Report of Canonical *BCR-ABL1* Fusion in Glioblastoma

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Introduction

Chimeric oncogenic fusion genes can arise because of somatic structural events including chromosomal translocation, amplification, deletion, or inversions.[1] A milestone in oncology research was the discovery of Philadelphia chromosome (Ph) in chronic myeloid leukemia with the identification of the oncogenic fusion kinase, breakpoint cluster region protein-ABL1 (BCR-ABL1), as a major tumorigenic factor of this disease and an important prognostic biomarker.[2,3] Imatinib, a tyrosine kinase inhibitor (TKI), is a US Food and Drug Administration–approved targeted therapy for Ph-positive leukemia.[4] Importantly, *BCR-ABL1* is one of the most reported fusion genes related to hematologic malignancies; however, no study has reported on nonhematologic solid tumors.[5]

Glioblastomas are the most common primary malignant brain tumors. They are incurable largely because of their heterogeneity and highly dynamic genomic landscape.[6] Therefore, identifying druggable oncogenic targets is critically important. Oncogenic fusion transcript is one of the most promising targets for glioblastomas, and up to 30%-50% of glioblastomas carry fusion transcripts, some of which are druggable, including neurotrophic tropomyosin receptor kinase (*NTRK*) fusions.[7-9] In addition, the KIAA1549-Serine/threonine-protein kinase B-raf (*BRAF*) fusion that commonly occurs in the pilocytic astrocytoma, a low-grade glioma, has also been reported in glioblastoma and can be targeted using BRAF inhibitors.[10] This study reports an unexpected finding of the actionable classical e1a2 *BCR-ABL1* fusion transcript in a glioblastoma.

Case Report

The patient, a 61-year-old woman, was referred to the National Institutes of Health for consultation after a right frontal lobe glioblastoma resection, concomitant chemoradiation, and one cycle of adjuvant temozolomide treatment. During the initial visit, a nonenhancing lesion in the right temporal lobe was noted (Fig 1A). Because of the concern for a malignant process of the lesion, a pathologic diagnosis was recommended. A gross total resection of the temporal lobe lesion was subsequently performed. The pathologic diagnosis was consistent with high-grade astrocytoma (Fig 1B). The patient's blood and tumor samples were submitted for whole-exome sequencing (WES) and whole transcriptome analysis (RNA-seq). The tumor was found to harbor a wild-type isocitrate dehydrogenase (*IDH*) gene, but genomic alterations including phosphatase and tensin homolog (*PTEN*) deletion, telomerase reverse transcriptase (*TERT*) promotor mutation (c.-146C>T), and chromosome 10 loss, which are consistent with the molecular features of de novo glioblastoma.[11,12] In addition, methylation profiling of the primary tumor sample (sample number V053) suggested that it was an *IDH*-wild-type glioblastoma of mesenchymal subclass (class assignment confidence calibrated score = 0.995; Fig 1C). The RNA-seq analysis revealed a classical *BCR-ABL1* fusion, which has never been reported in glioblastoma. Sanger sequencing of the RT-PCR fusion amplicon validated the fusion transcript as an in-frame fusion with an intact tyrosine kinase domain on *ABL1*. The breakpoint was after exon 1 of *BCR*, resulting in exon 1 of *BCR* being juxtaposed to exon 2 of *ABL1*, coding for the classical e1a2 *BCR-ABL1* fusion that usually occurs in acute lymphoblastic leukemia[13] (Figs 1D and 1E). To rule out the possibility of hematologic malignancy, we performed both RT-PCR and RNA-seq assays on patient's blood RNA. *BCR-ABL1* fusion transcript was not detected. The absence of *BCR-ABL1* fusion transcript in the blood sample is consistent with the lack of clinical or laboratory evidence of hematologic malignancy.

Subsequently, the patient received concurrent chemoradiation therapy with temozolomide followed by six cycles of temozolomide after tumor resection. Several months after the completion of adjuvant temozolomide, an magnetic resonance imaging brain examination revealed a fast-growing enhancing lesion around the resection cavity to the right temporal lobe, suggesting tumor progression (Fig 1A). A second surgical resection was performed, confirming the recurrence of the tumor (Fig 1B). Methylation profiling of the recurrent tumor

