Detection, Characterization, and Inhibition of FGFR–TACC Fusions in IDH Wild-type Glioma

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Abstract

Purpose: Oncogenic fusions consisting of fibroblast growth factor receptor (FGFR) and TACC are present in a subgroup of glioblastoma (GBM) and other human cancers and have been proposed as new therapeutic targets. We analyzed frequency and molecular features of FGFR–TACC fusions and explored the therapeutic efficacy of inhibiting FGFR kinase in GBM and grade II and III glioma.

Experimental Design: Overall, 795 gliomas (584 GBM, 85 grades II and III with wild-type and 126 with IDH1/2 mutation) were screened for FGFR–TACC breakpoints and associated molecular profile. We also analyzed expression of the FGFR3 and TACC3 components of the fusions. The effects of the specific FGFR inhibitor JNJ-42756493 for FGFR3–TACC3–positive glioma were determined in preclinical experiments. Two patients with advanced FGFR3–TACC3–positive GBM received JNJ-42756493 and were assessed for therapeutic response.

Results: Three of 85 IDH1/2 wild-type (3.5%) but none of 126 IDH1/2-mutant grade II and III gliomas harbored FGFR3–TACC3 fusions. FGFR–TACC rearrangements were present in 17 of 584 GBM (2.9%). FGFR3–TACC3 fusions were associated with strong and homogeneous FGFR3 immunostaining. They are mutually exclusive with IDH1/2 mutations and EGFR amplification, whereas they co-occur with CDK4 amplification. JNJ-42756493 inhibited growth of glioma cells harboring FGFR3–TACC3 in vitro and in vivo. The two patients with FGFR3–TACC3 rearrangements who received JNJ-42756493 manifested clinical improvement with stable disease and minor response, respectively.

Conclusions: RT-PCR sequencing is a sensitive and specific method to identify FGFR–TACC–positive patients. FGFR3–TACC3 fusions are associated with uniform intratumor expression of the fusion protein. The clinical response observed in the FGFR3–TACC3–positive patients treated with an FGFR inhibitor supports clinical studies of FGFR inhibition in FGFR–TACC–positive patients. Clin Cancer Res; 21(14); 3307–17. ©2015 AACR.

See related commentary by Ahluwalia and Rich, p. 3105

Introduction

The history of successful targeted therapy of cancer largely coincides with the inactivation of recurrent, oncogenic, and addicting gene fusions in hematologic malignancies and recently in some types of epithelial cancer (1, 2). Glioblastoma multiforme (GBM) is among the most lethal forms of human cancer, and targeted therapies against common genetic alterations in GBM have not changed the dismal outcome of the disease.

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Underlying biologic features, including infiltrative growth behavior, intratumoral heterogeneity, and adaptive resistance mechanisms, coupled with the unique challenges of intracranial location present significant problems in its effective management. Despite surgery and chemoradiotherapy, most patients rapidly recur and no effective treatment options are available at that stage. Besides GBM, which features the highest grade of malignancy among glioma (grade IV), lower grade gliomas, which include grades II and III, are a heterogeneous group of tumors in which specific molecular features are associated with divergent clinical outcome. The majority of grade II and III glioma (but only a small subgroup of GBM) harbor mutations in IDH genes (IDH1 or IDH2), which confer a more favorable clinical outcome. Conversely, the absence of IDH mutations is associated with the worst prognosis (5).

We have recently identified FGFR–TACC gene fusions (mostly FGFR3–TACC3 and rarely FGFR1–TACC1) as the first example of highly oncogenic and recurrent gene fusions in GBM. The FGFR–TACC fusions that have been identified so far include the tyrosine kinase (TK) domain of FGFR and the coiled-coil domain of TACC proteins, both necessary for the oncogenic function of FGFR–TACC fusions. We also tested tumor dependency on FGFR–TACC fusions that have been identified in glioblastomas as one of the chromosomal translocations. The molecular characterization of fusion-positive glioma revealed that FGFR–TACC is mutually exclusive with EGFR amplification but co-occurs with CDK4 amplification. FGFR–TACC–positive glioma displays strikingly uniform and strong expression of the fusion protein at the single-cell level. Preclinical experiments with FGFR3–TACC3–positive glioma cells treated with the fibroblast growth factor receptor (FGFR) inhibitor INI-42756493 showed strong antitumor effects, and treatment of two patients with recurrent GBM harboring FGFR3–TACC3 resulted in clinical improvement and radiologic tumor reduction. These findings validate the treatment of FGFR inhibitors of patients with glioma harboring FGFR–TACC chromosomal translocations.

