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A case series of extraneural metastatic glioblastoma at Memorial Sloan Kettering Cancer Center

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Abstract

Background. Extraneural metastasis of glioma is a rare event, often occurring in patients with advanced disease. Genomic alterations associated with extraneural glioma metastasis remain incompletely understood.

Methods. Ten patients at Memorial Sloan Kettering Cancer Center diagnosed with extraneural metastases of glioblastoma (9 patients) and gliosarcoma (1 patient) from 2003 to 2018 were included in our analysis. Patient characteristics, clinical course, and genomic alterations were evaluated.

Results. Patient age at diagnosis ranged from 14 to 73, with 7 men and 3 women in this group. The median overall survival from initial diagnosis and from diagnosis of extraneural metastasis was 19.6 months (range 11.2 to 57.5 months) and 5 months (range 1 to 16.1 months), respectively. The most common site of extraneural metastasis was bone, with other sites being lymph nodes, dura, liver, lung, and soft tissues. All patients received surgical resection and radiation, and 9 patients received temozolomide, with subsequent chemotherapy appropriate for individual cases. 1 patient had an Ommaya and then ventriculoperitoneal shunt placed, and 1 patient underwent craniectomy for cerebral edema associated with a brain abscess at the initial site of resection. Genomic analysis of primary tumors and metastatic sites revealed shared and private mutations with a preponderance of tumor suppressor gene alterations, illustrating clonal evolution in extraneural metastases.

Conclusions. Several risk factors emerged for extraneural metastasis of glioblastoma and gliosarcoma, including sarcomatous dedifferentiation, disruption of normal anatomic barriers during surgical resection, and tumor suppressor gene alterations. Next steps with this work include validation of these genomic markers of glioblastoma metastases in larger patient populations and the development of preclinical models. This work will lead to a better understanding of the molecular mechanisms of metastasis to develop targeted treatments for these patients.

Keywords

extraneural glioblastoma metastases | extracranial glioblastoma metastases | molecular evolution of glioblastoma | gliosarcoma

Glioblastoma remains one of the most lethal human cancers, with a median overall survival of just 14-16 months despite surgical resection, chemotherapy, and radiation.[1,2] As a predominantly intracranial disease, nearly all treatment in glioblastoma is focused on the primary site of disease within the brain. Small case series have also investigated therapy of spinal glioma. However, it is known that extraneural metastasis can also occur, with no current dedicated diagnostic or therapeutic guidelines for this disease entity.

It is estimated that extraneural metastasis occurs in about 0.4%-0.5% of glioblastoma patients, with the most frequent sites of involvement being lungs, pleura, lymph nodes, bone

marrow, bone, and liver.[3] It is generally thought that surgical intervention represents the strongest risk factor for metastasis, with hematogenous or lymphatic spread occurring at the time of surgery or peritoneal access occurring through placement of a ventriculoperitoneal shunt (VPS).[4,5] However, several cases of extraneural metastases have been identified even in the absence of any intracranial surgeries, supporting the theory that extraneural involvement may occur early in the disease process.[6–8]

In patients with extraneural glioblastoma metastasis, metastasis is generally diagnosed in the later stages of illness. Interestingly, a meta-analysis demonstrated no significant difference in overall survival between patients with and without extraneural metastases.[4] Though there may be several theories for this finding, it is likely that extraneural metastases are diagnosed when the patient's primary site of disease is also poorly controlled. Thus, concomitant intracranial and extraneural progression both culminate in the same overall survival.

The era of next-generation sequencing has allowed more accurate diagnosis, and in some cases, has led to the use of targeted therapies in glioblastoma patients. Though extraneural metastases are being increasingly recognized, mostly because of early diagnosis, there have not been any studies examining the genomic landscape of metastatic lesions in these patients. Many critical aspects of extraneural metastasis remain unanswered, including what mutations may facilitate hematogenous spread and growth in other tissue niches. As liquid biopsy becomes increasingly popular to determine tumor-associated mutations and to study tumoral heterogeneity, these studies may likewise shed light on genomic alterations that facilitate extraneural metastasis.

We present here the largest single-center series of 10 patients with extraneural metastases from glioblastoma and gliosarcoma. We also report the first mutational data from extraneural metastatic glioblastoma and gliosarcoma, which adds to our understanding of the molecular progression of this disease.

