Evaluating Cancer of the Central Nervous System Through Next-Generation Sequencing of Cerebrospinal Fluid

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ABSTRACT

Purpose
Cancer spread to the central nervous system (CNS) often is diagnosed late and is unresponsive to therapy. Mechanisms of tumor dissemination and evolution within the CNS are largely unknown because of limited access to tumor tissue.

Materials and Methods
We sequenced 341 cancer-associated genes in cell-free DNA from cerebrospinal fluid (CSF) obtained through routine lumbar puncture in 53 patients with suspected or known CNS involvement by cancer.

Results
We detected high-confidence somatic alterations in 63% (20 of 32) of patients with CNS metastases of solid tumors, 50% (six of 12) of patients with primary brain tumors, and 0% (zero of nine) of patients without CNS involvement by cancer. Several patients with tumor progression in the CNS during therapy with inhibitors of oncopgenic kinases harbored mutations in the kinase target or kinase bypass pathways. In patients with glioma, the most common malignant primary brain tumor in adults, examination of cell-free DNA uncovered patterns of tumor evolution, including temozolomide-associated mutations.

Conclusion
The study shows that CSF harbors clinically relevant genomic alterations in patients with CNS cancers and should be considered for liquid biopsies to monitor tumor evolution in the CNS.

INTRODUCTION

The treatment of human cancer has shifted toward a precision medicine paradigm that increasingly relies on the genomic annotation of each patient’s tumor tissue. This trend is supported by the clinical observation that tumor responses to signal transduction inhibitors often are greatest in tumors that harbor mutations in the targeted pathway, by the discovery of specific drug-resistance mutations in tumors that resume growth during therapy, and by the recent association between effective immunotherapy and tumor-specific missense mutations. Outgrowth of drug-resistant tumor cell clones during therapy can limit the clinical relevance of the initial tumor profile and has motivated the development of technologies that can track the evolution of the cancer genome in accessible body fluids.1

Cancers that affect the central nervous system (CNS) pose a particular challenge because of the difficulty in accessing tumor tissue and the inability to detect circulating tumor DNA in the plasma of affected patients.2 One potential source of tumor-derived DNA in patients with CNS tumors is cerebrospinal fluid (CSF), which circulates through the CNS. In patients whose primary tumor had disseminated to the CNS, several groups were able to identify selected mutations of the primary tumor in CSF by using polymerase chain reaction detection techniques.3-7 A recent study collected CSF during the resection of primary brain or spinal cord tumors and reported that 26 of 35 (74%) samples contained tumor DNA, which was defined as the presence of at least one mutation in the primary tumor.8 All patients were previously untreated, and the detection of mutations in the CSF was guided by prior profiling of the primary tumor. A separate
Fig 1. Comparison of tumor-derived DNA from cerebrospinal fluid (CSF) cell pellet and supernatant. (A) Schematic of separation of CSF pellet and supernatant. Cellular DNA is isolated from the pellet, and cell-free DNA (cfDNA) is isolated from the supernatant. (B) Variant allele frequencies for known mutations in CSF cfDNA and pellet DNA. (C) Log2 ratios of normalized sequence coverage for target exons in CSF cfDNA and pellet DNA for patient 8. Greater than 10-fold amplification of HER2 was observed in CSF cfDNA, whereas HER2 amplification was barely detectable in pellet DNA. (D) Evidence of EML4-ALK gene fusion in CSF cfDNA and pellet DNA for patient 6. Read pairs supporting the fusion (red) were visualized by using the Integrative Genomics Viewer. Pt, patient ID.
## Table 1. Characteristics of 53 Patients With CNS Involvement and Primary Tissue Cancer for Which CSF Cell-Free DNA Was Extracted and Analyzed

| Patient No. | Primary Tumor | CNS Involvement | Original Specimen (molecular pathology) | CSF (MSK-IMPACT) |
|-------------|--------------|----------------|----------------------------------------|-----------------|
| 1           | NSCLC        | Brain metastases | EGFR L858R (bone, Sequenom)             | EGFR L858R (56%) |
| 2           | NSCLC        | Brain metastases | EGFR L858R (lung, Sequenom)             | EGFR L858R (76%) |
| 3           | NSCLC        | Brain metastases | EGFR exon 19 del (cheast wall, Sequenom)| EGFR L858R (2.9%), EGFR L858R (10%); |
| 4           | NSCLC        | Brain metastases | EGFR L858R (lung, Sequenom)             | EGFR L858R (55%) |
| 5           | NSCLC        | Brain metastases | KRAS G12C (c34 G>T) (lung, Sequenom)    | KRAS G12C (96%), CDKN2B del (log2, −2.97) |
| 6           | NSCLC        | Brain metastases | ALK rearrangement (lung, ND)            | EML4-ALK fusion (39 reads) |
| 7           | NSCLC        | Leptomeningeal metastases | EML4-ALK fusion (lung, ND) | EML4-ALK fusion (102 reads) |
| 8           | Breast       | Brain metastases | HER2 AMP (breast, FISH)                 | PIK3CA H1047R (38%), HER2 AMP (log2, 3.5) |
| 9           | Breast       | Brain metastases | HER2 AMP (breast, FISH)                 | HER2 AMP (log2, 2.6) |
| 10          | Breast       | Brain metastases | HER2 positive (breast, IHC 3+)          | HER2 AMP (log2, 2.6) |
| 11          | Breast       | Brain metastases | No molecular profiling performed        | EGFR AMP (log2, 3.1), PIK3CA H1047R (28%) |
| 12          | Breast       | Brain metastases | TP53 V272M (56%), PTEN del (log2, −2.0; lymph node, MSK-IMPACT) | TP53 V272M (81%), PTEN del (log2, −2.97) |
| 13          | Breast       | Brain metastases | ER positive, PR/HER2 negative (thyroid metastases, IHC) | PIK3CA E545K (26%) |
| 14          | Melanoma     | Brain metastases | BRAF V600E (skin, ND)                   | BRAF V600E (24%) |
| 15          | Melanoma     | Leptomeningeal metastases | BRAF V600E (skin, ND) | BRAF V600E (log2, −2) |
| 16          | Melanoma     | Brain metastases | BRAF V600E (skin, ND)                   | BRAF V600E (log2, −0.5) |
| 17          | Bladder cancer | Brain metastases | No molecular profiling performed        | AKT2 AMP (log2, 3.37), TP53 R158L (43%) |
| 18          | Gastroesophageal metastases | No molecular profiling performed | HER2 AMP (log2, 2.4), FGFR2 (log2, 3.6) |
| 19          | Neuroendocrine | Brain metastases | No molecular profiling performed        | MYCN AMP (log2, 4.1) |
| 20          | Ovarian      | Brain metastases | BRCA1 insc (blood, Myriad Genetics laboratory) | BRCA1 Q1756 fs (53%), CDKN2B del (log2, −2.1) |
| 21          | Ovarian      | Leptomeningeal metastases | No molecular profiling performed        | Negative |
| 22          | Breast       | Brain metastases | No molecular profiling performed        | Negative |
| 23          | Breast       | Brain metastases | HER2 AMP (breast, FISH)                 | Negative |
| 24          | Breast       | Brain metastases | ESR1 YS375S (62%), CCND1 AMP (log2, 1.5; breast, MSK-IMPACT) | Negative |
| 25          | Breast       | Brain metastases | R857L435S-S*3 (liver, MSK-IMPACT)        | Negative |
| 26          | Breast       | Brain metastases | Pik3ca R108 del (39%), CCND1 AMP (log2, 1.0; soft tissue, MSK-IMPACT) | Negative |
| 27          | NSCLC        | Brain metastases | No molecular profiling performed        | Negative |
| 28          | NSCLC        | Brain metastases | No molecular profiling performed        | Negative |
| 29          | SCLC         | Brain metastases | No molecular profiling performed        | Negative |
| 30          | NSCLC        | None             | ALK rearrangement (lung, FISH)          | Negative |
| 31          | Melanoma     | Brain metastases | BRAF V600E (skin, Sequenom)             | Negative |
| 32          | Melanoma     | Brain metastases | BRAF V600K (lymph node, Sequenom)       | Negative |
| 33          | Melanoma     | None             | NRAS (lung, Sequenom)                   | Negative |
| 34          | Thyroid      | Brain metastases | NRAS TP53 (thyroid, PCR)                | Negative |
| 35          | Thyroid      | None             | No molecular profiling performed        | Negative |
| 36          | Rectal       | None             | No molecular profiling performed        | Negative |
| 37          | Prostate     | None             | No mutation found (prostate, MSK-IMPACT) | Negative |
| 38          | Prostate     | None             | NOTCH1 R1758H (13%; prostate, MSK-IMPACT) | Negative |
| 39          | Renal        | None             | No molecular profiling performed        | Negative |
| 40          | Renal        | None             | No molecular profiling performed        | Negative |
| 41          | Liposarcoma  | None             | No molecular profiling performed        | Negative |
| 42          | Anaplastic astrocytoma | None | IDH1 R132H (IHC), PIK3CA H1047R | IDH1 R132H (38%), PTEN R130* (25%) |
| 43          | Glioblastoma | None             | No molecular profiling performed        | PIK3CA V344M (6%) |
| 44          | Glioblastoma | None             | PTEN loss (IHC)                         | PTEN Y338F337 delins* (14%), EGFR AMP (log2, 3.4) |

