DNMT3A co-mutation in an *IDH1*-mutant glioblastoma

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Abstract Glioblastomas are highly aggressive, infiltrative, and genetically heterogeneous primary brain tumors that arise de novo or secondarily progress over time from low-grade tumors. Along with well-established signature mutational profiles, emerging research suggests that the epigenetic tumor landscape plays an important role in gliomagenesis via transcriptional regulation, DNA methylation, and histone modifications. The pursuit of targeted therapeutic approaches, based not only on expression profiles but also on somatic mutations, is fundamental to the effort of improving survival in patients with glioblastoma. Here, we describe a missense *DNMT3A* p.P904S mutation in an *IDH1*-mutant glioblastoma. Although never previously reported in gliomas, this mutation is predicted to be pathogenic and has been reported in several other malignancies. Our report suggests that elucidating epigenetic control is important to understanding glioblastoma biology and may likely unveil targets potentially important to glioblastoma treatment in an effort to improve survival.

[Supplemental material is available for this article.]

INTRODUCTION

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Ontology term: neoplasm of the nervous system

Glioblastoma multiforme (GBM) is a WHO Grade IV glioma, comprising more than one-half of primary brain tumors (Louis et al. 2016); these tumors are known for their highly aggressive and infiltrative nature as well as their genetic heterogeneity (Verhaak et al. 2010; Brennan et al. 2013). GBMs arise de novo or progress from low-grade gliomas (i.e., secondary GBMs), with corresponding molecular signatures, and can be further classified based on their molecular expression or genomic alteration profiles (Verhaak et al. 2010; Brennan et al. 2013; Sun et al. 2014). For instance, *IDH1* mutations and *1p19q* loss are associated with oligodendroglial histology, whereas *ATRX* mutations correlate with astrocytic tumors (Cancer Genome Atlas Research Network et al. 2015; Louis et al. 2016). Primary GBMs are associated with loss of *PTEN* and *CDKN2A* and with *EGFR* amplifications, whereas secondary GBMs frequently carry *IDH1* R132H mutations (Wong et al. 1992; Yan et al. 2009; Brennan et al. 2013; Ohgaki and Kleihues 2013; Bai et al. 2016).

Based on such expression profiles, along with clinical characteristics, patients with GBMs fall into four different subtypes: proneural, atypical, proliferative, and mesenchymal, with the proneural subgroup seemingly associated with better survival (Verhaak et al. 2010; Brennan et al. 2013; Sun et al. 2014). Despite the current standard of care treatment, including aggressive surgical resection and adjuvant radiation therapy (RT) combined with the alkylating agent temozolomide (TMZ), the prognosis for patients diagnosed with GBM remains poor, with an overall median survival of ~14 mo after initial diagnosis, and <10% of patients survive >5 yr (Lacroix et al. 2001; Stupp et al. 2005, 2015; Sanai et al. 2011; Chinot et al. 2014).

The pursuit of more targeted therapeutic approaches has become fundamental in an effort to prolong survival in patients with GBM. Whole-exome sequencing provides invaluable promise to guiding multidisciplinary treatments. Herein, we describe a patient with an *IDH1-*, *ATRX-,* and *TP53*-mutant GBM, who also harbored a somatic mutation in the *DNA methyltransferase 3 alpha* (*DNMT3A*) gene, all of which were preserved during progression and recurrence. *DNMT3A* gene alterations relate to aberrant DNA methylation and associated tumorigenesis in other malignancies. The somatic mutation found, p.P904S, has previously been reported in other cancers, but never in GBM, and has been predicted to be pathogenic (Forbes et al. 2017). The patient, who has been treated with multiple aggressive surgical resections and standard of care and novel therapies, remains alive nearly 3 yr after his initial diagnosis of GBM.