Methods

We conducted a retrospective, IRB-approved single-center search of patients at Memorial Sloan Kettering Cancer Center (MSKCC) diagnosed with extraneural metastatic glioblastoma between January 1, 2003 and August 1, 2018. In order to identify eligible patients, we performed a free text search of pathology reports using the search terms "glioblastoma OR GBM" AND "mets OR met OR metast." We retrieved 123 unique patients from this search. We further refined these records by removing those patients in whom the pathology reports described pathologies other than extraneural metastatic glioblastoma. From this review, we identified 10 unique patients with biopsy-confirmed extraneural metastatic glioblastoma and gliosarcoma.

We compiled data on age, gender, tumor type, site of primary and metastatic disease, date of diagnosis, time from diagnosis to detection of extraneural metastasis, time from detection of extraneural metastasis to death, overall survival, O-6-methylguanine-DNA methyltransferase (MGMT) methylation status, symptoms of metastasis, treatment history, and relevant intracranial surgeries. We obtained genomic data from 7 patients in this study using the MSK-IMPACT (Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets) platform, a custom FDA-authorized next-generation sequencing–based assay that captures all protein-coding exons of 468 cancer-associated genes.[9] We also obtained genomic data from an additional patient using the Sequenom platform. These data were organized and analyzed with cBioPortal.[10,11] Biopsies of lymph node metastases were localized by ultrasound guidance, and bone metastases were localized by MRI guidance.

For prior glioblastoma cases at MSKCC, we included all patients with a diagnosis of glioblastoma who were seen at 3 or more office visits between 2014 and 2016. For prior case reports of extraneural metastases of glioblastoma, we performed a free text search of PubMed using the search terms "glioblastoma OR GBM" AND "extraneural." Publications in other languages were included in some cases provided that an abstract written in English was available. We chose cases that included primary and secondary glioblastoma, as well as gliosarcoma, that invaded organs outside the brain or spinal cord. We excluded all non-glioblastoma and non-gliosarcoma histologies. We compiled data on overall survival, time from symptom onset to diagnosis, diagnosis to detection of extraneural metastasis, and detection of extraneural metastasis to death. We also recorded the age and sex of the patients, the location of the primary tumor and site of extraneural metastases, performance of surgery, radiation, chemotherapy, and/or cerebral spinal fluid (CSF) shunt procedures.

Results

In this retrospective case series of 10 patients, there were 7 men and 3 women, with an initial diagnosis of glioblastoma in 9 patients and mixed glioblastoma/gliosarcoma in 1 patient (Table 1). Two additional patients initially diagnosed with glioblastoma were found to have gliosarcoma at recurrence in the primary site of the disease. The diagnosis of gliosarcoma rested on demonstration of tumor components having a mesenchymal appearance, rich reticulin investment, and loss of glial fibrillary acidic protein (GFAP) expression. One other glioblastoma patient was found to have glioblastoma with a primitive neuroectodermal-like component in her subdural metastasis. This diagnosis rested on the finding within an otherwise conventional glioblastoma of undifferentiated-appearing small cell components exhibiting loss of GFAP expression, upregulated expression of synaptophysin, and highly elevated Ki-67 labeling indices (>70%), similar to prior reports.[12]

The median age at diagnosis was 37.5 (range 14-73 years) (Table 2). The median overall survival of this population was 19.6 months (range 11.2-57.5 months). The median time from initial diagnosis to extraneural metastasis was 15.6 months (range 5.2-41.4 months), and the median time from diagnosis of extraneural metastasis to death was

Table 1. Clinical characteristics of the 10 patients with metastatic glioblastoma or gliosarcoma

