW, Meyerson M, Getz G, Perou CM, Hayes DN. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell*. 2010; 17:98–110. [PubMed: 20129251]
9. Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, Pan F, Pelloski CE, Sulman EP, Bhat KP, Verhaak RG, Hoadley KA, Hayes DN, Perou CM, Schmidt HK, Ding L,

- Wilson RK, Van Den Berg D, Shen H, Bengtsson H, Neuvial P, Cope LM, Buckley J, Herman JG, Baylin SB, Laird PW, Aldape K. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. *Cancer Cell*. 2010; 17:510–522. [PubMed: 20399149]
10. Parsons D, Jones S, Zhang X, Lin J, Leary R, Angenendt P, Mankoo P, Carter H, Siu I, Gallia G, Olivi A, McLendon R, Rasheed B, Keir S, Nikolskaya T, Nikolsky Y, Busam D, Tekleab H, Diaz LJ, Hartigan J, Smith D, Strausberg R, Marie S, Shinjo S, Yan H, Riggins G, Bigner D, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu V, Kinzler K. An integrated genomic analysis of human glioblastoma multiforme. *Science*. 2008; 321:1807–1812. [PubMed: 18772396]
 11. Hegi M, Diserens A, Gorlia T, Hamou M, de Tribolet N, Weller M, Kros J, Hainfellner J, Mason W, Mariani L, Bromberg J, Hau P, Mirimanoff R, Cairncross J, Janzer R, Stupp R. *MGMT* gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med*. 2005; 352:997–1003. [PubMed: 15758010]
 12. Yan H, Parsons D, Jin G, McLendon R, Rasheed B, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins G, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler K, Velculescu V, Vogelstein B, Bigner D. IDH1 and IDH2 mutations in gliomas. *N Engl J Med*. 2009; 360:765–773. [PubMed: 19228619]
 13. Sanson M, Marie Y, Paris S, Idhah A, Laffaire J, Ducray F, Hallani SE, Boisselier B, Mokhtari K, Hoang-Xuan K, Delattre JY. Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. *J Clin Oncol*. 2009; 27:4150–4154. [PubMed: 19636000]
 14. Jansen M, Yip S, Louis DN. Molecular pathology in adult gliomas: diagnostic, prognostic, and predictive markers. *Lancet Neurol*. 2010; 9:717–726. [PubMed: 20610347]
 15. Dias-Santagata D, Akhavanfard S, David SS, Vernovsky K, Kuhlmann G, Boisvert SL, Stubbs H, McDermott U, Settleman J, Kwak EL, Clark JW, Isakoff SJ, Sequist LV, Engelman JA, Lynch TJ, Haber DA, Louis DN, Ellisen LW, Borger DR, Iafrate AJ. Rapid targeted mutational analysis of human tumours: a clinical platform to guide personalized cancer medicine. *EMBO Mol Med*. 2010; 2:146–158. [PubMed: 20432502]
 16. Stupp R, Mason W, van den Bent M, Weller M, Fisher B, Taphoorn M, Belanger K, Brandes A, Marosi C, Bogdahn U, Curschmann J, Janzer R, Ludwin S, Gorlia T, Allgeier A, Lacombe D, Cairncross J, Eisenhauer E, Mirimanoff R. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005; 352:987–996. [PubMed: 15758009]
 17. Palmisano W, Divine K, Saccomanno G, Gilliland F, Baylin S, Herman J, Belinsky S. Predicting lung cancer by detecting aberrant promoter methylation in sputum. *Cancer Res*. 2000; 60:5954–5958. [PubMed: 11085511]
 18. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature*. 2008; 455:1061–1068. [PubMed: 18772890]
 19. Chi A, Batchelor T, Kwak E, et al. Rapid radiographic and clinical improvement after treatment of a MET-amplified recurrent glioblastoma with a MET inhibitor (crizotinib). *J Clin Oncol*. 2011; 29(15_suppl):2072.
 20. Humphrey P, Wong A, Vogelstein B, Friedman H, Werner M, Bigner D, Bigner S. Amplification and expression of the epidermal growth factor receptor gene in human glioma xenografts. *Cancer Res*. 1988; 48:2231–2238. [PubMed: 3258189]
 21. Mizoguchi M, Betensky R, Batchelor T, Bernay D, Louis D, Nutt C. Activation of STAT3, MAPK, and AKT in malignant astrocytic gliomas: correlation with EGFR status, tumor grade, and survival. *J Neuropathol Exp Neurol*. 2006; 65:1181–1188. [PubMed: 17146292]
 22. Chaffanet M, Chauvin C, Laine M, Berger F, Chedin M, Rost N, Nissou M, Benabid A. EGF receptor amplification and expression in human brain tumours. *Eur J Cancer*. 1992; 28:11–17. [PubMed: 1567659]
 23. Gallia GL, Rand V, Siu IM, Eberhart CG, James CD, Marie SK, Oba-Shinjo SM, Carlotti CG, Caballero OL, Simpson AJ, Brock MV, Massion PP, Carson BS Sr, Riggins GJ. PIK3CA gene mutations in pediatric and adult glioblastoma multiforme. *Mol Cancer Res*. 2006; 4:709–714. [PubMed: 17050665]
 24. Knobbe CB, Reifenberger J, Reifenberger G. Mutation analysis of the Ras pathway genes *NRAS*, *HRAS*, *KRAS* and *BRAF* in glioblastomas. *Acta Neuropathol*. 2004; 108:467–470. [PubMed: 15517309]