Materials and Methods

Patients and tissue samples

This study includes a cohort of 746 untreated patients with histologic diagnosis of glioma from 5 institutions. Forty-nine recurrent gliomas from Pitie-Salpetriere Hospital and one recurrent glioma from the University of Calgary (Calgary, Canada) were also included. A summary of the patient cohort is provided in Table 1. Two recurrent patients with GBM harboring FGFR3–TACC3 were enrolled in the dose escalation part of JNJ-42756493 trial (NCT01962532) at the Gustave Roussy Institute (Paris, France).

Identification of fusion transcripts and analysis of genomic breakpoints

Total RNA was extracted from frozen tissues using TRIzol (Invitrogen) according to manufacturer instructions. Two to three hundred nanograms of total RNA was retrotranscribed with the Maxima First Strand cDNA Synthesis Kit (Thermo Scientific) or SuperScript II (Invitrogen). RT-PCR was performed using AccuPrime Taq DNA Polymerase (Invitrogen). Primer pairs used for the FGFR3–TACC3 fusions screening were: FGFR3ex12-FW: 5’-CGTGAAGATGCCTGAAGAAGCTGATG-3’ and TACCex14-RV: 5’-AAGCCGGTGAAGGTCGGAAG-3’. Amplification conditions were 94°C 3 minutes (94°C-30 seconds/61°C-30 seconds/68°C-1 minute).
Table 1. Frequency of FGFR3–TACC3 fusions in GBM and grade II–III glioma

<table>
<thead>
<tr>
<th>Tumor sample source</th>
<th>Cases (GBM), n</th>
<th>Detected fusions, n</th>
<th>Immunostaining FGFR3 positive/sample analyzed</th>
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<td>380</td>
<td>9</td>
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<tr>
<td>Besta Neurological Institute</td>
<td>85</td>
<td>5</td>
<td>2/2</td>
</tr>
<tr>
<td>University of Calgary</td>
<td>60 + 1R^a</td>
<td>2 + 1R^a</td>
<td>1/1 + 1/1R^a</td>
</tr>
<tr>
<td>Montreal Neurological Institute</td>
<td>51</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>University of British Columbia</td>
<td>8</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>584 (100%)^b</td>
<td>17 (2.9%)</td>
<td>—</td>
</tr>
</tbody>
</table>

NOTE: Distribution of the FGFR3–TACC3 fusions in GBM (top) and lower grade glioma (bottom) samples stratified according to the institution of origin. The table reports numbers of cases analyzed; number of tumors harboring FGFR3–TACC3 fusion transcripts, and results of FGFR3 immunostaining. Lower grade glioma samples are further classified according to IDH status (IDH2 and IDH2). The respective frequency of FGFR3–TACC3 in GBM, glioma grades II–III IDH wild type (wt), and IDH mutant (Mut) glioma is reported in parentheses.

^a Recurrent GBM

^b Twenty-five cases of 85 are unknown for IDH2 status.

minute 40 seconds) for 35 cycles, 68°C-7 minutes. FGFR1– TACC1 fusions were amplified with FGFR1ex16-FW: 5’-TGGC- TGTGGAGGAGACTTITCA-3’ and TACC1ex13-RV: 5’-CCCCAATCT- CACGACCTCTAAG-3’ primers (94°C-30 seconds/60°C-30 seconds/68°C-1 minute 40 seconds for 35 cycles). PCR products were subjected to Sanger sequencing.