Patient number	Age	Gender	Primary tumor histological diagnosis	Metastatic tumor histological diagnosis	Site of glioblastoma	Radiographic feature	Surgery, # craniotomies	Radiation	Chemotherapy	CSF shunt	Metastatic sites	Symptoms of metastatic site	MGMT methylation	IDH-1/2 mutation	Time to Met (mo)	Met to death (mo)	OS (mo)	Surgical complications	Sequenced Site
1	73	M	GBM	Gliosarcoma	Frontal	Cystic, solid, +CE, +hem	+, 2	+	TMZ, PDL-1i, Bev, Carmustine	–	Dura	None	–	–	5.2	6.0	11.2		Brain (primary); dura (met)
2	39	M	GBM	Gliosarcoma	Frontal	Cystic, solid, +CE, +hem	+, 1	+	TMZ, Bev	–	Spine, lung, liver, iliac bone	Low back pain	+	–	11.1	1.0	12.1		Brain (primary)
3	41	M	GBM	GBM	Temporal	Cystic, solid, +CE, +hem	+, 2	+	None	–	Cervical lymph nodes	Neck pain	Unknown	–	10.4	2.0	12.4	Pseudomeningocele	Brain (primary); LN (met)
4	35	M	GBM/ gliosarcoma	GBM/ gliosarcoma	Temporal	Unavailable	+, 2	+	Gliadel, TMZ	–	Sacrum	Back pain	Unknown	Unknown	12.1	5.6	17.7		Brain (primary)
5	44	F	GBM	GBM	Parietal	Unavailable	+, 2	+	TMZ	–	T7-8 dura, spinal cord, leptomeninges	Progressive paraparesis and ascending thoracic sensory loss	Unknown	Unknown	21.5	3.1	24.6		Not sequenced
6	28	M	GBM	GBM	Frontoparietal	Cystic, solid, +CE	+, 3	+	Nivo, TMZ, Bev, Lomustine, Carbo, Etoposide	–	Vertebrae and femurs	Back pain	–	R132H	32.5	5.0	37.5		Brain (primary); brain (recurrence); brain (recurrence); sacrum (met)
7	23	F	GBM	Poorly differentiated high-grade glioma	Occipital	Cystic, solid, +CE	+, 1	+	TMZ	–	Sacrum, L5 vertebra, hip	Low back pain	–	Unknown	41.4	16.1	57.5		Vertebrae (met); vertebrae (met)
8	14	M	GBM	Poorly differentiated high-grade glioma	Frontotemporal	Cystic, solid, +CE, +hem	+, 3	+	TMZ, MEKi, Bev	+	Lung, skin, thoracic lymph nodes	Pain, shortness of breath	Unknown	Unknown	18.3	2.4	20.7		Brain (recurrence)
9	36	M	GBM	GBM	Frontal	Unavailable	+, 2	+	TMZ	–	Humerus	Shoulder pain	Unknown	Unknown	19.2	25+#	44+#		Not sequenced
10	52	F	GBM	GBM with primitive neuroectodermal tumor-like component	Temporal	Cystic, solid, +CE	+, 2	+	TMZ, Bev	–	Subdural region	Headache	–	Unknown	13.0	6.6	19.6		Brain (primary); dura (met)

IDH, isocitrate dehydrogenase; Met, metastasis; OS, overall survival; CE, contrast enhancement; hem, hemorrhage; TMZ, temozolomide, PDL-L1, programmed cell death ligand 1 inhibitor; Bev, bevacizumab; Nivo, nivolumab; Carbo, carboplatin; MEKi, mitogen-activated protein kinase inhibitor.

5 months (range 1-16.1 months). The timeline for each patient is shown in Figure 1. The diagnosis of extraneural metastasis occurred in the latter half of illness in 9 patients. One patient remains alive as of the time of this publication.

In our series, the most common site of extraneural metastasis was bone (7 patients), comprised of metastases to vertebrae (5 patients), skull (1 patient), femur (1 patient), humerus (1 patient), and hip (1 patient). One patient had metastasis to the subdural region (patient 10), demonstrating glioblastoma with a primitive neuroectodermal tumor-like component. One patient had involvement of level 2 cervical draining lymph nodes (patient 3), and another had involvement of thoracic lymph nodes (patient 8). One patient had radiographically evident but not histologically confirmed leptomeningeal metastases. Symptoms from extraneural metastasis correlated to the site of metastatic involvement in all patients. Symptoms were typically described as pain in the case of bone involvement. One patient had progressive paraparesis and ascending thoracic sensory loss from her thoracic vertebral metastases (patient 5), 1 patient had shortness of breath due to lung metastasis (patient 8), and 1 patient had headache from her subdural metastasis (patient 10).

All patients underwent either subtotal (4 patients) or gross total (6 patients) resection. Two patients had a total of 1 craniotomy, 6 patients had 2 total craniotomies, and 2 patients had 3 total craniotomies for resection at the primary site throughout their treatment course. All but 1 patient received initial radiation, but this patient received radiation later on in treatment. Four patients received bevacizumab for recurrent disease, but 3 of these patients received bevacizumab after their diagnoses of metastases. One patient received a programmed cell death ligand 1 (PDL-1) inhibitor as part of a clinical trial. One patient had a dural metastasis, 1 patient had a pseudomeningocele with methicillin-sensitive *Staphylococcus aureus* infection followed by craniectomy (patient 3), and 1 patient underwent intracranial Ommaya and later VPS placement (patient 8). Five patients had MGMT methylation testing. Of these, 1 was methylated, and 4 were unmethylated.