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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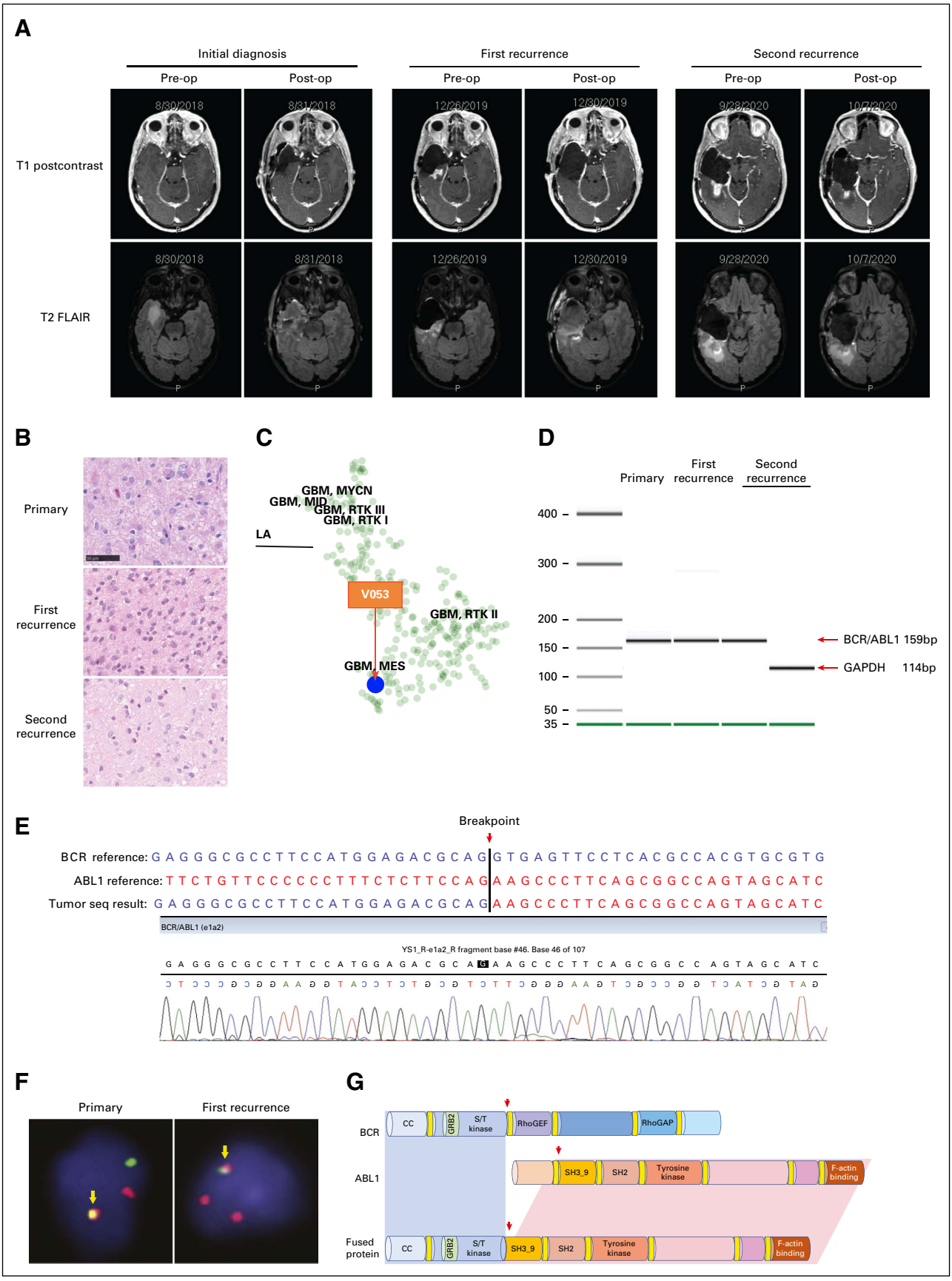
A
Initial diagnosis
First recurrence
Second recurrence
Pre-op
Post-op
Pre-op
Post-op
Pre-op
Post-op
T1 postcontrast
T2 FLAIR
8/30/2018
8/31/2018
12/26/2019
12/30/2019
9/28/2020
10/7/2020
B
Primary
First recurrence
Second recurrence
C
GBM, MYCN
GBM, MID
GBM, RTK III
GBM, RTK I
LA
V053
GBM, RTK II
GBM, MES
D
Primary
First recurrence
Second recurrence
400
300
200
150
100
50
35
BCR/ABL1 159bp
GAPDH 114bp
E
Breakpoint
BCR reference: G A G G G C G C C T T C C A T G G A G A C G C A G G T G A G T T C C T C A C G C C A C G T G C G T G
ABL1 reference: T T C T G T T C C C C C C T T T C T C T T C C A G A A G C C C T T C A G C G G C C A G T A G C A T C
Tumor seq result: G A G G G C G C C T T C C A T G G A G A C G C A G A A G C C C T T C A G C G G C C A G T A G C A T C
BCR/ABL1 (e1a2)
YS1_R-e1a2_R fragment base #46. Base 46 of 107
F
Primary
First recurrence
G
BCR
ABL1
Fused protein
CC
GRB2
S/T kinase
RhoGEF
RhoGAP
SH3_9
SH2
Tyrosine kinase
F-actin binding