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study used targeted next-generation sequencing to reveal oncogenic mutations in tumor-derived DNA from CSF in a limited number of patients. Together, these studies suggest that the shedding of tumor DNA into the CSF may be a frequent occurrence in CNS cancers, but it is unclear whether comprehensive sequencing of CSF can routinely provide clinically meaningful information without a need for surgery in a large series of patients. The goal of the current study was to explore whether routine lumbar puncture and high-throughput sequencing of CSF could identify tumor-associated DNA in patients with known or suspected CNS involvement and provide clinically meaningful insights into the biology of these tumors and their treatment response.

### MATERIALS AND METHODS

#### CSF Collection and Preparation

We collected CSF samples from 53 patients with cancer who underwent evaluation for leptomeningeal metastasis between August 2014 and February 2015. Fifty-two (98%) CSF samples were obtained by lumbar puncture and one from an Ommaya reservoir. All patients signed informed consent for use of leftover CSF for research purposes under protocols approved by the Memorial Sloan Kettering (MSK) Cancer Center Institutional Review Board. Within 2 to 3 hours of CSF collection, 5 mL of CSF was placed on ice and centrifuged at 1,000 g for 5 minutes. The supernatant was aseptically transferred to prelabeled cryotubes, and the cell pellet was resuspended in 1 mL of RPMI + 20% fetal bovine serum + 20% dimethyl sulfoxide. All tubes were stored at −70°C.

#### Extraction of Cell-Free DNA

The minimum amount of the CSF tested was 2 mL (mean, 5 mL; range, 2 to 7 mL). Stored CSF samples were thawed at room temperature and centrifuged at 10,000 × g for 30 minutes at 4°C to remove residual precipitated cellular components and various particles. The method applied for the extraction of cell-free DNA (cfDNA) was based on the manufacturer’s protocol for the QIAamp Circulating Nucleic Acid Kit (catalog #55114; QIAGEN, Valencia, CA). Briefly, 5 mL of CSF was mixed with 500 μL of protease K and 4 mL of buffer ACL. After incubation at 60°C for 30 minutes, 9 mL of buffer ACB was added and then incubated on ice for 5 minutes. The mixture was filtered through a minicolumn and rinsed by ACW1, ACW2, and ethanol. DNA was eluted in 100 μL of buffer AVE.

### Targeted Capture and Sequencing

All samples were subjected to molecular analysis by using the MSK-Integrated Molecular Profiling of Actionable Cancer Targets (IMPACT) assay, which captures all protein-coding exons of 341 cancer-associated genes as well as 33 introns in 14 recurrently rearranged genes. The Illumina libraries were constructed with KAPA Hyper Prep Kit followed by ligation with 5 μM adaptor concentration (catalog #KK8504; Kapa Biosystems, Wilmington, MA). Libraries of this targeted capture were pooled in equimolar concentrations and sequenced on an Illumina HiSeq 2500 system (Illumina, San Diego, CA) as paired end 100–base pair reads.

#### Genomic Analysis

Analysis for the targeted sequencing data was performed as described previously. In brief, demultiplexed FASTQ files were aligned to GRCh37 reference human genome assembly by using BWA-MEM (Burrows-Wheeler Aligner software version 0.7.5a, http://arxive.org/abs/1303.3997), and polymerase chain reaction duplicates were identified with use of the MarkDuplicates tool in Picard Tools software version 1.96 (https://github.com/broadinstitute/picard). Regions in the genome covered by more than 20X coverage were identified by using the FindCoveredIntervals from the Genome Analysis Toolkit and subjected to indel realignment by Assembly-Based ReAligner version 0.92 software. Variant calling was performed in paired tumor/normal mode by using MuTect software version 1.1.4 for single nucleotide variants and SomaticIndelDetector for small insertions and deletions. All variants were then annotated using ANNOVAR software version 20120718. For CSF cfDNA and cell pellets without a genetically matched normal, variants were called against a single pool of unmatched normal samples, and variants were filtered if the minor allele frequency was > 0.0004 in any subpopulation in the 1000 Genomes Project cohort or Exome Aggregation Consortium because these are more likely to be common population polymorphisms than somatic mutations. All candidate mutations and indels in the Data Supplement were called automatically by using the bioinformatics pipeline described previously and subsequently reviewed manually by using the Integrative Genomics Viewer to eliminate potential false-positive calls. The current framework can be found at https://github.com/rshah/impact-pipeline.