FGFR3–TACC3 genomic breakpoints were analyzed in 6 FGFR3–TACC3–positive samples, 5 of which from the Pitie-Salpetriere Hospital and 1 from Montreal Neurological Institute (Montreal, Canada). Three additional samples (MB-22, TCGA 27-1835, and TCGA 06-6390) available from our previous study (6) were also included in the analysis. Fifty nanograms of gDNA was used in the PCR reaction, performed with Accuprime Taq Polymerase (Invitrogen) and PCR products were Sanger sequenced. Primers used in genomic PCR were designed according to the breakpoint sequence in the mRNA; the list of primers used are: FGFR3ex17-FW 5’-TGAGCCCTGCTCCTATCCGAT-3’ (PCR samples 3048, 4373, 4867, 4451, MB-22, OPK 14, 06-6390, 27-1835 and sequencing sample 3048, 4373, 4872, 4451, MB-22, OPK14, 06-6390, 27-1835); FGFR3ex16-FW 5’-GGTCTCCTTTGGGGTCC- TGCT-3’ (PCR and sequencing sample 3808); TACC3ex6-RV 5’-CTCCITTTCAGCTCAGGCA-3’ (PCR and sequencing sample 3808); TACC3ex6-RV 5’-CTCCITTTCAGCTCAGGCA-3’ (PCR and sequencing sample 4451 and OPK14); TACC3ex6-RV 5’-CTCCITTTCAGCTCAGGCA-3’ (PCR and sequencing sample 3808); TACC3ex6-RV 5’-CTCCITTTCAGCTCAGGCA-3’ (PCR and sequencing sample 4451 and OPK14); TACC3ex6-RV 5’-CTCCITTTCAGCTCAGGCA-3’ (PCR and sequencing sample 3808); TACC3ex6-RV 5’-CTCCITTTCAGCTCAGGCA-3’ (PCR and sequencing sample 3808); TACC3ex6-RV 5’-CTCCITTTCAGCTCAGGCA-3’ (PCR and sequencing sample 3808). All reactions were performed in triplicate and the data are reported as fold change ± SD.

Immunofluorescence and immunohistochemistry

For immunofluorescent (IF) staining of FGFR3, 5-μm formalin-fixed, paraffin-embedded (FFPE) sections were subjected to antigen retrieval with citrate buffer for 8 minutes. Primary antibodies were: FGFR3-N (1:400, sc-13121, Santa Cruz Biotechnology), FGFR3-C: Forward 5’-TACCTGCTGAGCGTGAGG-3’ and N-terminal domain of TACC3, TACC3-N: Forward 5’- TCCCTTCCTGCCACCTCTTCAAGC-3’, Reverse 5’-TACCTGCTGAGCGTGAGG-3’ and N-terminal domain of TACC3, TACC3-N: Forward 5’-TACCTGCTGAGCGTGAGG-3’ and N-terminal domain of TACC3, TACC3-N: Forward 5’-TACCTGCTGAGCGTGAGG-3’ and N-terminal domain of TACC3, TACC3-N: Forward 5’-TACCTGCTGAGCGTGAGG-3’. All reactions were performed in triplicate and the data are reported as fold change ± SD.

Molecular characterization of tumor samples

Mutational status of IDH1, IDH2, TERT promoter, as well as the methylation status of the MGMT promoter was analyzed in the Pitie-Salpetriere cohort. Expression of IDH1-R132H mutant was analyzed by IHC in 500 cases as previously described (22). IDH1 and IDH2 gene mutations were identified by Sanger sequencing in 464 and 388 gliomas, respectively (5). IDH wild-type tumors are
defined according to the absence of IDH1-R132H immunopositivity and/or mutations in IDH1 and IDH2 genes. TERT promoter status was determined by the same technique in 277 samples (23). Hypermethylation of the MGMT promoter was tested in 242 samples by bisulfite pyrosequencing (24). The presence of EGFR-vIII was evaluated by RT-PCR in 118 samples using EGFR-FW 5'–CITCCCCGGGACCCACGACAG-3' and EGFR-RV 5'CCTGTCCTCAATCGAGGGGAGGAGGCA-3' primers (25).