RESULTS

Clinical Presentation

The patient is an otherwise healthy 39-yr-old male who initially presented in 2016 with progressively worsening headaches and clumsiness. Magnetic resonance imaging (MRI) of the brain showed a large heterogeneously enhancing right fronto-parietal mass with associated vasogenic edema and infiltrative tumor (Fig. 1A,D). He underwent subtotal resection (STR) at an outside hospital followed by standard 60-Gy fractionated conformal RT and concomitant TMZ (Stupp et al. 2005). Pathology was consistent with an *IDH1* R132H-mutant, MGMT-methylated GBM with a Ki67 of 10%–15%. The patient was referred to our institution
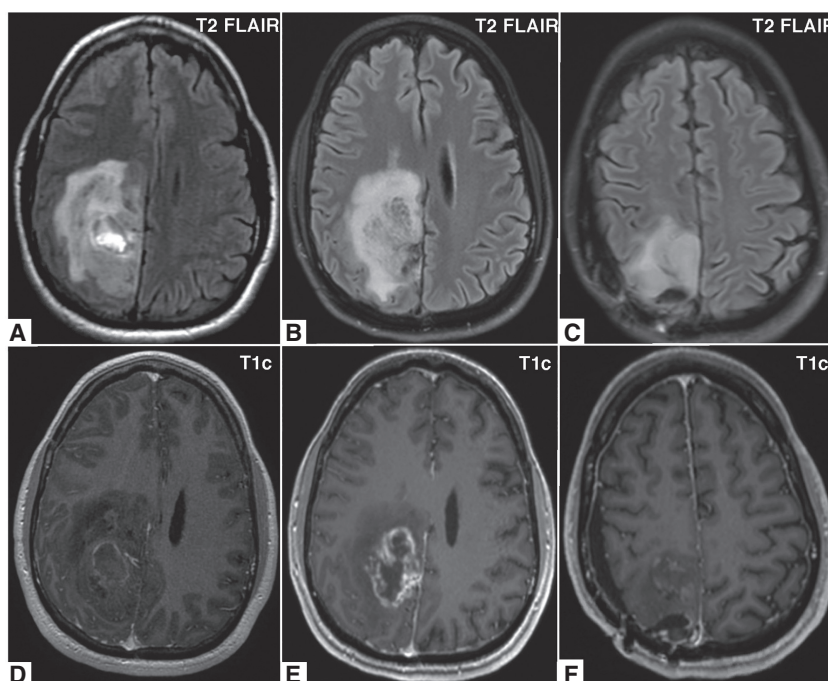
Figure 1. Magnetic resonance imaging (MRI) of a patient with *DNMT3a* co-mutation in an *IDH1*-mutant recurrent glioblastoma. (*A,D*) Preoperative imaging for the initial lesion operated on at the outside hospital 3/2016; (*B,E*) the first recurrence of the GBM 8 mo later; (*C,F*) the second recurrence of the GBM 23 mo thereafter. Axial T2-weighted FLAIR (*A–C*) and axial T1-weighted (*D–F*) with gadolinium contrast images are shown.