Copy number variation was identified by analyzing sequence coverage of targeted regions in a tumor sample compared with a standard diploid normal sample after performing sample-wide LOWESS normalization for guanine-cytosine percentage across exons and normalizing...

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**Table 1. Characteristics of 53 Patients With CNS Involvement and Primary Tissue Cancer for Which CSF Cell-Free DNA Was Extracted and Analyzed (continued)**

| Patient No. | Primary Tumor | CNS Involvement | Original Specimen (molecular pathology) | CSF (MSK-IMPACT) |
|-------------|---------------|-----------------|----------------------------------------|-----------------|
| 45          | Anaplastic oligodendroglioma | N/A | IDH1 R132H (IHC), 1p/19q del (FISH) | IDH1 R132H (44%), 1p/19q del (log2, −0.8) |
| 46          | Glioblastoma | N/A | PTEN loss, CDX4 AMP, CL11 AMP, TP53, TERT, SPTA1 (FISH) | CDX4 AMP (log2, 2.4) |
| 47          | Brainstem glioma | N/A | No molecular profiling performed | PDGFRA AMP (log2, 2.0), CDKN2B del (log2, −3.0) |
| 48          | Glioblastoma | N/A | No molecular profiling performed | Negative |
| 49          | High-grade glioma | N/A | No molecular profiling performed | Negative |
| 50          | Oligodendroglioma | N/A | No molecular profiling performed | Negative |
| 51          | Anaplastic ependymoma | N/A | CDKN2A Y44* (8%; MSK-IMPACT) | Negative |
| 52          | Anaplastic oligodendroglioma | N/A | 1p/19q del (FISH) | Negative |
| 53          | Anaplastic oligodendroglioma | N/A | IDH1 R132H (IHC), 1p/19q del (FISH) | Negative |

Abbreviations: ALK, anaplastic lymphoma kinase; AMP, amplification; CNS, central nervous system; CSF, cerebrospinal fluid; del, deletion; delins, deletion/insertion; EGFR, epidermal growth factor receptor; ER, estrogen receptor; FISH, fluorescent in situ hybridization; FM, Foundation Medicine; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ins, insertion; MSK-IMPACT, Memorial Sloan Kettering-integrated Mutation Profiling of Actionable Cancer Targets; N/A, not applicable; ND, not determined; NSCLC, non-small-cell lung cancer; PCR, polymerase chain reaction; PR, progesterone receptor; SCLC, small-cell lung cancer.
for global differences in on-target sequence coverage, as previously described. Somatic structural aberrations were identified by using DELLY software. All candidate somatic structural aberrations were filtered, annotated by using in-house tools, and manually reviewed with Integrative Genomics Viewer. Figures were created and modified by using R for statistical computing and graphics (R Development Core Team) and Adobe Illustrator (http://www.adobe.com/products/illustrator.html) by using free templates designed by Freepik (http://freepik.com).

RESULTS

Comparison of Mutation Detection in CSF cfDNA Versus Cell-Pellet DNA

As a first step toward developing a robust mutation detection method, we examined which component of CSF was most sensitive for the detection of the most common cancer-associated genetic alterations. Because CSF in healthy individuals contains a small number of WBCs (0 to 5/μL), we were concerned that germline DNA from normal or reactive WBCs would dilute the signal from tumor-derived DNA. To address this question, we centrifuged eight freshly collected CSF samples, separated the cell pellet from the supernatant (Fig 1A), and compared DNA yields and detection of cancer-associated mutations in each aliquot. To increase the likelihood of detecting tumor-derived DNA, these pilot experiments focused on eight patients with an established diagnosis of CNS metastasis from solid tumors on the basis of typical radiographic findings (n = 8) or detection of tumor cells in the CSF (n = 7). In each patient, the primary tumor was known to harbor a clinically relevant driver mutation. There was a trend toward higher DNA yields from CSF pellets (mean, 280 ng) than from the CSF supernatants (mean, 27 ng), but this difference was not statistically significant (P = .22), and we achieved high unique sequence coverage of sequencing libraries from both pellets (mean, 746×) and supernatants (mean, 444×).

We next compared our ability to detect sequence mutations, copy number alterations, and structural rearrangements in CSF pellet DNA and CSF cfDNA with our in-house sequencing assay (MSK-IMPACT), which interrogates 341 clinically relevant cancer genes. MSK-IMPACT has been extensively validated in a cohort of > 300 distinct positive-control tumors and has been approved as a clinical test by the New York State Department of Health. All four patients with known single nucleotide substitutions exhibited a higher percentage of sequence reads that harbored the mutant allele in the CSF cfDNA compared with the CSF pellet DNA. These included two patients with BRAF V600E mutant melanoma, one with non–small-cell lung cancer (NSCLC) with EGFR L858R mutation, and one with NSCLC with KRAS G12C mutation (Fig 1B). In terms of gene copy number alterations, we observed an 11-fold amplification of the HER2 gene locus in CSF cfDNA from one patient with HER2-amplified breast cancer. In contrast, the copy number plot obtained from
pellet DNA of the same CSF sample was markedly blunted, and the HER2 gene amplification was barely detectable (Fig 1C). In another patient with breast cancer with known loss of PTEN, we detected a homozygous deletion at the PTEN locus on chromosome 10q in the CSF cfDNA sample but not in the cell pellet DNA (Appendix Fig A1, online only). Two patients with NSCLC harbored rearrangements that involved anaplastic lymphoma kinase (ALK) in the primary tumor, which was supported by 39 and 102 DNA fragments in CSF cfDNA compared with 14 and 102 DNA fragments in the respective CSF pellet DNA (Fig 1D; Appendix Fig A2, online only). In summary, known molecular alterations from the primary tumors were readily detectable in all eight (100%) CSF cfDNA samples but only in five (63%) CSF pellet DNA samples. In every case, the evidence that supported the key alteration was greater in cfDNA than in pellet DNA (Appendix Table A1, online only), which suggests that a higher proportion of the cfDNA is tumor derived.

**CSF cfDNA in Various Types of CNS Cancer**

Based on results from our pilot study, we used cfDNA as the DNA source for all subsequent analyses of genomic alterations in CSF and extended the study to 45 additional CSF samples from patients with cancer referred to our neuro-oncology clinic. All patients underwent standard-of-care diagnostic testing with magnetic resonance imaging (MRI) of the brain and CSF cytology. Together with the samples from our pilot project, the current study comprised 53 CSF samples from 41 patients with solid tumors and 12 with primary brain tumors. Of the 41 with solid tumors, 32 had CNS involvement by cancer. Nine with a variety of cancer types had no evidence of CNS involvement (ie, MRI and CSF cytology negative) and had an organ-confined primary tumor (n = 6) or metastatic cancer to non-CNS sites (n = 3; Table 1).