Copy number variations analyses have been performed on 192 tissue samples using CGH arrays using BAC arrays (n = 187), Agilent 4 × 180 K (n = 2), Nimblegen 3 × 720 K (n = 2), and Agilent 8 × 60 K (n = 1). Results were normalized using control DNA from matched blood samples as previously described (26). Additional analyses of 193 tumor specimens were performed by SNP array, using Illumina Omni (n = 110), Illumina HumCore (n = 32), Illumina 370 K (n = 27), or Illumina 610 K (n = 24), as previously described (27). Array processing was outsourced to Integragen. Raw copy numbers were estimated at each of the SNP and copy number markers. Biodiscovery property SNP-FASST2 algorithm was then used to segment copy number data. Segments were mapped to hg18 genome assembly (28). Copy number alterations magnitudes called log-R ratio (LRR) were classified using simple thresholds: deletion (x ≤ 1), loss (−1 ≤ x < 0.2), gain (0.2 ≤ x ≤ 1), or amplification (x ≥ 1) according to default Nexus 7.5 software. For additional 56 gliomas, 10q loss was mapped to hg18 genome assembly at Broad.

Overall, 158 GBM (all with a wild-type TERT, were classified with the Pitié-Salpêtrière dataset). The molecular profiles obtained in the Pitié-Salpêtrière dataset were compared with those available in The Cancer Genome Atlas (TCGA) data portal. TCGA GBM segmented copy number variation profile was downloaded from The UCSC Cancer Genomics Browser (31). Copy number variations (CNV) were measured experimentally using the Affymetrix Genome-Wide Human SNP Array 6.0 platform at the Broad TCGA genome characterization center (32). Raw copy numbers were estimated at each of the SNP and copy number markers. Circular binary segmentation was then used to segment the copy number data (28). Segments were mapped to hg18 genome assembly at Broad.

For CNV analysis of the regions across FGFR3 and TACC3 genes, we considered samples for which RNAseq and CNV data were available or samples for which only CNV data were available and RT-PCR sequencing of FGFR3–TACC3 fusion had been performed. Overall, 158 GBM (all with a wild-type IDH1 gene) satisfied these criteria. Among them, 5 harbored an FGFR3–TACC3 fusion, whereas 153 were FGFR–TACC–negative. The CNV magnitudes, called LRR, were classified using the following thresholds: deletion (x < 1), loss (−1 ≤ x < −0.2), gain (0.2 ≤ x ≤ 1), or amplification (x ≥ 1), according to the Atlas-TCGA (32). The analysis of the genomic regions encompassing EGFR, MDM2, CDK4, CDKN2A, 7p, 10q, according to hg18 genome assembly, was performed to evaluate their CNV. EGFR/vIII mutation status was inferred according to Brennan and colleagues (32). The frequencies of the aberrations of these genes in FGFR3–TACC3–positive and -negative samples were calculated and the obtained data were then combined with the Pitié-Salpêtrière Hospital dataset.

Statistical analysis

Differences in the distribution on categorical variables were analyzed using the Fisher exact test. The P values were adjusted for multiple testing according to Benjamini and Hochberg false discovery rate (FDR). A q value ≤ 0.05 (2-sided) was considered to be statistically significant.

Overall survival (OS) was defined as the time between the diagnosis and death or last follow-up. Patients who were still alive at the last follow-up were considered as censored events in the analysis. Progression-free survival (PFS) was defined as the time between the diagnosis and recurrence or last follow-up. Patients who were recurrence-free at the last follow-up were considered as censored events in the analysis. Survival curves were calculated by the Kaplan–Meier method and differences between curves assessed using the log-rank test. A log-rank test P ≤ 0.05 (2-sided) was considered to be statistically significant.

Cell culture and cell growth assay

GIC-1123 gliomospheres were cultured in neurobasal medium (Invitrogen) supplemented with B27, N2 (Invitrogen), EGF, and FGF2 (20 ng/mL, PeproTech). Mouse astrocytes Inh4A-Arf+/−/− were cultured in DMEM supplemented with 10% FBS. Cells were seeded at 1,000 cells per well in a 96-well plate and treated with JNJ-42756493 (Active Biochem, #A-1278). After 72 hours, cell viability was assessed using the MTT assay. Data are mean ± SEM of 6 replicates. Experiments were performed 3 times.