25. Jeuken J, van den Broecke C, Gijzen S, Boots-Sprenger S, Wesseling P. RAS/RAF pathway activation in gliomas: the result of copy number gains rather than activating mutations. *Acta Neuropathol.* 2007; 114:121–133. [PubMed: 17588166]
26. Basto D, Trovisco V, Lopes JM, Martins A, Pardo F, Soares P, Reis RM. Mutation analysis of *B-RAF* gene in human gliomas. *Acta Neuropathol.* 2005; 109:207–210. [PubMed: 15791479]
27. Yip S, Miao J, Cahill DP, et al. MSH6 mutations arise in glioblastomas during temozolomide therapy and mediate temozolomide resistance. *Clin Cancer Res.* 2009; 15(14):4622–4629. [PubMed: 19584161]
28. Snuderl M, Fazlollahi L, Le LP, et al. Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma. *Cancer Cell.* 2011; 20(6):810–817. [PubMed: 22137795]
29. Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, Upadhyay R, Maira M, McNamara K, Perera SA, Song Y, Chirieac LR, Kaur R, Lightbown A, Simendinger J, Li T, Padera RF, Garcia-Echeverria C, Weissleder R, Mahmood U, Cantley LC, Wong KK. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med.* 2008; 14:1351–1356. [PubMed: 19029981]
30. Bamford S, Dawson E, Forbes S, Clements J, Pettett R, Dogan A, Flanagan A, Teague J, Futreal PA, Stratton MR, Wooster R. The COSMIC (catalogue of somatic mutations in cancer) database and website. *Br J Cancer.* 2004; 91:355–358. [PubMed: 15188009]
31. Peraud A, Watanabe K, Schwechheimer K, Yonekawa Y, Kleihues P, Ohgaki H. Genetic profile of the giant cell glioblastoma. *Lab Invest.* 1999; 79:123–129. [PubMed: 10068201]
32. Peraud A, Watanabe K, Plate KH, Yonekawa Y, Kleihues P, Ohgaki H. p53 Mutations versus EGF receptor expression in giant cell glioblastomas. *J Neuropathol Exp Neurol.* 1997; 56:1236–1241. [PubMed: 9370234]
33. Meyer-Puttlitz B, Hayashi Y, Waha A, Rollbrocker B, Bostrom J, Wiestler OD, Louis DN, Reifenberger G, von Deimling A. Molecular genetic analysis of giant cell glioblastomas. *Am J Pathol.* 1997; 151:853–857. [PubMed: 9284834]
34. Duerr EM, Rollbrocker B, Hayashi Y, Peters N, Meyer-Puttlitz B, Louis DN, Schramm J, Wiestler OD, Parsons R, Eng C, von Deimling A. PTEN mutations in gliomas and glioneuronal tumors. *Oncogene.* 1998; 16:2259–2264. [PubMed: 9619835]
35. Wang SI, Puc J, Li J, Bruce JN, Cairns P, Sidransky D, Parsons R. Somatic mutations of PTEN in glioblastoma multiforme. *Cancer Res.* 1997; 57:4183–4186. [PubMed: 9331071]
36. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliareis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science.* 1997; 275:1943–1947. [PubMed: 9072974]

Fig 1.

Representative examples of SNaPshot tumor genotyping. The *top panels* show genotyping data obtained for normal male genomic DNA (Promega, Madison, WI) and the *bottom panels* show data derived from patient GBM tumors. Mutant and wild-type alleles are distinguished based on the slightly different positions and on the distinct colors of their corresponding peaks. *Arrows* indicate mutant alleles. **a** Identification of the IDH1 R132H (cDNA nucleotide change 395C>A) mutation in one GBM specimen. Assays: (1) IDH1 R132; (2) EGFR E746_A750; (3) EGFR L858; (4) CTNNB1 S45; (5) PIK3CA E542; (6) NRAS G12; (7) EGFR L861 and (8) AKT1 E17. **b** Identification of the PIK3CA H1047R (3140A>G) mutation in another GBM tumor. Assays: (1) PIK3CA H1047; (2) CTNNB1 G34; (3) BRAF V600; (4) NRAS G13; (5) PIK3CA Q546; (6) APC R1450 and (7) APC R1114

Fig 2. Detection of gene amplification by fluorescence in situ hybridization (FISH). Tumor cells show *EGFR* amplification in **a** (orange signals) and *MET* amplification in **b** (pink signals). The CEP7 chromosome (centromere control) is represented by aqua signals (white arrowheads). Nuclei are stained with 4',6-diamidino-2-phenylindole