CSF from patients with brain metastases and positive CSF cytology (n = 13) showed significantly higher DNA yields, sequence library complexity, and unique sequence coverage than CSF from patients with negative CSF cytology (Appendix Fig A3, online only). Overall, we achieved $\geq 100\times$ coverage in 12 of 16 patients with positive CSF cytology (average unique median coverage, 397×) and in four of 25 with negative CSF cytology (average unique median coverage, 175×; P < .001). The patients who did not have detectable levels of CSF cfDNA had lower overall DNA yields (13 v 90 ng) and lower unique sequence coverage.

### Table A1: Patients With Acquired Resistance to Molecularly Targeted Therapies

| Patient No. | Tumor Type | Genotype-Directed Therapy | First Tumor Profile | Recurrent Tumor Profile | CSF Profile (IMPACT) |
|------------|------------|---------------------------|---------------------|-------------------------|----------------------|
| 1          | NSCLC      | Erlotinib                 | EGFR L858R (bone, Sequenom) | EGFR L858R and T790M (bone, PCR) | EGFR L858R (56%) |
| 2          | NSCLC      | Erlotinib                 | EGFR L858R (lung, Sequenom) | EGFR L858R and T790M (lung, Sequenom) | EGFR T790M (2.5%), EGFR L858R (76%) |
| 3          | NSCLC      | Erlotinib, Second-generation TKI | EGFR exon 19 del (chest wall, Sequenom) | EGFR exon 19 del and T790M (bone, Sequenom) | EGFR T790M (2.8%), EGFR T790M (37%) |
| 4          | NSCLC      | Erlotinib                 | EGFR L858R (lung, Sequenom) | EGFR T790M (lung, PCR) | KRAS G12A (19%), EGFR L858R (65%) |
| 5          | NSCLC      | Crizotinib                | ALK rearrangement (lung, ND) | EGFR T790M (lung, PCR) | EML4-ALK fusion (39 reads) |
| 6          | NSCLC      | Crizotinib                | EML4-ALK fusion (lung, Foundation Medicine) | EML4-ALK fusion (102 reads) | EML4-ALK fusion (102 reads) |
| 7          | NSCLC      | Crizotinib                | EML4-ALK fusion (lung, Foundation Medicine) | Pik3ca H1047R (38%), ERBB2 AMP (log2, 3.5) | Pik3ca H1047R (38%), ERBB2 AMP (log2, 3.5) |
| 8          | BrCa       | Trast, pert, lapatinib, T-DM1 | HER2 AMP (breast, FISH) | ERBB2 AMP (log2, 2.6) | ERBB2 AMP (log2, 2.6) |
| 9          | BrCa       | Trast, lapatinib          | HER2 AMP (breast, FISH) | ERBB2 AMP (log2, 2.6) | ERBB2 AMP (log2, 2.6) |
| 10         | BrCa       | Trast, lapatinib, pert, T-DM1 | HER2 positive (breast, IHC 3+) | ERBB2 AMP (log2, 2.6) | ERBB2 AMP (log2, 2.6) |
| 11         | Melanoma   | Dabrafenib, trametinib    | BRAF V600E (skin, ND) | PNET del (log2, –3.5), BRAF V600E (96%) | PNET del (log2, –3.5), BRAF V600E (96%) |
| 12         | Melanoma   | Dabrafenib                | BRAF V600E (skin, ND) | PNET del (log2, –3.5), BRAF V600E (96%) | PNET del (log2, –3.5), BRAF V600E (96%) |
| 13         | Melanoma   | Dabrafenib                | BRAF V600E (skin, ND) | PNET del (log2, –3.5), BRAF V600E (96%) | PNET del (log2, –3.5), BRAF V600E (96%) |
| 14         | Melanoma   | Dabrafenib                | BRAF V600E (brain metastasis, Sequenom) | BRAF V600E (24%) | BRAF V600E (24%) |
| 15         | Melanoma   | Dabrafenib, trametinib    | BRAF V600E (brain metastasis, Sequenom) | NRAS G12K (3%), PNET del (log2.3), BRAF V600E (47%) | NRAS G12K (3%), PNET del (log2.3), BRAF V600E (47%) |

![Fig 3](https://www.jco.org) Drug-resistance mutations in patients whose central nervous system (CNS) disease progresses during kinase inhibitor therapy. (A) Summary of genomic profiling results from cerebrospinal fluid (CSF) and other tumor sites in patients in whom progressive CNS disease developed during treatment with the indicated kinase inhibitors. (B) Disease timeline and brain magnet resonance images (MRI) from a patient with EGFR-mutant NSCLC (patient 3) who presented with leptomeningeal metastasis (baseline MRI, arrows), responded to erlotinib follow-up MRI at 26 months), was found to have a secondary EGFR mutation (T790M) in a bone metastasis, and developed progressive CNS disease (brain MRI and CSF cytology negative) and had an organ-confined primary tumor (n = 6) or metastatic cancer to non-CNS sites (n = 3; Table 1).
Next-Generation Sequencing of CSF in CNS Cancer

(42× 9 464×), which reflected the smaller quantities of tumor-derived DNA and low background levels of nontumor-derived DNA in CSF. For patients with primary brain tumors, we achieved ≥ 100× coverage in five of 12 patients (average unique median coverage, 391×). CSF from most of these patients (11 of 12) had negative cytology.

With MSK-IMPACT, we detected high-confidence somatic alterations in 63% of patients with CNS metastases and solid tumors and 50% of patients with primary brain tumors. None of the CSF samples from patients with cancer without CNS involvement (n = 9) showed tumor-derived molecular alterations. Within the group with CNS metastases, high-confidence somatic alterations were found in all 16 (100%) patients with positive CSF cytology and four of 16 (25%) with negative CSF cytology with radiographic evidence for CNS metastases (P = .1; Fig 2). No statistically significant relationship was found between the presence of tumor-associated DNA in CSF and patient age or sex, histology of the primary tumor, presence of metastases outside the CNS, prior treatment, or site of the CNS metastases (Appendix Table A2, online only).