Subcutaneous xenografts and drug treatment

GIC-1123 cells (5 × 105) were injected subcutaneously in the flank of athymic nude (nu/nu) mice (Charles River Laboratories). Mice carrying about 200 mm3 subcutaneous tumors were randomized to receive 12 mg/kg JNJ-42756493 (Active Biochem, #A-1278) or dimethyl sulfoxide (DMSO) in 1% Tween 80 by oral gavage. Tumor diameters were measured with caliper and tumor volumes estimated using the formula: 0.5 × length × width2. Data are mean ± SD of 9 mice in each group. Mice were sacrificed when tumors in the control group reached the maximal size allowed by the IACUC Committee at Columbia University (New York, NY).

MRI imaging and evaluation of clinical response to JNJ-42756493

Baseline and follow-up imaging assessments were performed on 1.5-Tesla MR imaging systems, including at least axial T1-weighted images before gadolinium injection, Axial or 3D FLAIR (fluid-attenuated inversion recovery), dynamic susceptibility contrast MR perfusion (0.1 mmol/kg of gadobutrol), axial and 3D T1-weighted images after gadolinium injection. Tumor response was assessed according to the RANO criteria (33). Contrast-enhancing lesion volume was assessed with the help of a semiautomated volumetry tool (SegmentX), based on shape detection and thresholding, with control and manual correction of edges when necessary. Because exclusion of cystic or necrotic portions of the lesion may be affected by operator subjectivity, we included them both for volumetric and axial measurements.

DSC (dynamic susceptibility contrast) perfusion datasets were processed with vendor’s software suite (Neuroperfusion, Philips), including coregistration and tCBV (relative cerebral blood volume) parametric maps generation with 3 different algorithms (Gamma-variate fitting, Arterial Input Function–based deconvolution and Model Free).
Results

Detection of FGFR1–TACC1 and FGFR3–TACC3 fusions in GBM and grade II–III glioma

To determine the frequency and molecular features of FGFR–TACC fusions in human patients with glioma, we screened a cohort of 584 GBM and 211 grade II–III glioma treated at 5 neuro-oncology centers (Table 1). One hundred eight were grade III (49 IDH wild-type, 52 IDH1 mutant, and 7 IDH2 mutant) and 103 were grade II (36 IDH wild-type, 63 IDH1 mutant, and 4 IDH2 mutant). We also established the IDH mutational status of 333 GBM and determined that 303 harbored wild-type IDH1/2 and 30 were mutated at codon 132 of IDH1. We designed a RT-PCR assay for the detection of all known and possibly new variants of FGFR1–TACC1 and FGFR3–TACC3 fusions that retain the mRNA sequences coding for the key FGFR-TK and TACC domains required for the oncogenic activity of the fusion protein (Figs. 1 and 2A–D). Overall, we found 20 tumors with an FGFR3–TACC3 fusion, of which 17 were GBM (2.9% positives) and 3 lower grade glioma harboring wild-type IDH1/2 genes (3.5% positives). The size of the FGFR3–TACC3 RT-PCR amplicons ranged from 928 bp (for FGFR3ex18-TACC3ex13) to 1,706 bp (for FGFR3ex18-TACC3ex4). The FGFR1–TACC1 fusion was detected in one grade II IDH wild-type glioma (Fig. 1). Conversely, we did not find any IDH1/2-mutant glioma harboring FGFR–TACC fusions (P < 0.02). Sanger sequencing of the fusion amplicons revealed that each FGFR–TACC cDNA joined in-frame the sequence coding for the entire TK domain upstream of TACC-coding sequences that invariably include the coiled-coil TACC domain (Fig. 1). However, we detected a notable variability among FGFR3–TACC3 fusion isoforms, whereby 5 of the identified variants occurred only in individual cases (Fig. 1). Furthermore, 6 fusion transcripts emerged as new variants that have not been reported before in human cancer (marked in red in Fig. 1).

Next, we designed suitable PCR primers to map the genomic breakpoint coordinates for 9 FGFR3–TACC3–positive samples for which gDNA was available (Supplementary Figs. S1 and S2). We successfully reconstructed the genomic breakpoints by Sanger sequencing and found that they differ for each of the 9 positive cases. Interestingly, even cases harboring the same FGFR3–TACC3 transcript splice variants (#4451 and #0P14 joining exon 17 of FGFR3 to exon 6 of TACC3; #3048 and #4373 joining exon 17 of FGFR3 to exon 8 of TACC3; #3808 and #27-1835 joining exon 17 of FGFR3 to exon 11 of TACC3) had different genomic breakpoints (Supplementary Fig. S2). Taken together, the above findings indicate that the noticeable variability among FGFR3–TACC3 fusion transcripts and genomic breakpoints is efficiently resolved by the RT-PCR screening assay.