3 months after his initial surgery, when he was started on the PARP inhibitor, veliparib (ABT-888), along with adjuvant TMZ (Alliance trial A071102; NCT02152982).
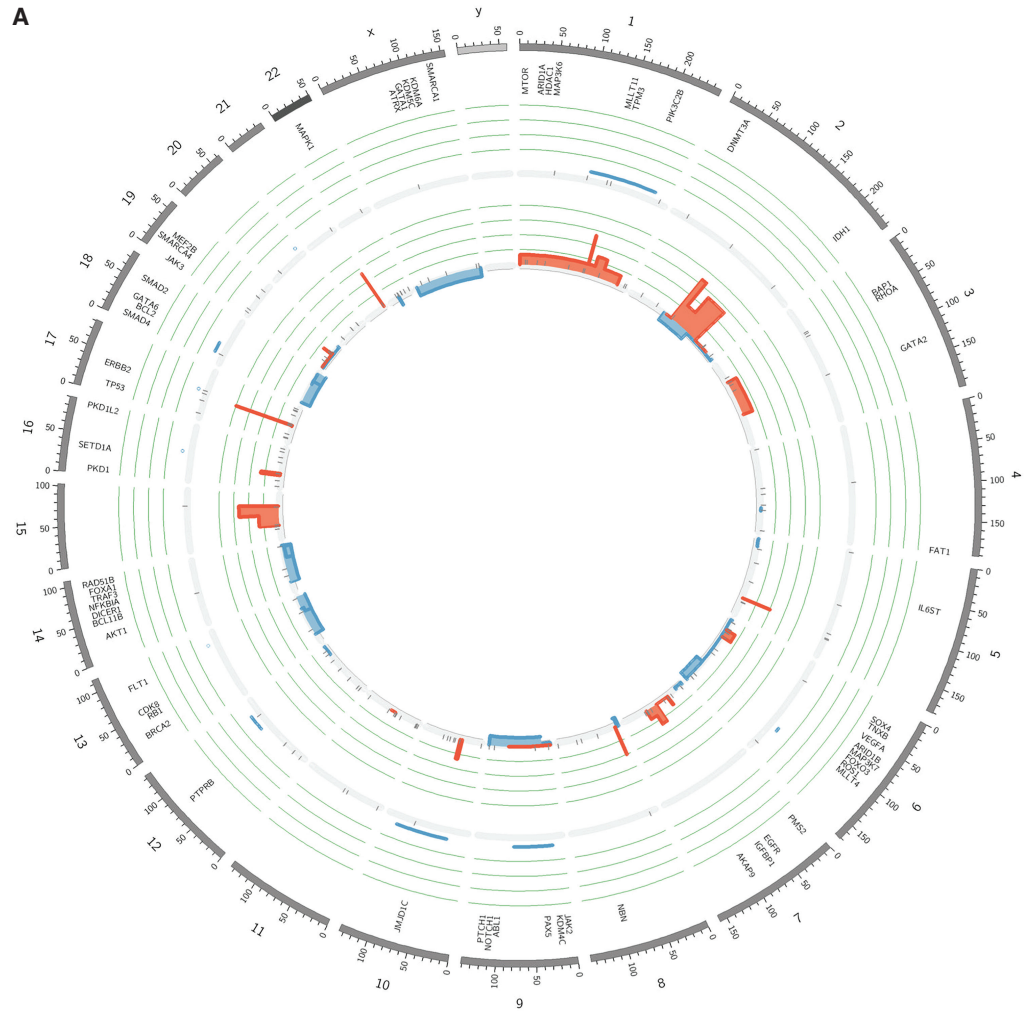
The brain MRI after four cycles of veliparib/TMZ and 8 mo after his original presentation was concerning for relapse (Fig. 1B,E). Concurrently, the patient began exhibiting weakness and left agraphesthesia. He underwent repeat STR, with a small amount of residual tumor left along the motor strip. The pathology was consistent with the initial surgery. Postoperatively, he underwent treatment with INC280, a highly potent selective cMet inhibitor, and the VEGFR/PDGFR inhibitor, bevacizumab, and remained neurologically and radiographically stable through 22 cycles of treatment. However, a brain MRI showed disease progression again 30 mo after initial diagnosis. An fMRI was obtained and showed the anterolateral displacement of the motor tracts and tumor proximity to the lower extremity somatosensory region. The patient underwent his third STR, 23 mo after his second surgery, with expected anterior inferior residual disease (Fig. 1C,F). Pathology again returned as *IDH1*-mutant, *MGMT*-methylated, GFAP-positive GBM without *EGFR* or *cMet* amplifications. Ki67 was elevated at 10%–15%. The patient then was started on off-label nivolumab and underwent 35-Gy reirradiation to the tumor bed.

His overall survival to date is 32 mo after the initial diagnosis of GBM, with progression-free survival of 8 mo after initial resection, postoperative RT with concurrent TMZ, and veliparib/TMZ on the Alliance trial, and 22 mo after repeat resection/INC280/bevacizumab followed by reirradiation with nivolumab.