![Diagram of patient 3: Lung Adenocarcinoma](image)

![Diagram of patient 4: Lung Adenocarcinoma](image)

Fig 3. (Continued).
Fig 4. Tumor evolution in patients with primary brain tumors. (A) Spatial and temporal heterogeneity among samples obtained at diagnosis, at recurrence, and from cerebrospinal fluid (CSF) in patient 42 with recurrent glioblastoma. CSF cell-free DNA harbors a PTEN R130* mutation (variant allele frequency, 0.25), whereas resection 2 harbors a PIK3CA H1047R mutation (variant allele frequency, 0.441). (B) CSF molecular profile for patient 45 with anaplastic oligodendroglioma contains the IDH1 R132H mutation and 1p/19q deletion found in tissue resection 2 as well as 454 nonsilent somatic mutations. Four hundred forty-eight SNVs represent C>T/G>A mutations that demonstrate TMZ-induced mutagenesis. Carbo, carboplatin; CCNU, lomustine; rhuMAB VEGF, bevacuzumab; RT, radiotherapy, SNV, single nucleotide variant; TMZ, temozolomide.
Clinical Significance of Mutations in CSF cfDNA

To interpret the genetic alterations in CSF cfDNA within their clinical context, we compared them to molecular profiling results of earlier biopsy specimens taken from the same patient. For many patients, the primary tumor sample had been examined by using a range of clinically approved laboratory tests. Mutations in CSF cfDNA were called de novo on the basis of a bioinformatics pipeline without prior knowledge of the alterations seen in tissue. In all available patients in whom CSF cfDNA was detectable, the CSF cfDNA sample was concordant with the previously identified molecular alteration (Table 1; Appendix Table A3, online only).

An important potential application of CSF genomic profiling is the identification of drug-resistance mechanisms in patients whose primary tumor responded to genotype-directed targeted cancer therapy but then progressed in the CNS. Of note, secondary kinase mutations can confer drug resistance at low allelic frequency and, therefore, can be difficult to detect.20 We hypothesized that the coverage of the MSK-IMPACT sequencing assay is sufficiently deep and broad to uncover a range of drug-resistance mutations in CSF cfDNA. In a group of 12 cohort patients in whom progressive CNS disease developed during treatment with inhibitors of oncogenic kinases (epidermal growth factor receptor [EGFR], ALK, human epidermal growth factor receptor 2 [HER2], or BRAF), we identified drug-resistance mutations in the CSF in four (one third). CSF cfDNA from two patients with EGFR-mutant lung cancer (patients 2 and 3 in Fig 3A) showed an EGFR T790M mutation, the most common cause of acquired resistance to first-generation EGFR tyrosine kinase inhibitors (TKIs) in NSCLC.21 Tumors obtained from both patients before EGFR TKI therapy were negative for the EGFR T790M mutation in a Sequenom-based genotyping assay, and the absence of the T790M mutation was confirmed in the pre-treatment tissue by MSK-IMPACT in patient 2. In a third patient with EGFR-mutant NSCLC and acquired erlotinib resistance in the CNS, we identified an activating KRAS G12A mutation, another common cause of EGFR TKI resistance in NSCLC22,23 (Fig 3B). In a patient with BRAF V600E–mutant (and NRAS-negative) melanoma, we identified an NRAS G12R mutation in the CSF (patient 16), a genetic alteration known to promote acquired resistance to BRAF inhibition in melanoma.24-28 Both the KRAS G12A and the NRAS G12R mutations were not detected by Sequenom before therapy.

Sequencing of CSF from two additional patients identified candidate drug-resistance mutations, but the relationship to clinical drug resistance was more ambiguous. Progressive CNS disease eventually developed in one patient (patient 8) with HER2-amplified breast cancer who received multiple HER2-targeted therapies (trastuzumab, rhUMab 2C4, and lapatinib) and who was found to harbor an activating mutation in the catalytic subunit of phosphatidylinositol 3-kinase (PI3K; PIK3CA H1047R) in CSF cfDNA, a potential cause of trastuzumab resistance in breast cancer.27,28 The allelic frequency of the PIK3CA mutation was considerably higher in the CSF sample (38%) than in the pretreatment primary tumor (4%). CSF from patient 15 with BRAF V600E–mutant melanoma showed a homozygous deletion of PTEN, but the primary tumor as not available to ascertain whether this genetic alteration occurred only in the CSE.

We also identified clinically relevant genomic alterations in CSF cfDNA from patients whose primary tumors could not be profiled because of limited access to tumor tissue, insufficient tumor content, or DNA quantity. For example, the CSF cfDNA from patient 18 with gastrointestinal carcinoma whose available tumor tissue was not adequate for molecular profiling (Appendix Fig A4, online only) harbored multiple somatic copy number alterations, including the loci for the receptor tyrosine kinases HER2 and fibroblast growth factor receptor 2, which are established drug targets in cancer and frequently amplified in this cancer type.29 Similarly, we identified PDGFRα amplification in a patient with a brainstem glioma30 that could not be biopsied because of the precarious tumor location (Appendix Figs A5 and A6, online only).

Sequencing of CSF cfDNA identified mutations in six of 12 (50%) patients with primary brain tumors despite that the CSF was negative for malignant cells in most (n = 11) of these patients. In one of these patients (Fig 4A), we were able to compare the pattern of mutations in CSF cfDNA with tumor profiling results obtained from the original tumor specimen and a second specimen obtained 3 weeks after CSF collection. Mutations in four genes (IDH1, TP53, ATRX, and TGFBR1) were detected in all three samples, but only the later samples harbored additional, but distinct mutations that activate the PI3K pathway, namely a truncating mutation in the PTEN tumor suppressor gene (CSF cfDNA) and an oncogenic hotspot mutation in the catalytic subunit of PI3K (recurrence tumor specimen). These data illustrate spatial heterogeneity within the CNS with evolutionary convergence on the PI3K signaling pathway during glioma progression. In another patient with 1p/19q codeleted oligodendroglioma31,32 (Fig 4B), CSF cDNA profiling 7.4 years after the initial diagnosis identified > 400 nonsynonymous single nucleotide variants. These mutations almost entirely represented C>T/G>A nucleotide changes, a mutation pattern that has been associated with exposure to temozolomide therapy in glioma.33

Discussion

Cancers involving the CNS are associated with exceptional morbidity and mortality. The development of a precision medicine paradigm for these cancers is hampered by difficulty in accessing tumor tissue. The current study expands on recent technical reports9 and demonstrates the feasibility of deriving a comprehensive molecular profile from CSF collected through lumbar puncture, a procedure that often is done in the physician’s office. Key aspects of our approach included the use of CSF cfDNA, which obviated the need for additional steps to enrich for tumor cells and a clinically validated next-generation sequencing platform capable of identifying all classes of cancer-associated mutations (base substitutions, insertions, deletions, fusions, gene copy number alterations). Mutation calling and copy number analysis were performed by using an automated bioinformatics pipeline with consistent criteria and thresholds across all samples. These modifications from prior approaches help us to identify tumor-associated DNA without the need for invasive surgery, in patients with cytology-negative CSF, and without prior knowledge of molecular alterations in the primary tumor. This approach could be implemented in most health care environments that already collect and process CSF as part of routine clinical practice.
CSF represents just one of many bodily fluids from which tumor-derived cfDNA can be isolated for molecular profiling. Blood plasma has received the greatest attention in recent years for its potential to serve as a "liquid biopsy" for patients with solid tumors.34,35 Many groups have demonstrated the utility of plasma cfDNA to facilitate noninvasive mutation profiling, monitoring response to therapy, and the identification of emergent resistance mutations in patients with advanced disease.2,36-38 These studies have encompassed multiple tumor types, including breast cancer, lung cancer, and prostate cancer.39,40 Furthermore, plasma DNA shows promise at earlier stages of disease as a prognostic for the risk of recurrence after surgery and even as a means for early detection of cancer.41,42 whereas early studies used targeted assays to longitudinally monitor individual prespecified mutations, such as BEAMing43 and digital droplet PCR, next-generation sequencing technologies have demonstrated the potential to reveal not only novel mutations, but also gene amplifications and fusions.44-46