Immunostaining analysis of FGFR3–TACC3–positive tumors

We analyzed the expression of the FGFR3 fusion protein by IHC or IF using an antibody that recognizes the N-terminal region of FGFR3 (FGFR3-N) in 12 GBM and 3 lower grade glioma harboring FGFR3–TACC3 fusions for which sufficient tissue was available. Remarkably, each of the 15 positive tumors but none of those that had scored negative in the RT-PCR assay, displayed strong positivity for FGFR3 in the vast majority of tumor cells but
not endothelial cells throughout the analyzed tumor section (Fig. 2A–H). Notably, IF using an antibody that recognizes an epitope at the C-terminus of TACC3, which is invariably retained within FGFR3–TACC3 variants (TACC3-C), reproduced the staining pattern of the FGFR3-N antibody in FGFR3–TACC3–positive tumors. Conversely, negative or very weak staining was obtained in FGFR3–TACC3–positive tumors with antibodies recognizing the regions of FGFR3 (FGFR3 C-terminal region, FGFR3-C) and TACC3 (TACC3 N-terminal region, TACC3-N) constantly excluded from FGFR3–TACC3 fusion proteins (Supplementary Fig. S3A). Consistently, quantitative RT-PCR of GBM harboring FGFR3–TACC3 fusions showed that the expression of the N-terminal coding region of FGFR3 and the C-terminal coding region of TACC3 (which are included in the fusion genes) is markedly higher than the expression of the C-terminal coding region of FGFR3 and the N-terminal coding region of TACC3, which are excluded from the fusion transcripts (Supplementary Fig. S3B). We also analyzed one recurrent GBM from a patient whose tumor had been found positive for FGFR3–TACC3 at the initial diagnosis and who had recurred after concurrent radiotherapy and temozolomide treatment. The recurrent tumor retained the same FGFR3–TACC3 fusion gene and protein that was present in the untreated GBM as determined by RT-PCR sequencing and FGFR3 IF, respectively (Supplementary Fig. S4). Although this requires additional evaluation, the retained uniform positivity for FGFR3 in this recurrent GBM suggests that targeting the FGFR3–TACC3 fusion protein at relapse is a valid therapeutic strategy.

Clinical and molecular characteristics of glioma patients with FGFR3–TACC3 fusions