Genomic Analyses

Whole-exome sequencing of the first recurrence, which had been treated with 60-Gy RT and TMZ, identified 37 somatic mutations in the captured protein coding regions (Fig. 2A; Supplemental Table 1), including *IDH1* p.R132H, *TP53* p.C176F, and *ATRX* p.Q2108R mutations. Curiously, a missense *DNMT3A* p.P904S mutation affecting a highly conserved catalytic methyltransferase domain responsible for DNA/cofactor binding was identified in this tumor (Table 1). The *DNMT3A* p.P904S mutation was previously reported in AML, DLBCL, and esophageal squamous cell carcinoma, and mutations in *DNMT3A* residue 904 also have been reported in 25 cases in multiple other malignancies (Sawada et al. 2016; Forbes et al. 2017; Thol et al. 2017; Wong et al. 2018). Of note, no *DNMT3A* mutations affecting residue 904 were reported in gliomas in COSMIC. Analysis of the TCGA database identified eight *DNMT3A* mutations in six TCGA-GBM patients and 10 *DNMT3A* mutations in 10 TCGA-LGG patients in residues other than 904; two TCGA-GBM patients carried two *DNMT3A* mutations each, similar to AML patients (Grossman et al. 2016). Of note, four of eight mutations in TCGA-GBM patients and five of 10 mutations in TCGA-LGG patients localized to the DNMT3A methyltransferase domain, suggestive of their functional relevance; three of 10 mutations in TCGA-LGG patients were premature STOP codons resulting in truncated dysfunctional protein, including one localized to methyltransferase domain. Prevalence of *DNMT3A* mutations in 3106 CNS malignancies and in 2421 gliomas reported in COSMIC, all in residues other than P904, was 26, including 18 missense, three nonsense, and one frameshift; 10 of these localized to the methyltransferase domain (Forbes et al. 2017). No LOH events, or large-scale chromosomal aberrations, were noted in this recurrent tumor.

The second recurrent specimen, after multiple cycles of chemotherapy and targeted therapy treatment, was more heterogeneous, with 104 somatic mutations in the captured protein coding regions (Fig. 2A,B; Supplemental Table 2). Only 19 mutations found in the first recurrent GBM were preserved in the second recurrent tumor. Of these, only four mutations, *IDH1* p.R132H, *TP53* p.C176F, *ATRX* p.Q2146R, and *DNMT3A* p.P904S, are reported to be drivers in various cancer types. Interestingly, *DNMT3A* p.P904S, which was not reported in GBMs before, had high variant allele frequency in the second recurrent tumor,

Figure 2. CIRCOS plot representing the genomic profile of patient's first recurrent and second recurrent glioblastoma, including somatic mutations and copy-number variations. The outermost circle represents the first recurrent glioblastoma; the innermost circle represents the second recurrent glioblastoma. Dark gray hash marks represent individual somatic mutations; selected mutations are labeled on the graph. Dark red and blue bars represent copy-number variations, gains and losses, respectively. (*B*) Summary table annotating somatic mutations detected in patient's second recurrent glioblastoma, previously associated with cancer; mutations with VAF > 9% are included. M stands for missense mutations; STOP stands for STOP gained.

B

Type	Gene	Chr	Pos	Ref	Alt	Coding variant	AA change
M	*DNMT3A*	2	25457177	G	A	c.C2710T	p.P904S
M	*IDH1*	2	209113112	C	T	c.G395A	p.R132H
M	*TP53*	17	7578403	C	A	c.G527T	p.C176F
M	*ATRX*	X	76814207	T	C	c.A6323G	p.Q2108R
FS	*ATRX*	X	76849257	TAACA	T	c.5901_5904del	p.F1967fs
M	*PRAMEF11*	1	12887174	C	T	.	p.R228H
FS	*PMS2*	7	6017239	AG	A	c.2424delC	p.A808fs
M	*FAT1*	4	1.88E+08	T	C	c.A12139G	p.T4047A
SPL	*AKAP9*	7	91669987	G	T	c.4693-1G>T	.
M	*IL6ST*	5	55247363	A	G	c.T1586C	p.V529A
M	*PTPRB*	12	70928667	C	A	c.G5226T	p.M1742I
SPL	*NBN*	8	90967505	A	C	c.1397+6T>G	.