One major challenge associated with plasma cfDNA sequencing is the need to detect mutations at very low allele frequencies. Tumor-derived DNA typically constitutes only a small fraction of all cfDNA in plasma due to the relatively high background of normal DNA from nonmalignant cells. Consequently, key oncogenic mutations may occur in only 1% or even 0.1% of molecules from a given genomic locus, requiring very deep sequence coverage to achieve sufficient sensitivity for detecting these mutations. This has also necessitated modifications to sequencing assays in order to maintain high specificity in the presence of sequencing errors and artifacts. Examples include Safe-SeqS57 and Duplex Sequencing,48 both of which use unique molecular identifiers to reduce errors through replicate sequence reads from the same template molecule. Importantly, we found that these approaches were not necessary for CSF cfDNA profiling. Although cfDNA yields were generally low after nucleic acid extraction from CSF, we observed that the fraction of tumor-derived cfDNA was generally high due to the relative absence of background normal DNA. As a result, we were able to readily detect somatic mutations even in cases where we only achieved modest sequence coverage (< 100×).

We found that tumor-associated DNA can be detected in CSF in a substantial number of patients with primary brain tumors, which agrees with a recent study that collected CSF intraoperatively from patients with primary brain tumors5 and suggests that collection and genomic profiling of CSF should be considered more broadly in patients with these tumors because it might provide new insights into tumor evolution and drug response. Because brain metastases and primary brain tumors are often inaccessible to surgery, we focused on an approach that did not require surgery for CSF collection. The current study also points to other scenarios where liquid biopsies and genomic profiling of CSF might guide clinical decision making, such as with leptomeningeal metastasis. This condition is notoriously difficult to diagnose with current methods and is associated with extraordinary morbidity and mortality.49,50 Similarly, CSF liquid biopsies could be informative in patients with multiple brain metastases, which are rarely biopsied but can harbor mutations not observed in the primary tumor.51

Tumor growth in the CNS despite systemic disease control remains a major clinical challenge. It is often unclear whether the CNS represents a sanctuary site, shielding malignant cells from target therapies, or whether select mutations render malignant cells more likely to successfully colonize the CNS. Our data suggest caution when using systemic genotyping results to predict the clinical response of CNS disease to targeted therapies. For extracranial sites, on-treatment biopsy specimens have shown several resistance mechanisms, including mutations that can restore signaling downstream of the target kinase, activate an alternative signaling pathway, or impair drug binding to the target kinase.52 The molecular basis of kinase inhibitor resistance in the CNS is unknown and widely attributed to inadequate drug penetration into the CNS.28-33,49-58 The current data suggest a more nuanced view of this common clinical problem because we identified a genetic explanation for drug resistance in at least four of 12 patients (33%). The current findings may explain the clinical experience that only a fraction of patients with acquired kinase inhibitor resistance in the CNS respond to an increase in the drug dose or intrathecal drug administration designed to overcome reduced penetration of drug into the CNS.59-62 They are also consistent with the recent identification of mutations in surgically resected brain tumor metastases that were not found in the primary tumors of the same patients.51

Our findings suggest that CSF sequencing could substantially increase the number of patients who are eligible for genotype-directed cancer therapy, including patients whose primary tumor could not be successfully sequenced and patients who are poor candidates for neurosurgical surgery due to medical comorbidities or tumor location in neurologically critical areas (eg, brainstem). Our study also suggests that cfDNA analysis in the CSF could be a suitable biomarker to monitor clinical response to therapy, analogous to plasma cfDNA, an area that will warrant further prospective evaluation. Together with the cited studies, this study demonstrates that genomic analysis of CSF by using a sufficiently sensitive and comprehensive platform may be useful in facilitating the diagnosis of tumor in the CNS, monitor the evolution of the cancer genome during treatment of CNS cancers, guide the choice of second-line agents, and perhaps identify pathways that are uniquely associated with cancer spread to the CNS.

Disclosures provided by the authors are available with this article at www.jco.org.
Cerebrospinal fluid (CSF) offers a unique medium for studying the genomics of cancer. Early detection of leptomeningeal metastasis by PCR detection of tumor-derived p53 DNA in cerebrospinal fluid (CSF) has been shown to be a promising approach. A recent study published in *Nature* (2014) used next-generation DNA sequencing to detect tumor-derived p53 DNA in cerebrospinal fluid (CSF) and demonstrated that this approach can detect early-stage leptomeningeal metastases.

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Appendix

Fig A1. Copy number plot for patient 12. cfDNA, cell-free DNA; CSF, cerebrospinal fluid; Pt, patient ID.

Fig A2. Detection of EML4-ALK gene fusion in cerebrospinal fluid (CSF) cell-free DNA (cfDNA) and pellet DNA for patient 7. Pt, patient ID.
Next-Generation Sequencing of CSF in CNS Cancer

(A) DNA input, (B) library yield, and (C) unique mean sequence coverage.

Fig A3.
Fig A4. Patient 18: the copy number plot (left panel) showing multiple somatic copy number alterations, including the loci for the receptor tyrosine kinases HER2 and FGFR2 and a brain MRI (right panel) with arrows pointing toward leptomeningeal metastases. CSF, cerebrospinal fluid; FGFR2, fibroblast growth factor receptor 2; HER2, human epidermal growth factor receptor 2.