Table 2. Patient Characteristics

Median age at diagnosis (y)	39 (14–73)
Median OS (mo)	19.6 (11.2-57.5)
Median time to metastasis (mo)	18.3 (5.2-41.4)
Median metastasis to death (mo)	5 (1-16.1)

To compare the clinical features of extraneural metastatic vs nonmetastatic glioblastoma and gliosarcoma, we performed a review of 342 patients with nonmetastatic glioblastoma and gliosarcoma at MSKCC. This population exhibited a median overall survival of 16.9 months (2.5-68 months) (Table 3) vs 19.6 months in our metastatic population. The majority of these patients were male (59%), and the most common site of primary tumor was temporal lobe, followed by frontal, parietal, and occipital lobes. 20.5% of these patients were positive for MGMT promoter methylation, 64.3% were negative, and 15.2% had unknown MGMT status. 95.9% of these patients were IDH-1/2 (isocitrate dehydrogenase-1/2) wild type. This MGMT promoter methylation frequency is slightly lower than the 32%-60% observed in prior cohorts,[13] but the *IDH1* mutation frequency percentage is similar to large datasets.[14]

In prior series of patients with extraneural metastatic glioblastoma, the median age at diagnosis was 40, very similar to the young median age of 39 in our series. These data are summarized in Table 4.[6,7,15–120] The most common sites of extraneural metastases were bone, lymph nodes, lung, leptomeninges, soft tissue, and liver. The majority of these patients were male (75%), and the most common site of primary tumor was temporal lobe, followed by parietal lobe, occipital lobe, and frontal lobe. 93% of these patients underwent surgery prior to the diagnosis of their

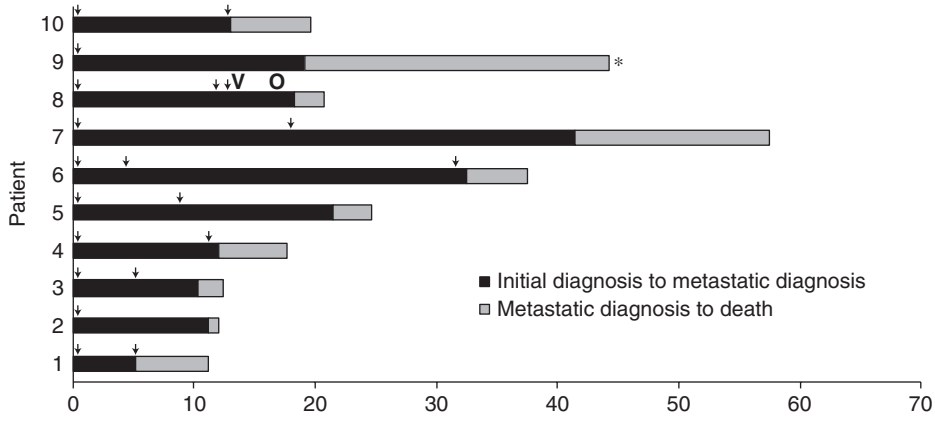

Figure 1. Timeline of illness from glioblastoma or gliosarcoma for each patient. The amount of time in months from the initial diagnosis to the diagnosis of the extraneural metastasis and from the diagnosis of the extraneural metastasis to death is shown for each patient. *Patient is alive as of the time of this publication; down arrows indicate the timing of craniotomies; V indicates the timing of ventriculoperitoneal shunt; O indicates the timing of Ommaya reservoir placement.