sample suggested it to be glioblastoma (sample number W879 and calibrated scores: 0.842), *IDH*-wild-type, subclass mesenchymal (Appendix Fig A1), which was similar to the primary tumor (sample number V053). *BCR-ABL1* fusion was also detected from this recurrent tumor sample by RNA-seq and fluorescence in situ hybridization analysis (Figs 1D-1G), suggesting that the fusion gene might have contributed to the rapid disease progression. Given the well-known safety profile of imatinib, an US Food and Drug Administration–approved drug for BCR-ABL–positive leukemia, therapy with this drug was offered to the patient after a short course of radiation therapy. The patient was able to tolerate 400 mg once a day imatinib, and her condition remained stable for several months. Ten months after the surgical resection, a follow-up magnetic resonance imaging revealed a new enhancing lesion posterior to the resection cavity (Fig 1A, second recurrence). Since the new enhancing lesion area was in an area of overlap of the three previous radiation treatments, radiation necrosis was highly suspected. Following surgical resection, the pathologic examination revealed a hypercellular parenchyma with atypical glial cells, histiocytes, and gliosis. In addition, the actively proliferating cells, indicated by Ki-67 staining, decreased from 40% in the first recurrent tumor before imatinib treatment to only 6%-8% in the second recurrent tumors after imatinib treatment (Fig 1B, middle and lower panels), suggesting that the imatinib treatment dampened the rate of tumor progression. The WES of the second recurrent tumor sample post-treatment of imatinib did not reveal any imatinib-resistant mutation (at 150× sequencing coverage) within the kinase domain. Therefore, imatinib treatment was planned to be restarted after this third surgical resection. However, the patient was found to have disease relapse from a previous diagnosis of breast cancer during the perioperative time. The timely initiation of imatinib was complicated by the extensive diagnostic or staging tests and treatment for breast cancer. Unfortunately, patient's overall condition rapidly declined after < 1 month of imatinib treatment and the patient decided on hospice.

The patient gave informed consent to present information and/or images from the patient.

Discussion

To our knowledge, this study reports the first case of glioblastoma with the classical hematologic *BCR-ABL1* fusion. The canonical fusion was confirmed in multiple tumor samples from the initial diagnosis and the two subsequent recurrences.

BCR-ABL1 tyrosine kinase fusion is caused by the reciprocal translocation t(9;22). On the basis of alternative breakpoints on the *BCR* gene, different BCR-ABL1 fusion proteins are formed, containing the ABL1 protein kinase domain. Since the presence of the *BCR-ABL1* might have contributed to glioma pathogenesis in this patient, we prescribed imatinib, which is a first-generation inhibitor of BCR-ABL1 shown to significantly increase the overall survival of patients with chronic myeloid leukemia carrying this kinase fusion.[14,15] Although imatinib is used to treat Ph+ leukemia, it has marginal effects in preventing or treating CNS leukemia when the blood-brain barrier (BBB) is intact.[16] However, in the case of glioblastoma, the BBB within the tumor is usually significantly disrupted and the intratumoral concentration of imatinib can be comparable with the level in plasma.[17,18] Therefore, we postulated that therapy with imatinib may be still beneficial. Compared with imatinib, second generation of TKIs (dasatinib and nilotinib) has improved BBB penetration and increased survival benefit.[19,20] Unfortunately, the second generation of TKI was not available to this patient. As a second option for a TKI, imatinib was offered. Furthermore, one third of patients have developed resistance to imatinib, mostly because of point mutations within the *BCR-ABL1* kinase domain, including T315I, Y253F/H, E255K/V, M351T, G250E, F359C/V, H396R/P, M244V, E355G, F317L, M237I, Q252H/R, D276G, L248V, and F486S. This has led to the development of second- and third-generation inhibitors to overcome the resistance.[21] In our study, we did not find evidence of any *BCR-ABL1* resistance mutation in the tumors by WES at a sequencing depth of 150× coverage (data not shown). Despite the aggressiveness of disease, a significantly lower proliferation index of tumor cells following radiation and imatinib treatment suggested a potential role of imatinib in control of tumor growth. Unfortunately, the evaluation of the imatinib response after the third tumor resection was challenging because of the complicated disease course. Although imatinib demonstrated some antiglioma activities in this patient, an alternative would have been the use of a second-generation TKI with a better BBB penetration. Despite the detection of *BCR-ABL1* fusion in the tumor sample from the initial diagnosis, the patient was initially not offered imatinib because of the unavailability of the drug.