Fig A5. CSF cell-free DNA profiling of glioblastoma for patient 46. CSF, cerebrospinal fluid; RT, radiotherapy; TMZ, temozolomide.
Patient 47: the copy number plot (left panel) showing amplification of the PDGFRA gene locus and a brain MRI (right panel) with arrows pointing toward the enhancing tumor in the brainstem.
### Table A2. Variables Included in Statistical Analysis

| CSF Tumor-Derived cfDNA Cohort (n = 20), No. (%) | Absent Tumor-Derived CSF cfDNA Cohort (n = 21), No. (%) | P |
|-----------------------------------------------|-------------------------------------------------------|---|
| Age, mean (SD) | 55.0 (11.5) | 55.4 (11.6) | .92 |
| Sex | | | |
| Male | 9 (45) | 8 (38) | .65 |
| Female | 11 (55) | 13 (62) | |
| Tumor histology | | | |
| Breast | 6 (30) | 5 (24) | .42 |
| Lung | 7 (35) | 4 (19) | |
| Melanoma | 3 (15) | 3 (14) | |
| Others | 4 (20) | 9 (43) | |
| Anatomic location | | | |
| Brain metastasis | | | |
| Supratentorial | 7 (35) | 7 (33) | .65 |
| Infratentorial | 3 (15) | 0 (0) | |
| Supra- and infratentorial | 7 (35) | 4 (19) | |
| No brain metastasis | 3 (15) | 10 (48) | |
| Distant metastasis | | | |
| Yes | 19 (95) | 16 (76) | .09 |
| No | 1 (5) | 5 (24) | |
| Prior CNS surgery | | | |
| Yes | 8 (40) | 3 (14) | .06 |
| No | 12 (60) | 18 (86) | |
| Chemotherapy at the time of CSF collection | | | |
| Yes | 17 (85) | 14 (67) | .17 |
| No | 3 (15) | 7 (33) | |
| Radiation therapy to CNS before CSF collection | | | |
| Yes | 12 (60) | 9 (43) | .27 |
| No | 8 (40) | 12 (57) | |

NOTE. This cohort excluded patients with primary brain tumors. See text and Tables 1 and A1 for details. Abbreviation: cfDNA, cell-free DNA.

### Table A3. Characteristics of 53 Patients, Including CSF Cytology and Primary Tissue Diagnosis, for Which CSF cfDNA Was Extracted and Analyzed

| Patient No. | Age | Sex | Tumor Type | CSF Cytology | CNS Involvement | Other Mets | Original Specimen (Molecular Path) | CSF (MSK-IMPACT) |
|-------------|-----|-----|------------|--------------|----------------|-----------|------------------------------------|-----------------|
| Solid tumors |
| 1 | 40 | M | Lung adenocarcinoma | Negative | BM | Yes | EGFR L858R (bone, Sequenom) | EGFR L858R (56%) |
| 2 | 52 | M | Lung adenocarcinoma | Positive | BM | No | EGFR L858R (lung, Sequenom) | EGFR T790M (2.5%), EGFR L858R (76%) |
| 3 | 51 | F | Lung adenocarcinoma | Positive | BM | Yes | EGFR exon 19 del (chest wall, Sequenom) | EGFR T790M (2.8%), EGFR 745,750 del (37%) |
| 4 | 75 | M | Lung adenocarcinoma | Negative | BM | Yes | EGFR L858R (lung, Sequenom) | KRA S G12C (96%), CDKN2B del (log2, −2.9) |
| 5 | 71 | F | Lung adenocarcinoma | Positive | BM | Yes | KRAS G12C (c34 G > T) (lung, Sequenom) | KRAS G12C (96%), EGFR L858R (85%) |
| 6 | 30 | M | Lung adenocarcinoma | Positive | BM | Yes | ALK rearrangement (lung, ND) | EML4-ALK fusion (39 reads) |
| 7 | 73 | F | Lung adenocarcinoma | Positive | LM | Yes | EML4-ALK fusion (lung, FM) | EML4-ALK fusion (102 reads) |
| 8 | 38 | F | Breast cancer | Positive | BM | Yes | HER2 AMP (breast, FISH) | PIK3CA H1047R (38%), HER2 AMP (log2, 3.5) |
| 9 | 56 | F | Breast cancer | Positive | BM | Yes | HER2 AMP (breast, FISH) | HER2 AMP (log2, 2.6) |
| 10 | 53 | F | Breast cancer | Positive | BM | Yes | HER2 positive (breast, IHC 3+) | HER2 AMP (log2, 2.6) |
| 11 | 59 | F | Breast cancer | Positive | BM | Yes | No molecular profiling performed | EGFR AMP (log2, 3.1), PIK3CA H1047R (28%) |
| 12 | 60 | F | Breast cancer | Positive | BM | Yes | TP53 V272M (56%), PTEN del (log2, −2.0; lymph node, MSK-IMPACT) | TP53 V272M (61%), PTEN del (log2, −2.97) |
| 13 | 59 | F | Breast cancer | Positive | BM | Yes | ER positive, PR/HER2 negative; thyroid metastasis, IHC) | PIK3CA E545K (26%) |
| 14 | 45 | M | Melanoma | Positive | BM | Yes | BRAF V600E (skin, ND) | BRAF V600E (24%) |
| 15 | 68 | M | Melanoma | Positive | LM | Yes | BRAF V600E (skin, ND) | PTEN del (log2, −XYY), BRAF V600E (96%) |
| 16 | 57 | F | Melanoma | Positive | BM | Yes | BRAF-V600E (skin, ND) | NRAS G132R (3%), PTEN del (log2, −3.0), BRAF V600E (47%) |

(continued on following page)
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### Table A3. Characteristics of 53 Patients, Including CSF Cytology and Primary Tissue Diagnosis, for Which CSF cfDNA Was Extracted and Analyzed (continued)