Clinical and molecular profiling data were available for 591 patients, including 380 GBM (9 with FGFR3–TACC3 fusions) and all 211 lower grade glioma (3 with FGFR3–TACC3 fusions). Of these 12 patients, 5 are males and 7 females, aged 48 to 82 years (median = 61 years). We sought to determine the molecular
profile of FGFR3–TACC3–positive glioma. To do so, we combined the analysis of CNVs and somatic mutations of key GBM genes in our dataset with the SNP6.0 high-density genomic array analysis of 158 TCGA-derived GBM samples fully annotated for FGFR3–TACC3 fusion genes (the RNA-seq and/or RT-PCR analysis of these samples had revealed that 5 of them harbor FGFR3–TACC3 fusions; ref. 6). Patients with FGFR3–TACC3 fusions displayed unique characteristics (Table 2). FGFR3–TACC3 fusions were mutually exclusive with EGFR amplification (0 of 16 vs. 166 of 411; $P = 0.0004$, FDR $q$ value corrected for multiple comparisons = 0.0012) and showed a clear trend against the presence of the EGFRvIII transcript variant (0 of 16 vs. 37 of 219; $P = 0.083$). Conversely, CDK4 amplification was significantly more frequent in FGFR3–TACC3–positive tumors (7 of 16 vs. 41 of 408; $P = 0.0008$; FDR $q$ value = 0.0024). A less significant association of FGFR3–TACC3 fusions was also seen with amplification of MDM2, which as CDK4, maps to chromosome 12q (4 of 16 vs. 24 of 408, $P = 0.016$; FDR $q = 0.048$). We found no statistical association between FGFR3–TACC3 fusions and other genetic and epigenetic alterations that commonly occur in gliomas harboring wild-type IDH genes (CDKN2A deletion, TERT promoter mutations, gain of chromosome 7p, loss of chromosome 10q, and wild-type MGMT promoter; Table 2). When compared with the IDH wild-type patient population of grade II and III glioma and GBM, there was no significant difference in PFS or OS between patients positive or negative for FGFR3–TACC3–positive cells, we treated with JNJ-42756493 mouse astrocytes expressing FGFR3–TACC3, FGFR3–TACC3 containing a mutation that inactivates the kinase activity of FGFR3 (FGFR3–TACC3-KD) or the empty vector. We also studied the effect of JNJ-42756493 on human glioma stem cells GIC-1123 that harbor the FGFR3–TACC3 gene fusion (6). These experiments revealed that both mouse astrocytes and GIC-1123 that express FGFR3–TACC3 but not cells expressing the KD mutant fusion or the empty vector are highly sensitive to FGFR inhibition by JNJ-42756493 with an IC$_{50}$ of 3.03 and 1.55 nM, respectively (Fig. 3A and B). Next, we tested whether oral treatment with JNJ-42756493 of mice-bearing xenografts of human GIC-1123 affects tumor growth. Mice were randomized to receive vehicle or JNJ-42756493 (12 mg/kg). Mirroring the in vitro results, JNJ-42756493 elicited a potent growth inhibition of GIC-1123 tumor xenografts (Fig. 3C and D) with a statistically significant tumor regression after 2 weeks ($P$ value of the slope calculated from the treatment starting point = 0.04). The above findings provide a strong foundation for the treatment of patients with GBM harboring FGFR–TACC rearrangements with JNJ-42756493.

Two patients with recurrent GBM harboring FGFR3–TACC3 fusions were treated with JNJ-42756493 in a first-in-man phase I trial. Patient 1, male aged 52 years, underwent partial surgical resection of a right parietal GBM, followed by fractionated radiotherapy and concomitant temozolomide as first-line treatment (36). The RT-PCR sequencing analysis of the GBM specimen revealed positivity for the FGFR3–TACC3 fusion (FGFR3-exon17-TACC3-exon 6, sample 4451, Supplementary Figs. S1 and S2) and the immunostaining using FGFR3 antibody on paraffin-embedded sections showed strong positivity in a large fraction of tumor cells (not shown). After 5 cycles of temozolomide, the patient presented with dizziness and headache and brain MRI revealed tumor progression (Fig. 4A). At this time, the patient was enrolled in the JNJ-42756493 trial and received JNJ-42756493 (12 mg/d administered in cycles of 7 days followed by 7 days off-treatment). After 3 weeks, the patient reported a marked clinical improvement (complete regression of dizziness and headache). On MRI, the sum of product diameters (RANO criteria, Fig. 4B) and volumetry (Fig. 4C) measured without excluding cystic and necrotic components showed disease stabilization. However, the tumor mass underwent significant decrease of the enhancing

<table>
<thead>
<tr>
<th>FGFR3–TACC3 fusion</th>
<th>EGFR amplification</th>
<th>CDK4 amplification</th>
<th>MDM2 amplification</th>
<th>CDKN2A deletion</th>
<th>Chr. 7p gain</th>
<th>Chr. 10q deletion</th>
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<td>50.0%</td>
</tr>
<tr>
<td>FGFR3–TACC3 negative, $n$</td>
<td>166/411</td>
<td>41/408</td>
<td>24/408</td>
<td>188/411</td>
<td>242/374</td>
<td>253/420</td>
<td>128/165</td>
<td>73/760</td>
</tr>
<tr>
<td>% of FGFR3–TACC3 negative</td>
<td>40.4%</td>
<td>10.0%</td>
<td>5.9%</td>
<td>45.7%</td>
<td>64.7%</td>
<td>60.2%</td>
<td>78.5%</td>
<td>45.6%</td>
</tr>
<tr>
<td>$P$ (Fisher test)</td>
<td>4.E–04</td>
<td>8.E–04</td>
<td>0.016</td>
<td>0.085</td>
<td>0.28</td>
<td>0.3</td>
<td>0.8</td>
<td>1</td>
</tr>
<tr>
<td>$q$ (FDR)</td>
<td>0.0012</td>
<td>0.0024</td>
<td>0.048</td>
<td>0.25</td>
<td>0.84</td>
<td>0.9</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 2.** Molecular alterations in IDH wild-type glioma harboring FGFR3–TACC3 fusions.