FIG 1. Case presentation: (A) serial brain MRIs of the patient with T1 postcontrast (upper panel) and T2 FLAIR (lower panel) images showing the right temporal lobe lesion before and after the surgical resections at the time of initial diagnosis, disease recurrent, and postimatinib treatment; (B) hematoxylin and eosin staining of tumor samples collected at initial diagnosis and subsequent recurrences; bar = 50 μm; imatinib was given after the first recurrence; (C) a t-distributed stochastic neighbor embedding plot showing clustering of the *BCR-ABL1* fusion primary tumor sample with main glioblastoma cluster (V053, red arrow); (D) agarose gel separation of the *BCR-ABL1* fusion–specific RT-PCR product from the patient's primary tumor, recurrent tumor, and blood samples; *GAPDH* was amplified as the positive control; (E) Sanger sequencing of *BCR-ABL1* fusion–specific RT-PCR amplicons; (F) FISH assay showing *BCR-ABL1* translocation in patient's primary and recurrent tumor samples (arrow); and (G) schematic representation of the *BCR-ABL1* fusion transcript by detection. FISH, fluorescence in situ hybridization; FLAIR, fluid attenuated inversion recovery; GBM, glioblastoma; MRI, magnetic resonance imaging; RT-PCR, reverse transcription polymerase chain reaction; T1, T1-weighted image; T2, T2-weighted image.

However, the patient was able to receive imatinib after the second surgical resection. Although the potential antitumor effect of imatinib is suggested in this case report, the definitive treatment response to TKIs in glioblastoma with *BCR-ABL* fusion needs to be tested in a larger patient population. Although *BCR-ABL1* fusion has never been reported in glioblastomas, it may be more common than appreciated in this patient population. However, a search of the literature and The Cancer Genome Atlas Program data from brain tumors failed to show evidence of the presence of *BCR-ABL1*.

In conclusion, our case study shows that *BCR-ABL1* fusion should be considered as an albeit rare but druggable event in GBM. Thus, our report describes that the first *BCR-ABL1* fusion gene in glioblastoma led to a targeted therapeutic option. The potential treatment response that resulted from targeting the BCR-ABL1 fusion oncoprotein underscores the importance of identifying oncogenic fusion genes, especially those that harbor intact druggable kinase domains. Importantly, we recommend *BCR-ABL1* fusion be considered as an additional biomarker for clinical molecular testing panels to be further investigated in a larger cohort of patients with glioma.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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APPENDIX

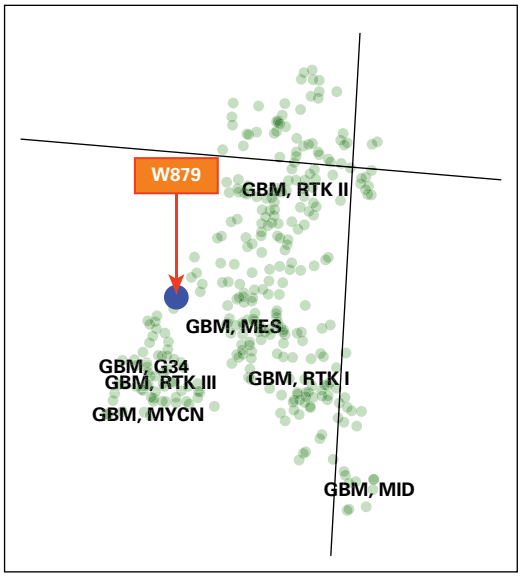

FIG A1. A t-distributed stochastic neighbor embedding plot showing clustering of the *BCR-ABL1* fusion in the first recurrent tumor sample with main glioblastoma cluster (W879, red arrow). GBM, glioblastoma.