Table 3. Patient Characteristics of Nonmetastatic GBM Cases at MSKCC

Median age at diagnosis (y)	61 (21–90)
Gender	
Male	59%
Female	41%
Site of glioblastoma	
Temporal	35.7%
Frontal	33.9%
Parietal	25.4%
Occipital	4.7%
Corpus callosum	1.5%
Thalamus	2.3%
Brainstem	0.9%
Multifocal	0.9%
Basal ganglia	0.6%
Cerebellum	0.3%
Insula	0.6%
Optic nerve	0.3%
Number of surgeries	
1	69.3%
2	26.6%
3	3.8%
4	0.3%
Radiation	
Yes	99.1%
No	0.9%
Chemotherapy	
Yes	98.8%
No	1.2%
CSF shunt	
Yes	6.1%
No	93.9%
MGMT-methylated	
Yes	20.5%
No	64.3%
Unknown	15.2%
IDH-mutated	
Yes	4.1%
No	95.9%
Median OS	16.9 (2.5-68)

metastatic lesion, and 87% of these patients received radiation. The median time to symptom onset was 2 months (range 0-60 months), median time from diagnosis to metastasis was 8 months (range 0-84), and median time from diagnosis of metastasis to death was 2.5 months (range 0-30 months). The overall survival of this population was 11 months (range 1-92 months). This slightly reduced survival time likely reflects the inclusion of cases dating back to 1935, before the advent of standard chemoradiation.

Genomic data were obtained from 8 patients in our study using the MSK-IMPACT or Sequenom platforms. The MSK-IMPACT protocol uses next-generation sequencing to identify cancer-causing mutations in tumor-containing specimens. MSK-IMPACT data or Sequenom data from the primary site of disease were obtained from all 8 of these patients, mutational data from recurrent disease at the primary site were obtained from 2 patients, and genomic data from sites of metastatic disease were obtained from 5 patients (Figure 2 and Supplementary Figure 1). *TP53* (5 patients), *RB1* (5 patients), *PTEN* (4 patients), *TERT* (4 patients), *ATRX* (4 patients), and *NF1* (3 patients) were the most common alterations identified. *IDH1* was mutated in 1 patient, and *EGFR* was amplified in 2 patients and mutated in another patient.

In 2 patients with genomic data available from the brain and dural metastatic site, all mutations from the primary site were shared in the dural metastatic side. In the first case, *TERT*, *TP53*, *ATRX*, *PTEN*, *RB1*, *MST1R*, and *SETD2* were shared between both sites. In the second case, *TP53*, *PTEN*, *RB1*, and *TERT* were shared between both sites. However, in this case, the subdural site was found to have 8 additional alterations, including *EGFR*, *MET*, and *CDKN2A/2B* amplification. In another patient with genomic data available from 3 separate craniotomies and from his sacral site of metastatic disease, all 4 samples contained 6 out of the 7 mutations (*IDH1*, *TP53*, *ARID2*, *ATRX*, *RB1*, and *RPTOR*) from the initial primary tissue. However, there were several genomic alterations that were found in the second and third brain tumor specimens as well as the sacral specimen, which were not found in his initial tumor tissue. In the third patient with genomic data available from the primary site as well as from a cervical lymph node, the 2 samples share 5 of the original 8 alterations (*TERT* promoter mutation, *PTEN* deletion, and *EGFR*, *MDM2*, and *MYCN* amplification). *PDGFRA* amplification and an *NF1-TP53BP1* fusion were found only in the primary site. There were no mutations shared in all 5 patients in whom genomic data were available from metastatic sites. However, in 4 out of 5 patients, *TP53* and *RB1* alterations were present at the metastatic site.

Discussion

Extraneural metastasis of glioblastoma is a rare event during the progression of this type of brain tumor, often being diagnosed in patients with advanced disease and with a reported incidence of 0.4%-0.5% of all glioblastoma patients.[3] Prior case series have investigated patterns of metastasis of glioblastoma outside the central nervous system, with common sites including bone, lymph nodes, lung, and liver.[4,5] Hematogenous spread during surgical resection, biopsy, VPS, or Ommaya placement have been described as etiological factors in the development of extraneural metastases. However, others have postulated that extraneural metastasis is an early event in the course