Table 1

Genetic abnormalities detected by molecular profiling and cancer therapies in clinical development

Gene	Amino acid residue/gene alteration interrogated	Relevant drugs available or in clinical development
AKT1	E17	PKB/AKT inhibitors (perifosine, MK-2206)
APC	R1114	None currently
	Q1338	
	R1450	
	T1556	
BRAF	G466 ^a	Raf inhibitors (sorafenib, PLX4032, GSK2118436)
	G469 ^a	MEK inhibitors (AZD6244, ARRY-162, GDC-0973)
	L597 ^a	
	V600	ERK inhibitors
CTNNB1 (beta-catenin)	D32	None currently
	S33	
	G34	
	S37	
	T41	
	S45	
EGFR	G719 ^a	EGFR inhibitors (gefitinib, erlotinib, BIBW2992, PF-00299804, lapatinib, vandetanib, cetuximab)
	T790	
	L858	
	L861 ^a	
	E746_A750 deletions	
	Exon 19 deletions	
	Exon 20 insert/deletions	
	Gene amplification (FISH)	
HER2	Exon 20 insertions	HER2 inhibitors (lapatinib, trastuzumab, PF-00299804)
IDH1	R132	None currently
KIT	D816	Kit inhibitors (imatinib, sorafenib, sunitinib, cediranib)
KRAS	G12	PI3K inhibitors (XL765, BKM120, BEZ235, GDC-0941)
	G13	MEK inhibitors (AZD6244, ARRY-162, GDC-0973)
	Q61 ^a	ERK inhibitors
MEK1	Q56 ^a	MEK inhibitors (AZD6244, ARRY-162, GDC-0973)
	K57 ^a	
	D67 ^a	
NOTCH1	L1575	Notch1/gamma-secretase inhibitors
	L1601	
NRAS	G12	PI3K inhibitors (XL765, BKM120, BEZ235, GDC-0941)
	G13	MEK inhibitors (AZD6244, ARRY-162, GDC-0973)
	Q61	ERK inhibitors

Gene	Amino acid residue/gene alteration interrogated	Relevant drugs available or in clinical development
PIK3CA	R88	PI3K inhibitors (XL765, BKM120, BEZ235, GDC-0941)
	E542	
	E545	PKB/AKT inhibitors (perifosine, MK-2206, GSK2110183)
	Q546	
	H1047	mTOR inhibitors (rapamycin, temsirolimus, everolimus, tipifarnib, BEZ235, XL765)
	G1049	
PTEN	R130	PI3K inhibitors (XL765, BKM120, BEZ235, GDC-0941)
	R173	
	R233	PKB/AKT inhibitors (perifosine, MK-2206, GSK2110183)
	K267	mTOR inhibitors (rapamycin, temsirolimus, everolimus, tipifarnib, BEZ235, XL765)
TP53	R175	None currently
	G245	
	R248	
	R273	
	R306	
PDGFRA	Gene amplification (FISH)	PDGFRA inhibitors (imatinib, sunitinib, sorafenib, cediranib)
MET	Gene amplification (FISH)	MET inhibitors (XL184, crizotinib, AMG102)
Chromosome arms 1p and 19q	Deletion/loss (FISH)	Procarbazine, CCNU, vincristine (PCV) Temozolomide
MGMT	Promoter DNA methylation (MS-PCR)	Temozolomide

^aLoci added to the SNaPshot assay in September 2010

Molecular profiling results

Table 2

	Number	Number of tumors with abnormality ^a													
		AKT1 mut ^b	BRAF mut ^b	CTNNB1 mut ^b	EGFR mut ^b	IDH1 mut ^b	KRAS mut ^b	PIK3CA mut ^b	PTEN mut ^b	TP53 mut ^b	EGFR Amp ^c	MET Amp ^c	PDGFRA Amp ^c	MGMT Meth ^d	1p/19q Code ^e
Astrocytic tumors															
Glioblastoma	164		1	1	1	17	3	9	4	20	42/102	4/102	2	61/114	4/30
GBM-O ^f	17					4	1		1	2	4/10			5/11	3/14
Secondary GBM	8					5				3	1/5			5/8	1/1
Anaplastic astrocytoma	23	1				9		1	1	8	3/6	0/5		1/4	1/7
Diffuse astrocytoma	5		1			2				1				0/1	0/1
Oligodendroglial tumors															
Anaplastic oligodendroglioma	7					6		1	1					0/1	5/5
Oligodendroglioma grade II	4					4								1/1	3/3
Oligoastrocytic tumors															
Anaplastic oligoastrocytoma	10					6				4				0/2	1/6
Oligoastrocytoma grade II	5					3				2	0/1			1/1	2/4
Total	218	1	2	1	1	47	3	11	6	35	45/109	4/107	2	64/123	16/56

^aOnly SNaPshot genes with detected mutations are shown

^bSNaPshot mutation

^cGene amplification

^dPromoter DNA methylation

^e1p/19q Co-deletion

^fGBM with oligodendroglial component