Table 1. Variant table

Gene	Chromosome	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect (substitution, deletion, etc.)	dbSNP/ dbVar ID	Genotype (heterozygous/ homozygous)
DNMT3A	2	c.C2710T	p.P904S	Missense	TDPDDDDMDD		Heterozygous

suggesting that it is in the founder clone preserved during tumor progression. In addition to the *ATRX* p.Q2108R mutation in the first recurrent tumor, a second frameshift mutation was identified in *ATRX* p.F1967fs. In comparison to the first recurrence, the second recurrent tumor harbored multiple LOH events, including Chromosome 13 region containing *RB1*. In addition, second recurrent GBM acquired a variety of large-scale chromosomal events, including gains in Chromosome 1, Chromosome 3, Chromosome 6p, Chromosome 7, Chromosome 17, and Chromosome 19p and losses of Chromosome 6, Chromosome 9, Chromosome 13, Chromosome 14, Chromosome 18, and Chromosome X (Fig. 2A; Supplemental Tables 3, 4). Chromosome 2 CNVs and LOH events were noted as well, none involving chromosomal regions containing *DNMT3A* (Supplemental Tables 3 and 4). There was no *1p19q* codeletion in either the first or second recurrent specimen. CNV analysis has been repeated using publicly available tools yielding similar results.

DISCUSSION

The 130-kDa protein product of *DNMT3A*, a member of the DNA methyltransferase family, is responsible for de novo DNA methylation of cytosine residues within CpG sites independent of DNA replication (Ley et al. 2010; Russler-Germain et al. 2014). DNMT3A contains the regulatory domain comprised of the Pro-Trp-Trp-Pro (PWWP) domain involved in DNA/histone binding, the ATRX-DNMT3-DNMT3L (ADD) domain responsible for H3 histone tail interactions, and the adenosyl methionine (SAM)-dependent catalytic methyltransferase C5-type domain responsible for methyl group transfer to five position of cytosine. During normal development, DNMT3A has been shown to play a role in early embryogenesis, imprinting, and spermatogenesis via its interactions with DNMT3L, which regulates its oligomerization and methyltransferase activity (Jia et al. 2007; Holz-Schietinger and Reich 2010). Mutations in any of these domains have been shown to be tumorigenic and particularly associated with AML, as well as other hematologic malignancies (Okano et al. 1999; Brunetti et al. 2017). We describe a patient with an *IDH1*-mutant recurrent glioblastoma, carrying a somatic mutation in *DNMT3A* p.P904S localizing to the highly conserved catalytic domain, never previously reported in glioblastoma patients. Although most *DNMT3A* mutations affect R882 residue within the catalytic domain, resulting in 80% reduction in DNMT3A enzymatic activity and dominant negative effects on the wild-type DNMT3A by blocking the formation of active tetramers, other somatic mutations including frameshift, splice site, missense, and nonsense mutations at non-R882 residues result in expression of nonfunctional or truncated protein and a haploinsufficient phenotype (Ley et al. 2010; Yamashita et al. 2010; Yan et al. 2011; Hájková et al. 2012; Russler-Germain et al. 2014). Studies suggest significantly diminished de novo methyltransferase activity with focal hypomethylation in R882-mutant AML and support worse overall survival and higher recurrences in AML patients carrying *DNMT3A* mutations (Hájková et al. 2012). Moreover, expression profiling of patient-derived primary AML samples have shown decreased DNMT3A activity with up-regulation of *HOX* genes that play a role in hematopoiesis and AML oncogenesis (Yan et al. 2011).