Table 4. Patient Characteristics of Previously Reported Cases

Median age at diagnosis (y)	40 (4–80)
Gender	
Male	74%
Female	26%
Site of glioblastoma	
Temporal	28%
Parietal	23%
Occipital	15%
Frontal	23%
Brainstem	3%
Subcortical	3%
Cerebellum	2%
Hemisphere	2%
Basal ganglia	1%
Thalamic	0.5%
Surgery preceding metastatic diagnosis	
Yes	93%
No	7%
Radiation	
Yes	88%
No	12%
Chemotherapy	
Yes	20%
No	80%
CSF shunt	
Yes	11%
No	89%
Site of extracranial metastasis	
Bone	12.4%
Lymph node	11.5%
Lung	10.3%
Leptomeninges	5.7%
Soft tissue	3.8%
Liver	4.1%
Dural sinus	3.8%
Surgical site	2.3%
Skin	2.3%
Spinal cord	1.8%
Systemic vasculature	1.8%
Neck	1.2%
Parotid gland	0.9%
Diaphragm	0.6%
Peritoneum	0.6%
Spleen	0.3%
Kidney	0.6%
Pancreas	0.6%
Pericardium	0.6%
Abdominal wall	0.3%
Adrenal gland	0.3%
Arm	0.3%
Bowel	0.3%
Breast	0.3%
Chest wall	0.3%
Heart	0.3%
Mediastinum	0.3%
Mesentery	0.3%
Pleura	0.3%
Pleural fluid	0.3%
Orbit	0.3%
Nasal cavities	0.3%
Muscle	0.3%
Median time from symptom onset to diagnosis	2 (0-60)
Median time from diagnosis to metastasis	8 (0-84)
Median time from metastasis to death	2.5 (0-30)
Median OS	11 (1–92)

of glioblastoma and occurs independently of surgical intervention.[6–8] Though the site of extraneural metastasis has been shown to correlate with overall survival (with lung being the least favorable site),[1] there have not been any in-depth studies using next-generation sequencing to investigate molecular genetics of extraneural metastasis.

In our series, we present 10 patients at MSKCC diagnosed with metastatic extraneural glioblastoma or gliosarcoma from 2003 to 2018. These cases demonstrate several potential risk factors for extraneural metastasis of glioblastoma and gliosarcoma, namely sarcomatous dedifferentiation and disruption of normal anatomic barriers during surgical resection. We found that the median age of patients in our study was 39, comparable to the median age in prior case series. This young age could relate to the fact that the 3 youngest patients in our study all had *RB1* alterations, either in their primary sites or in their metastatic sites for those whose primary sites were not sequenced. One of these patients (Patient 6) also had an *IDH1* mutation. In a prior case series, *RB1* deletion was found in 71% of pediatric patients who exhibited malignant transformation of diffuse low-grade glioma.[121] Likewise, *RB1* alteration tends to be more prevalent in children with low-grade glioma whose tumors do not have alterations in *IDH1/2* or *TP53*, or those without *1p/19q* loss.[122] Therefore, it is possible that the young age of patients in our case series is related to *RB1*-mediated transformation of low-grade gliomas and to an *IDH1* mutation in one patient. These young patients also had correspondingly long overall survivals of 37.5 months (Patient 6), 57.5 months (Patient 7), and 20.7 months (Patient 8). We also found in our series that the diagnosis of metastatic glioblastoma is typically made in the later stages of patients' illnesses, often in the final months of life. The relatively long 19.6-month median overall survival of patients in this series is likely a result of a larger percentage of these cases receiving chemotherapy

