Identifying brain tumor precursor cells and their “-omic” signatures is a major challenge in the field of neuro-oncology that holds tremendous promise for increasing survival by customizing treatment paradigms. Evidence suggests that tumor location plays an important role in prognosis¹ and is likely linked to the genetic profile of tumor cells of origin.²,³ In support of this hypothesis, region-specific brain tumor cells of origin have been identified in oligodendrogliomas,⁴ medulloblastomas,⁵ and ependymomas,⁶ and compelling evidence has suggested that this is the case for IDH1 mutated gliomas.⁷ Further, studies have identified clear links between radiologic features, including the volume and extent of edema and/or invasion⁸ as well as contrast enhancement,⁹ and -omic signatures. On the basis of these findings, we have constructed a set of radiographic atlases specifying the probability of tumor location and volumetric information for important demographic, -omic, and interventional phenotypes, with the goal of providing new insight into the possible niche locations of cells of origin in glioblastoma. The radiographic atlases of glioblastoma phenotypes presented in the current study quantify the probability of tumor occurrence in 507 glioblastomas stratified by
age, extent of resection, MGMT promoter methylation, IDH1 mutation, gene expression subclassification, PTEN loss, PTEN deficiency, EGFR amplification, EGFR variant 3 expression, time to progression from the start of radiochemotherapy, and overall survival from initial diagnosis, along with some interactions between these phenotypes.

**MATERIALS AND METHODS**

**Patients**

Five hundred seven patients with de novo histologically confirmed glioblastoma between April 2000 and December 2011 were enrolled in the current retrospective, institutional review board–approved, Health Insurance Portability and Accountability Act–compliant study. All patients had presurgical T2/FLAIR and post-contrast T1-weighted images available. Subsets of these patients were used in other studies.⁷,¹⁰,¹¹ The extent of resection was noted for the initial surgery. Overall survival was defined as the time from initial pathologic diagnosis until death.

**MR Imaging**

Data were collected on either a 1.5T or 3T MR imaging scanner. Standard anatomic MR imaging sequences consisted of T2-weighted FSE or FLAIR images, and gadolinium-diethylene-triamine pentaacetic acid– (Magnevist; Berlex, Wayne, New Jersey; 0.1 mmol/kg) or gadobenate dimeglumine– (MultiHance; Bracco, Milan, Italy; 0.1 mmol/kg) enhanced axial T1-weighted images (ie, T1+C). All images were 3- to 5-mm thick with a 0- to 1-mm intersection gap.

**Image Registration**

All images for each patient were registered to a 1.0-mm isotropic brain atlas (Montreal Neurological Institute 152) by using a mutual information algorithm and a 12 df transformation by using the Functional MR Imaging of the Brain Software Library (http://www.fmrib.ox.ac.uk/fsl/) followed by visual inspection and a consensus by 2 independent raters (W.B.P. and B.M.E.) to ensure adequate alignment.

**ROIs**

After image registration, T2/FLAIR and T1+C images were segmented by using a semiautomated procedure previously documented.⁶,¹² Briefly, the general regions of tumor on T2/FLAIR and T1+C images were first defined manually. Then, T2/FLAIR and T1+C images were thresholded by using an empiric threshold combined with a region-growing algorithm confined to the manually defined ROIs in the first step. Last, tumor regions were manually edited to exclude any obvious errors in segmentation. Regions of central necrosis were included in the study to outline the entire extent of the tumor. Tumor volume was calculated with respect to preregistration image resolution.

**ADIFFI**

ADIFFI analysis consisted of first constructing a $2 \times 2$ contingency table comparing 2 differential phenotypes (eg, phenotypes A and B) and tumor versus nontumor for each image voxel. Next, a 2-tailed Fisher exact test was performed on a voxelwise basis. According to the Fisher exact test, the probability of obtaining an observed pattern in the $2 \times 2$ contingency table is given by

$$p = \frac{(a + b)(c + d)(a + c)(b + d)}{a!b!c!d!n!}$$

where $a$ is the frequency of tumor occurrence in a particular voxel for phenotype A; $b$, the frequency of tumor occurrence in a particular voxel for phenotype B; $c$, the frequency of no tumor occurring in a particular voxel for patients with phenotype A; $d$, the frequency of no tumor occurring in a particular voxel for patients with phenotype B; $n$, the total number of patients included; and the exclamation point represents the factorial operation. To calculate the significance of the observed pattern in the contingency table corresponding to the total probability of observing a pattern in the contingency table as extreme or more extreme, we recalculated the $P$ value from each voxel for all cases in which the marginal totals were the same as the observed tables and only for cases in which the arrangement was as extreme as the observed pattern. We performed this iteratively so that the values were incremented to calculate a more extreme pattern, adding the previous $P$ value in each image voxel each time until the most extreme pattern was achieved (which may vary from voxel to voxel). The final $P$ value represents the probability of observing the given pattern in the contingency table by chance. $P < .05$ was considered significant. Additional details are presented in a previous publication.¹⁰