| Patient No. | Age | Sex | Tumor Type | CSF Cytology | CNS Involvement | Other Mets | Original Specimen (Molecular Path) | CSF (MSK-IMPACT) |
|-------------|-----|-----|------------|--------------|----------------|-----------|----------------------------------|------------------|
| 17          | 55  | M   | Bladder cancer | Positive     | BM             | Yes       | No molecular profiling performed | AKT2 AMP (log2, 3.37), TPS3 R158L (43%) |
| 18          | 50  | M   | Gastroesophageal | Positive     | LM             | Yes       | No molecular profile (MSK-IMPACT failure) | HER2 AMP (log2, 2.4), FGFR2 (log2 3.6) |
| 19          | 54  | M   | Neuroendocrine, unknown primary | Negative | BM             | Yes       | No molecular profiling performed | MYCN AMP (log2, 4.1) |
| 20          | 55  | F   | Ovarian cancer | Negative     | BM             | Yes       | BRCAl insC (blood, Myriad Genetics laboratory) | BRCAl Q1756fs (53%), CDX2N2B del (log2, –2.1) |
| 21          | 70  | F   | Ovarian cancer | Negative     | BM             | Yes       | No molecular profiling performed | Negative |
| 22          | 38  | F   | Breast cancer | Negative     | BM             | Yes       | No molecular profiling performed | Negative |
| 23          | 50  | F   | Breast cancer | Negative     | BM             | Yes       | HER2 AMP (breast, FISH) | Negative |
| 24          | 49  | F   | Breast cancer | Negative     | BM             | Yes       | ESR1 Y537S (62%), CCND1 AMP (log2, 1.5; breast, MSK-IMPACT) Negative |
| 25          | 32  | F   | Breast cancer | Negative     | BM             | Yes       | RB1 L343Sfs*3 (liver, MSK-IMPACT) Negative |
| 26          | 40  | F   | Breast cancer | Negative     | BM             | Yes       | PIK3CA R108 del (39%), CCND1 AMP (log2, 1.0; soft tissue, MSK-IMPACT) Negative |
| 27          | 63  | F   | Lung adenocarcinoma | Negative | BM             | Yes       | No molecular profiling performed | Negative |
| 28          | 66  | M   | Lung adenocarcinoma | Negative | BM             | Yes       | No molecular profiling performed | Negative |
| 29          | 66  | F   | Small-cell lung cancer | Negative | BM             | Yes       | No molecular profiling performed | Negative |
| 30          | 58  | F   | Lung adenocarcinoma | Negative     | BM             | Yes       | BRAF V600E (skin, Sequenom) Negative |
| 31          | 56  | M   | Melanoma | Negative | BM             | Yes       | BRAF V600K (lymph node, Sequenom) Negative |
| 32          | 54  | M   | Melanoma | Negative | BM             | Yes       | NRAS (lung, Sequenom) Negative |
| 33          | 37  | F   | Melanoma | Negative | BM             | Yes       | NRAS TP53 (thyroid, PCR) Negative |
| 34          | 55  | F   | Thyroid cancer | Negative     | BM             | Yes       | No molecular profiling performed | Negative |
| 35          | 54  | F   | Thyroid cancer | Negative | BM             | Yes       | No molecular profiling performed | Negative |
| 36          | 76  | M   | Rectal cancer | Negative | BM             | Yes       | No molecular profiling performed | Negative |
| 37          | 58  | F   | Prostate cancer | Negative     | BM             | Yes       | No mutation found (prostate, MSK-IMPACT) Negative |
| 38          | 67  | M   | Prostate cancer | Negative | BM             | Yes       | NOTCH1 R1758H (13%; prostate, MSK-IMPACT) Negative |
| 39          | 64  | M   | Renal cell carcinoma | Negative | BM             | No       | No molecular profiling performed | Negative |
| 40          | 58  | M   | Renal cell carcinoma | Negative | BM             | No       | No molecular profiling performed | Negative |
| 41          | 57  | F   | Liposarcoma | Negative | BM             | No       | No molecular profiling performed | Negative |
| 42          | 24  | M   | Anaplastic astrocytoma | Negative | N/A            | No       | IDH1 R132H (IHC), PIK3CA H1047R | IDH1 R132H (38%), PTPNR130* (25%) |
| 43          | 65  | M   | Glioblastoma | Negative     | N/A            | No       | No molecular profiling performed | PIK3CA V344M (6%) |
| 44          | 63  | M   | Glioblastoma | Negative     | N/A            | No       | PTEN loss (IHC) | PIK3CA V344M (6%) |
| 45          | 39  | F   | Anaplastic oligodendroglioma | Negative | N/A            | No       | IDH1 R132H (IHC), 1p/19q del (FISH) | IDH1 R132H (44%), 1p/19q del (log2, –0.8) |
| 46          | 66  | M   | Glioblastoma | Negative | N/A            | No       | PTEN loss, CDK4 AMP, CL11 AMP, TP53, TERT, SPTA1 (FISH) | CDK4 AMP (log2, 2.4) |
| 47          | 29  | M   | Brainstem glioma | Positive     | N/A            | No       | No molecular profiling performed | PDGFR4 AMP (log2, 2.0), CDX2N2B del (log2, –3.0) |
| 48          | 78  | M   | Glioblastoma | Negative     | N/A            | No       | No molecular profiling performed | Negative |
| 49          | 78  | F   | High-grade glioma | Negative | N/A            | No       | No molecular profiling performed | Negative |
| 50          | 38  | M   | Oligodendroglioma | Negative | N/A            | No       | No molecular profiling performed | Negative |
| 51          | 35  | F   | Anaplastic ependymoma | Negative | N/A            | No       | CDX2N2A Y44* (8%); MSK-IMPACT | Negative |
| 52          | 71  | M   | Anaplastic oligodendroglioma | Negative | N/A            | No       | 1p/19q del (FISH) | Negative |
| 53          | 65  | M   | Anaplastic oligodendroglioma | Negative | N/A            | No       | IDH1 R132H (IHC), p19q del (FISH) | Negative |

**Primary brain tumors**

| Patient No. | Age | Sex | Tumor Type | CSF Cytology | CNS Involvement | Other Mets | Original Specimen (Molecular Path) | CSF (MSK-IMPACT) |
|-------------|-----|-----|------------|--------------|----------------|-----------|----------------------------------|------------------|
| 42          | 24  | M   | Anaplastic astrocytoma | Negative | N/A            | No       | IDH1 R132H (IHC), PIK3CA H1047R | IDH1 R132H (38%), PTPNR130* (25%) |
| 43          | 65  | M   | Glioblastoma | Negative | N/A            | No       | No molecular profiling performed | PIK3CA V344M (6%) |
| 44          | 63  | M   | Glioblastoma | Negative | N/A            | No       | PTEN loss (IHC) | PIK3CA V344M (6%) |
| 45          | 39  | F   | Anaplastic oligodendroglioma | Negative | N/A            | No       | IDH1 R132H (IHC), 1p/19q del (FISH) | IDH1 R132H (44%), 1p/19q del (log2, –0.8) |
| 46          | 66  | M   | Glioblastoma | Negative | N/A            | No       | PTEN loss, CDK4 AMP, CL11 AMP, TP53, TERT, SPTA1 (FISH) | CDK4 AMP (log2, 2.4) |
| 47          | 29  | M   | Brainstem glioma | Positive | N/A            | No       | No molecular profiling performed | PDGFR4 AMP (log2, 2.0), CDX2N2B del (log2, –3.0) |
| 48          | 78  | M   | Glioblastoma | Negative | N/A            | No       | No molecular profiling performed | Negative |
| 49          | 78  | F   | High-grade glioma | Negative | N/A            | No       | No molecular profiling performed | Negative |
| 50          | 38  | M   | Oligodendroglioma | Negative | N/A            | No       | No molecular profiling performed | Negative |
| 51          | 35  | F   | Anaplastic ependymoma | Negative | N/A            | No       | CDX2N2A Y44* (8%); MSK-IMPACT | Negative |
| 52          | 71  | M   | Anaplastic oligodendroglioma | Negative | N/A            | No       | 1p/19q del (FISH) | Negative |
| 53          | 65  | M   | Anaplastic oligodendroglioma | Negative | N/A            | No       | IDH1 R132H (IHC), p19q del (FISH) | Negative |

**Abbreviations:** AMP, amplification; BM, brain metastasis; del, deletion; delins, deletion/insertion; EGFR, epidermal growth factor receptor; FISH, fluorescent in situ hybridization; FM, Foundation Medicine; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; LM, leptomeningeal metastasis; Mets, metastases; MSK-IMPACT, Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets; N/A, not applicable; ND, not determined; PCR, polymerase chain reaction.