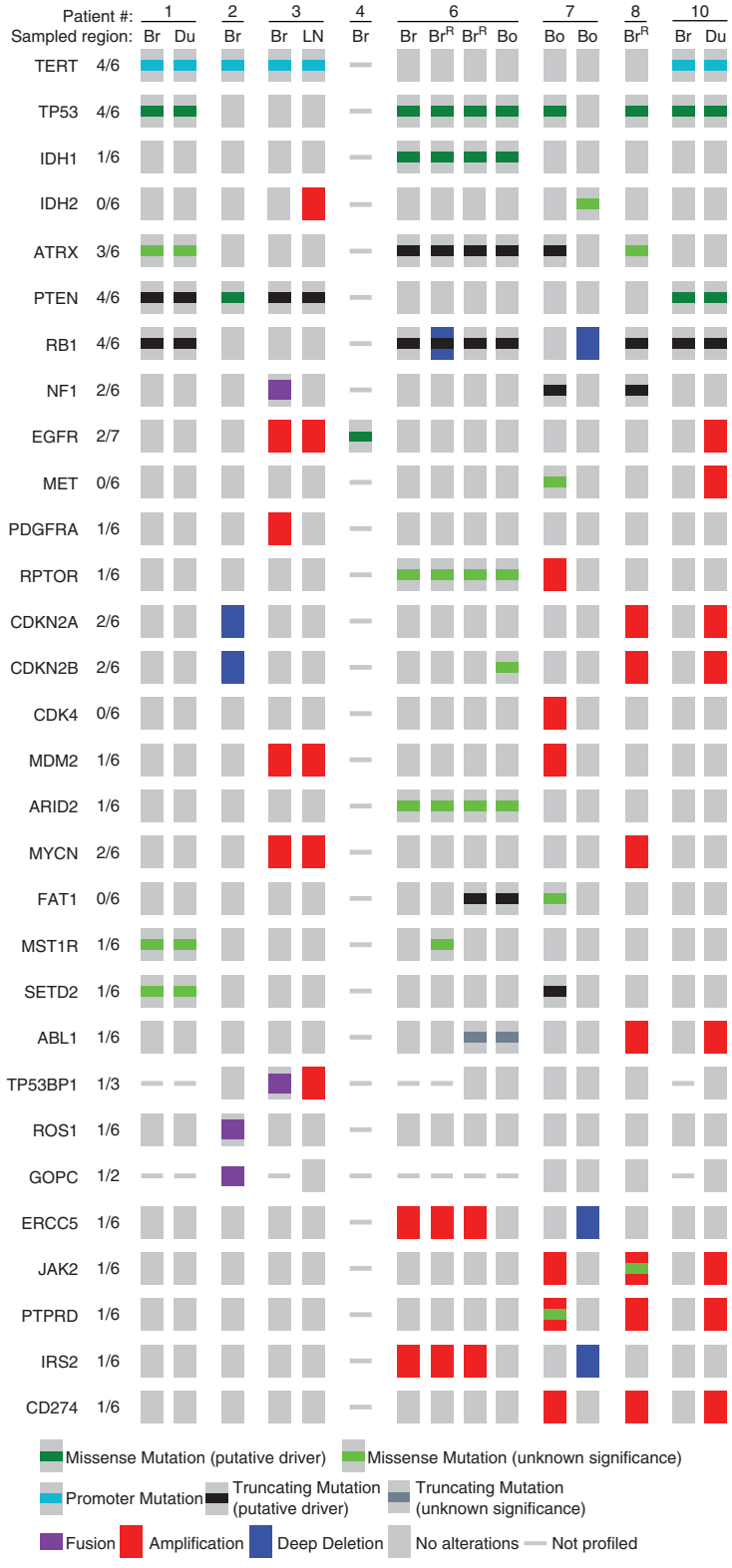

Figure 2. Genomic profiles from MSK-IMPACT or Sequenom sequencing for 8 patients with extraneural metastatic glioblastoma or gliosarcoma. Tumor tissue from primary and metastatic sites of disease was obtained from 8 patients with extraneural metastatic glioblastoma or gliosarcoma through standard-of-care surgical resection or biopsy. Next-generation sequencing of cancer-causing genes using the MSK-IMPACT protocol or Sequenom platform was performed on these specimens, and the most common alterations from this analysis are presented in Oncoprint format. The frequency of genomic alterations is displayed as the number of alterations present in primary tumor specimens out of all sequenced primary tumor specimens. Br, original brain tumor specimen; Du, dura; LN, lymph node; Bo, bone; Br^R, brain tumor recurrence.

and radiation at diagnosis as compared to prior cases that were diagnosed and treated prior to the availability of these regimens.

Most of the patients in our study had symptoms referable to their extraneural metastases. In addition, in patients with VPS placement and/or with systemic symptoms, such as bone pain, respiratory changes, transaminitis, or cytopenias, which are not easily explained, clinicians should maintain a high index of suspicion for extraneural metastases. The most common site of metastatic glioblastoma and gliosarcoma in this study was bone, with bony involvement including skull, vertebrae, and long bones. Other metastatic sites included lymph nodes and adjacent dura.

It is interesting to note that bone metastases, in particular, are common sites of extraneural involvement in glioblastoma. This predilection for bone may come from both tumor-derived and extracellular niche-derived cues. For example, glioblastoma cells express many of the same hematopoietic stem cell proteins that are critical for growth within bone marrow, including stromal cell-derived factor 1 alpha (SDF-1α), C-X-C chemokine receptor type 4 (CXCR4), osteopontin (OPN), and cathepsin K (CATK).[123] Glioblastoma cells also recruit bone marrow-derived progenitor cells to support tumor-associated vasculature through the secretion of pro-angiogenic signals.[124] In addition, CXCR4, OPN, CATK, and CD44 are induced by hypoxia-inducible factor-1α and vascular endothelial growth factor (VEGF), two proteins known to increase glioma aggressiveness and invasion.[125] In our series, 4 out of 10 patients received the VEGF-directed agent, bevacizumab, but 3 out of 4 of these patients received bevacizumab after their diagnoses of metastases. Though our patient population is small in this study, it will be important to determine whether bevacizumab treatment influences the development or progression of extraneural metastases.