**Correction for Cluster Size by Using Random Permutations**

A cluster-based permutation correction was performed as outlined by Bullmore et al.¹³ A total of 500 permutations were performed, the resulting ADIFFI-defined clusters were retained, and the 95% confidence intervals for significant cluster size occurring by chance were documented for each phenotype. The cluster-size thresholds used for each phenotype had a <5% probability of occurring by chance.

**Molecular and Genetic Analysis**

MGMT methylation analysis was performed by methylation-specific polymerase chain reaction or real-time methylation-specific polymerase chain reaction (LabCorp, Santa Monica, California), according to previously published protocols.¹⁰,¹⁴,¹⁵ IDH1 mutation status was determined by subjecting DNA to amplification by polymerase chain reaction by using primers specific for the IDH1 gene, followed by DNA sequencing analysis of a region surrounding codon 132 as described in Lai et al.² Gene-expression subclassification was performed according to standard protocols and categories.¹⁶ EGFR amplification was performed by the UCLA Cytogenetics Laboratory (Los Angeles, California) by using fluorescence in situ hybridization (Abbott/Vysis, Des Plaines, Illinois; LSI EGFR/CEP7 probe cocktail specific for the 7p12 EGFR locus and centromere of chromosome 7). The PTEN copy number was analyzed by using fluorescence in situ hybridization (Abbott/Vysis; LSI PTEN/CEP10 probe cocktail specific for the 10q23 PTEN locus and centromere of chromosome 10). PTEN deficiency was determined by immunohistochemistry (Sarkaria Biomarker Innovations Laboratory or Brain Tumor Translational Resource at UCLA, Los Angeles, California) by using standard protocols.¹⁷ Tissue sections were stained with monoclonal antibodies for
PTEN (clone 6H2.1; Cascade Bioscience, Winchester, Massachusetts). EGFR variant 3 expression was determined by immunohistochemistry or real-time polymerase chain reaction.

**Time to Progression for Radiochemotherapy Interventional Phenotype**

Patients treated with upfront radiochemotherapy (radiation therapy and concomitant TMZ temozolomide followed by adjuvant TMZ temozolomide as described by Stupp et al) showing progression before the last date of censorship (n = 374) were stratified on the basis of the time to treatment failure. Centralized review of progression-free survival was performed for all patients with available scans as previously described. The date of death was used as the data of progression if the patient had a stable scan finding 2 months before death.

**RESULTS**

A substantial number of glioblastomas grew into the periventricular white matter regions adjacent to the subventricular zone (Fig 1A, -B), and 91.9% of glioblastomas had T2/FLAIR signal abnormality contiguous with the ventricular system. Tumor frequency results stratified by age suggested frontal lobe predominance in younger patients (a in Fig 2A) compared with older patients (b in Fig 2B). ADIFFI statistical analysis performed on glioblastomas stratified by the extent of resection (gross total resection versus subtotal resection) identified a single spatially distinct cluster encompassing both Broca and Wernicke areas that was statistically more likely to be subtotal resection (Fig 3).