In the recent years, the epigenetic tumor landscape has been shown to play an increasingly important role in the tumor biology via transcriptional regulation, because of the changes in methylation of CpG islands within gene promoter regions, and histone modifications, with resulting changes in chromatin structure and accessibility to transcriptional activators or repressors. The association between altered DNA methylation and gliomagenesis has been well-described (Pajtler et al. 2015; Sturm et al. 2016; Capper et al. 2018). Although *IDH1*-mutant gliomas, similar to AML, show global genome hypomethylation proposed to be associated with genomic instability, CpG islands within promoters of tumor-suppressor genes are frequently methylated, resulting in their inactivation of transcription (Cancer Genome Atlas Research Network et al. 2013; Bai et al. 2016). DNMT3A can affect chromatin modifications via its interactions with histone modifiers including EZH2, SUV39H1, SETDB1, and EHMT2 (Epsztein-Litman et al. 2008; Karimi et al. 2011; Yang et al. 2013). Conditional *DNMT3A* deletion in hematopoietic stem cells results in a marked increase in self-renewal in lieu of differentiation, possibly via its effects on *Ctnnb1* with its concomitant promoter hypomethylation, β-catenin overexpression, and up-regulation of target gene expression (Challen et al. 2014); *DNMT3A* has likewise been implicated in neural stem cell differentiation (Wu et al. 2012). Existence of *DNMT3A* mutations in TCGA-LGG (low-grade glioma) and TCGA-GBM patients, 50% of which affect methyltransferase domain containing *DNMT3A* P904S mutation, supports the notion that it likely is a premalignant driver event. Indeed, *DNMT3A* mutations are present in founder AML clones, have been shown to be important in AML oncogenesis, and often confer resistance to conventional chemotherapy. Multiple *DNMT3A* mutations can cooccur in the same AML patient, similarly seen in the TCGA-GBM patient cohort carrying *DNMT3A* mutations (Thol et al. 2017; Wong et al. 2018). It is possible that *DNMT3A* mutations are premalignant events that skew neural stem/progenitor cells toward self-renewal rather than differentiation, possibly via epigenetic mechanisms, contributing to glioma initiation.

In this case, we describe a patient harboring a GBM with somatic co-mutations in *IDH1*, *TP53*, and *ATRX*, as well as *DNMT3A*. *IDH1*- or *IDH2*-mutant gliomas have been well-studied and associated with nearly twice the median overall survival as nonmutated counterparts (Cairncross et al. 2013, 2014; van den Bent et al. 2013; Ostrom et al. 2015). The *IDH1* p.R132H mutation, in particular, has molecularly been well-described and clinically shown to carry a better prognostic significance for GBM patients. *IDH1*-mutant GBMs, as compared to *IDH1* wild-type GBMs, demonstrate hypermethylation of CpG islands, with the mechanism of action thought to be competitive inhibition of α-ketoglutarate-dependent dioxygenases, including TET2 and JMJ C hydroxylases by D2HG, which disrupts normal patterns of DNA and histone methylation in a process similar to AML (Noushmehr et al. 2010; Lu et al. 2012; Turcan et al. 2012). More recently, the biological importance of DNA methylation profiles in CNS tumors, characterizing various GBM subtypes, has been demonstrated (Capper et al. 2018). According to TCGA, up to 44% of AML patients carry mechanistically and prognostically important mutations in genes regulating genome methylation, including frequent mutations in *DNMT3A* in as much as 30% normal karyotype AML patients, *Tet oncogene family member 2 deoxygenase* (*TET2*), and *isocitrate dehydrogenases 1/2* (*IDH1/2*) (Figueroa et al. 2010; Yan et al. 2011; Cancer Genome Atlas Research Network et al. 2013; Thol et al. 2017). In AML patients, *DNMT3A* somatic mutations are commonly present together with mutations in *IDH1/2*, *FLT3*, *NPM1*, and *cKit* and are mutually exclusive with mutations in *TET2* (Ley et al. 2010; Im et al. 2014; Thol et al. 2017). It has been proposed that the type of *DNMT3A* mutation and gene dosage effects, combined with secondary mutations, may dictate the type of hematologic disease (Yang et al. 2015). Interestingly, *IDH* and *DNMT3A* mutations were shown to have mutually exclusive, opposing effects on the epigenome, with cooccurrence of both mutations resulting in epigenetic antagonism, in which CpG sites affected by either *IDH* or *DNMT3A* mutations alone are no longer affected