With the limited genomic data presented in this series, we found that many genomic alterations are shared in both primary and metastatic specimens. Similar to the *TP53*-positive case presented in Zhen et al.,[5] 5 of our 8 sequenced patients had *TP53* mutations. In 3 of these 5 cases, the *TP53* mutation was found in both the primary and metastatic sites. One patient had the mutation in his primary site, but metastatic sites were not sequenced. One patient developed a *TP53* mutation in her metastatic site. However, the overall survival in these 5 patients was quite disparate, likely due in large part to the presence of an *IDH1* mutation in 1 patient and an *IDH2* mutation in another patient. Other common alterations in glioblastoma, including in *ATRX*, *PTEN*, *RB1*, *TERT*, *IDH1*, and *NF1*, were found in both primary and metastatic sites. Tumor suppressor gene alterations were particularly common in this series, with *TP53* and *RB1* both mutated in 4 out of 7 (57%) sequenced primary tumors, as opposed to 23% and 7% in The Cancer Genome Atlas (TCGA), respectively.[14] Prior studies suggest that alterations in tumor suppressor genes may be associated with long-term survival in glioblastoma, particularly in younger patients, which may also help to explain the relatively long survival we observed in our study.[126,127] We also found private mutations in metastatic sites, suggesting possible evolutionary changes that allowed tumor cells to metastasize or to grow once arriving at metastatic sites. The metastatic potential of glioblastoma may have less to do with the duration of the disease than the primary biology. For example, sarcomatous dedifferentiation in 3 patients along with relatively uncommon (3 out of 8 sequenced patients) EGFR alterations (compared to 56% alteration rate in large glioblastoma datasets[14]) in this group suggests that glioblastoma with sarcomatous or mesenchymal features may encourage extraneural metastasis. A recent study from our institution showed that 7.8% of patients with leptomeningeal metastases from primary glial brain tumors had gliosarcoma on histological examination.[128]

The study of patients with extraneural metastases may also shed light on glioblastoma heterogeneity, which is often thought to account for resistance to targeted therapy. A tumor's mutational landscape may change over time, making one targeted agent obsolete when a new driver mutation develops. Approaches to sample glioma heterogeneity noninvasively to select the most appropriate targeted agent are being heavily investigated. Such noninvasive approaches use CSF ctDNA and/or plasma-derived circulating tumor DNA (ctDNA) or extracellular vesicles to collect genomic data on a patient's tumor. In patients with intracranial glioblastoma, CSF-derived ctDNA is more abundant and better represents the treatment course and tumor heterogeneity than plasma-derived ctDNA.[129] Other groups have used plasma ctDNA, with generally inferior detection of tumor-associated alterations and tumor evolution.[130] In patients with extraneural glioma metastases, however, plasma-derived ctDNA may be more easily detected and could recapitulate the mutational profile of these metastases. In addition, the prospective collection of plasma-derived ctDNA in glioma patients may help better define the time-course of extraneural metastasis. In this small subset of patients, prospective collection of ctDNA from the time of extraneural metastasis could help define the evolution of the mutational landscape.

This study has several limitations. This study is limited by its small size and retrospective nature. The genomic findings are limited by the incomplete sequencing of primary and metastatic samples in some patients as well as the lack of multiregional sampling, which may have led to the identification of additional alterations in some patients. Nonetheless, this is the largest single-institution case series of patients with metastatic glioblastoma and is the first study to report next-generation sequencing results in a case series of primary and extraneural glioblastoma. This is also the first study to explore the relationship between genomic alterations in patients with extraneural metastases of glioblastoma and cell signaling pathways that may be associated with extraneural metastasis.

Future directions with this work include analysis of the molecular features of extraneural glioblastoma metastases with respect to multiregional sequencing of the tumor, which we anticipate will lead to a better understanding of the molecular mechanisms of metastasis and may improve treatments for these patients. In addition, further study of this patient population is needed in order to develop standardized treatment regimens in the setting of extraneural metastatic glioblastoma.

Supplementary material

Supplementary material is available online at *Neuro-Oncology* (http://neuro-oncology.oxfordjournals.org/).

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