**MGMT Promoter Methylation and IDH1 Mutation**

Four hundred thirty-three patients had MGMT promoter methylation data available, for which 38% had MGMT promoter methylation. A subset of patients was also included from a previous study investigating MGMT promoter methylation. ADIFFI analysis of glioblastomas based on MGMT promoter methylation status identified a cluster in the right temporal lobe as most frequently associated with MGMT promoter unmethylated tumors and a similar cluster in the left temporal lobe most frequently associated with MGMT promoter methylated tumors (a in Fig 4A), similar to previous data. MGMT promoter methylated tumors had a lower volume of contrast enhancement (Fig 4C; t test, P = .038) and T2/FLAIR hyperintensity (Fig 4C; t test, P = .004) compared with unmethylated tumors. Four hundred patients with glioblastoma had IDH1 mutation status information available, for which 8.5% of glioblastomas were IDH1-mutated. A spatially distinct region in the frontal lobe occurred at a significantly higher frequency in IDH1-mutant tumors (Fig 4B), similar to previously presented data. IDH1 wild-type glioblastomas had larger volumes of contrast enhancement (Fig 5B; t test, P = .005) but no difference in T2/FLAIR volume (Fig 5B; t test, P = .645). ADIFFI statistical analysis of the interaction between MGMT and IDH1 phenotypes identified 2 independent clusters when IDH1 mutation status was compared within MGMT promoter methyl-
unmethylated tumors (the frontal lobe, corresponding to regions with a significantly higher frequency of MGMT promoter unmethylated tumors did not show any significant difference between topographic MGMT methylation and higher frequency of MGMT promoter methylated tumors (methylated status illustrating cluster a in the left temporal lobe corresponding to regions with a significantly higher likelihood of being MGMT deficient running along the posterior aspect of the left lateral ventricle (Fig 6B). No differences in lesion volumes were found between MGMT-deficient and intact tumors (t test, P > .05).

**EGFR Amplification and EGFR variant 3 Expression**

EGFR amplification information was available on 136 patients, of which 42% showed amplification. EGFR variant 3 expression was observed in 25% of the 48 patients with data available. ADIFFI analysis isolated 2 clusters both containing a significantly higher proportion of tumors exhibiting EGFR amplification (Fig 7A). ADIFFI analysis of EGFR variant 3 expression also identified 1 of the 2 clusters (a in Fig 7B). EGFR-amplified tumors had a significantly higher T1+C (t test, P = .049) and T2/FLAIR hyperintense volume (t test, P = .032) compared with tumors lacking EGFR amplification. No difference in tumor volumes was detected between EGFR variant 3-expressing tumors and tumors lacking EGFR variant 3 expression (t test, P > .05).

**Radiochemotherapy Interventional Phenotypes**

Three hundred seventy-four patients with glioblastomas were initially treated with radiochemotherapy and showed progression at the time of censor. ADIFFI analysis of tumor location between nonresponders (<6-month time to progression) and long-term responders (>12-month time to progression) identified 2 clusters (a and b in Fig 8C), both associated with a favorable response. No relationships between tumor volume and time to progression were identified (T1+C and T2/FLAIR, 1-way ANOVA; <6-month time to progression, 6- to 12-month time to progression, and >12-month time to progression; P > .05). When examining the interaction between MGMT promoter methylation and time to progression, ADIFFI statistical analysis identified 2 distinct clusters (a and b in Fig 8D) in a similar location in all glioblastomas; however, the lateral portion of cluster a and the anterior lateral portion of cluster b were associated with long-term responders, whereas medial regions in cluster a and posterior regions in cluster b were regions associated with nonresponders.
Overall Survival
ADIFFI analysis identified 2 distinct clusters on separate hemispheres associated with either a short overall survival (<12-month overall survival; right hemisphere, a in Fig 9C) or a long overall survival (>36-month overall survival; left hemisphere, b in Fig 9C). Both T1+C and T2/FLAIR tumor volumes were not significantly different among different overall survivals (T1+C and T2/FLAIR, 1-way ANOVA; <12-month overall survival, 12–36-month overall survival, >36-month overall survival; \( P > .05 \)).

DISCUSSION
The current study demonstrates the utility of a comprehensive atlas of stereotactic tumor locations composed of >500 de novo glioblastomas and 10 different demographic, -omic, and interventional phenotypes. Restricted patterns in the topographic distribution of glioblastomas appear to arise from specific phenotypes, which appear consistent with the hypothesis of distinct glioma cells of origin. Our results appear to support the hypothesis that glioblastomas may arise from neural stem cells near the subventricular zone\(^2\) and may migrate along periventricular white matter tracts.

Atlas Stratifying the Extent of Resection Highlights Broca and Wernicke Areas
The extent of surgical resection is a significant prognostic factor in glioblastoma\(^2\), which also reflects surgical practices of the attending neurosurgeons. At our institution, fMRI is performed to spare eloquent function and limit morbidity; therefore, we expected a high frequency of tumors in language functional areas to occur more often in subtotal resection. Consistent with this hypothesis, results from the current study clearly outlined a cluster of tissue connecting Broca and Wernicke areas as the primary region occurring most frequently in subtotal resection compared with gross total resection.