in the presence of both, with the overall loss of differential methylation within gene compartments (Glass et al. 2017). Our findings underscore the importance of better elucidating the role of epigenetic mechanisms in the initiation and progression of GBMs, with the possibility that targeting them may be helpful to improve outcomes and survival.

METHODS

This study was approved by Yale University's Human Investigations Committee and Human Research Protection Program. Informed consent was obtained from the patient prior to collection of blood and tumor samples. Pathology and imaging were evaluated by board-certified neuropathologists per WHO classification criteria. Genomic DNA was isolated using standard phenol–chloroform techniques. Sequencing was performed at the Yale Center for Genome Analysis (YCGA) as previously described using Illumina platform. Coding sequences were captured using xGEN Exome Research Panel v1.0 (Integrated DNA Technologies); reads were aligned to GRCh37 human genome using BWA-MEM (v0.7.10) and processed with Picard to exclude PCR duplicates (v1.18). Multisequence realignment, base quality recalibration, and HaplotypeCaller were available through Genome Analysis Toolkit (GATK; v3.2-2). Somatic SNVs were identified using MuTect (v2.7.1); somatic insertion or deletions (indels) were identified using Indelocator (v36.3). Raw SNVs were filtered using "GATK-Best Practice" parameters (https://software.broadinstitute.org/gatk/) and annotated with ANNOVAR (v.2015-12-14) and VEP (v.83). Somatic calls were filtered based on their presence in control databases ExAC, 1000 Genomes, and NHLBI (<1%). Copy-number variations (CNVs) were assessed using tumor over normal coverage ratios, normalized by total coverage variation. DNAcopy in house R script was used for segmentation. Copy-number neutral loss of heterozygosity (LOH) was computed using VAF shift for germline heterozygous mutations in tumor versus blood. Tumor cell purity of 78.8% was reported for the second recurrent GBM, based on deviation of tumor VAF for LOH regions as compared to blood; computational prediction of the tumor cell purity is not available because of the lack of LOH in the initial recurrence GBM. Mean coverages of 165.5×/96.5× and 266×/96.5× were achieved for tumor versus blood from the initial recurrence GBM (second resection) and second recurrence GBM (third resection), respectively (Supplemental Table 5). CNV analysis has been repeated using ExomeCNV package, with the log ratio of depth of coverage for tumor and blood calculated by the Depth of Coverage tool by GATK.

ADDITIONAL INFORMATION

Data Deposition and Access

Variant described in this manuscript has been submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) (SUB5780842) under accession number SCV000924296. Raw sequencing data is available by contacting the authors.

Ethics Statement

The whole-exome sequencing performed in this study was approved by Yale University's Human Investigations Committee and Human Research Protection Program, HIC Protocol Number 9406007680. Informed consent was obtained from the patient prior to collection of blood and tumor samples.

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Author Contribution

E.I.F. developed the manuscript, along with the assistance of A.Z., and created the clinical summary and figures and tables. E.Z.E.-O. performed and interpreted whole-exome sequencing analysis and also developed the manuscript and edited tables. A.H. performed histologic analysis of pathology specimens and grading according to WHO criteria. R.K.F. performed imaging review and interpretation and critically evaluated and edited the imaging figure. J.M. directed the study with interpretation of clinical and scientific findings and developed the manuscript. R.S.B. reviewed and edited the manuscript regarding aspects of RT and *IDH1/2*-mutant biology. All authors critically edited the manuscript. The authors report no pertinent disclosures, and no conflict of interest concerning the materials or methods used in this study or findings described in this paper.

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