Atlas of MGMT Promoter Methylation Detects Temporal Lobe Asymmetry, Which May Be Dependent on Mutation of IDH1
A recent report containing a subset of patients from the current study demonstrated, in patients with glioblastoma, significant lateralization of MGMT promoter methylated tumors to the left temporal lobe and lateralization of MGMT unmethylated tumors to the right hemisphere.\(^10\) These findings were also consistent with those in the current study with more patients. Hemispheric asymmetry in brain structures is well documented,\(^21,22\) particularly in the temporal lobe.\(^23\) Hemispheric differences in gene expression have also been identified,\(^24\) including the suppressor of the fused homolog gene in the sonic hedgehog pathway that regulates brain tumor proliferation.\(^25\) Most interesting, localization of MGMT promoter methylated tumors was also present when examining the interaction between IDH1 mutation status and MGMT promoter methylation, suggesting that a significant number of IDH1 mutant tumors are localized to the frontal lobe only in MGMT promoter methylated tumors. The topographic link between IDH1 mutation and MGMT promoter methylation status supports findings that these 2 phenotypes are tightly correlated.\(^26\)

Radiographic Atlases Identify a Subset of Tumors with Frontal Lobe Predominance
Results from the current study suggest that glioblastomas occurring in young
radiographic atlases identifying the location and frequency of glioblastoma tumor occurrence for specific demographic, -omic, and interventional phenotypes. These findings can provide new insight into patient response to therapy, survival, and molecular phenotypes, which are known to have a survival advantage, illustrated.

Radiographic Atlases Identify Regions Associated with Treatment Response and Survival

Results from the current study suggest that the periventricular white matter regions in the right hemisphere were associated with a short overall survival from the time of diagnosis. Similarly, this region was also shown to be contiguous with regions possessing the mesenchymal gene expression profile, lack of MGMT promoter methylation, and IDH1 wild-type classification, all of which are known to have a poor prognosis. In the radiochemotherapeutic interventional phenotype and when stratified by overall survival, tumors in the left temporal lobe had the best prognosis. When examining the response to radiotherapy in only the MGMT promoter methylated tumors, a similar region in the left temporal lobe was associated with a favorable response.

Bihemispheric clusters identified for EGFR amplification may explain contradicting results as to the prognostic relevance of EGFR amplification. When one considers both EGFR amplification and EGFR variant 3 expression, a common cluster occurs in the left temporal lobe anterior to the region identified by MGMT promoter methylation. As previously mentioned, this particular region was associated with a statistically longer progression-free survival and overall survival, supporting the findings from other investigators as to the negative prognostic value of EGFR amplification. Nevertheless, the relationship between tumor response to therapy, survival, and molecular phenotypes likely involves complex interactions that still need to be further delineated. We hypothesize that radiographic atlases illustrating the spatial correlations among these different phenotypes, as presented in the current study, may be important for deconvolving these interactions.

Limitations

There are a number of critical limitations to the current study. Because of the retrospective nature of the current study, we were unable to standardize the imaging data, which included heterogeneity with respect to field strength, type of gadolinium chelate used, imaging-section thickness, and the type of T2-weighted sequence (T2 FSE versus FLAIR). Second, image registration of anatomically distorted brains into standard stereotactic atlas space can be challenging. Despite our best effort to align images manually (when necessary) and verification of alignment by 2 independent observers, this lack of accuracy is a potential limitation.

Conclusions

Radiographic atlases identifying the location and frequency of glioblastoma tumor occurrence for specific demographic, -omic, and interventional phenotypes can provide new insight into po-
tential overlap between prognostic variables and may help identify niche locations for glioma cells of origin. Results from the current study suggest that tumor laterality (left versus right hemisphere) and frontal lobe involvement may play a role in the particular molecular and genetic profile of tumors and their response to cytotoxic treatment and overall survival. Future studies aimed at determining the possible biologic mechanisms for these findings are warranted.

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FIG 9. Atlas of overall survival. Frequency of T1+C tumor occurrence for short-term (<12 months, n = 106) (A) and long-term survivors (>36 months, n = 86) (B). C, ADITIFF statistical analysis isolates 2 clusters in the temporal lobes, a and b, associated with a high frequency of tumor occurrence in short-term and long-term survivors, respectively.

OS < 12 mo. (n = 106) OS >36 mo. (n = 86)
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