The table reports the absolute number and frequency (percentage) of individual glioma-specific molecular alterations in tumors scoring positive or negative for FGFR3–TACC3 fusions. The analysis is done on the Union dataset (TCGA and “Pitié-Salpêtrière Hospital” datasets, see Materials and Methods for details).

Statistically significant associations are indicated in bold (Fisher exact test, $q$ values adjusted with FDR).
parenchyma (~44%) with formation of a cystic portion in the central core (33). The objective response was further corroborated by the marked reduction of the extent of tumor vascularity estimated by quantitative analysis of rCBV (relative cerebral blood volume) from dynamic susceptibility MR perfusion maps (37) (Fig. 4D). Stabilization lasted for 115 days. During JNJ-42756493 treatment, mild and manageable toxicity was observed (grade I hyperphosphatemia, asthenia, dysgueusia, dry mouth, keratitis, and grade II nail changes). After 4 months, tumor progressed on MRI locally both on T1 contrast-enhanced area and T2/FLAIR hypersignal. The patient was reoperated and subsequently treated with CCNU. He is still alive, but in progression after 21 months from diagnosis and 287 days from the start of the anti-FGFR therapy.

Patient 2 is a 64-year-old woman, affected by left parietal GBM, diagnosed by stereotactic biopsy. The tumor was positive for FGFR3–TACC3 gene fusion by RT-PCR sequencing and showed diffuse FGFR3 expression in most tumor cells (Fig. 2A, C and E, sample 4620). The patient received as first-line treatment fractionated radiotherapy and temozolomide according to the Stupp protocol (36), but after 2 cycles of monthly temozolomide, she presented with clinical deterioration including progressive headaches, visual field defect, and memory impairment. Brain MRI performed 3 and 4 months after the completion of concomitant chemoradiation revealed tumor progression with increase of the left parietal mass and the appearance of a small contralateral lesion (Fig. 4E). The patient was thus enrolled in the JNJ-42756493 trial (12 mg/d administered in cycles of 7 days followed by 7 days off-treatment) and showed clinical improvement after 4 weeks (regression of headaches, visual field defect, and memory impairment). Best response was observed after 104 days of treatment with a 22% reduction of tumor size according to the RANO criteria (Fig. 4F) and 28% according to volumetry (Fig. 4G). Grade I hyperphosphatemia, nail changes, and mucositis were observed. Clinical status remained stable until disease progression occurring 134 days after the start of the anti-FGFR therapy. The patient is still alive and is receiving a third-line chemotherapy with nitrosoureas and bevacizumab.

Discussion

FGFR–TACC fusions are potent oncogenic events that when present in brain tumor cells confer sensitivity to FGFR inhibitors (6). Since our original identification of recurrent FGFR–TACC fusions in GBM, small subgroups of patients harboring FGFR–TACC translocations have been identified in several other tumor types (7–15). Here, we report an unbiased RT-PCR-sequencing analysis for the identification of all possible functional FGFR–TACC fusion transcripts. The screening of a large glioma dataset from multiple institutions not only confirmed that FGFR–TACC...
FGFR–TACC Identification and Inhibition in Glioma Patients

Figure 4.
Baseline and posttreatment MRI of patients treated with JNJ-42756493. Patient 1 (A–D). A, post-gadolinium T1-weighted images show the target lesion on the right parietal lobe. The interval (days) from the beginning of follow-up is indicated above each MRI. B, analysis of sum of product diameters (SPD) before and during the anti-FGFR treatment (RANO criteria). C, analysis of tumor volume (cm³) before and during the anti-FGFR treatment. During anti-FGFR treatment, a stabilization of the tumor was observed according to RANO criteria and volumetry. D, perfusion images at baseline and after 20 days of anti-FGFR treatment. rCBV post-gadolinium T1-weighted images with color overlay of rCBV are shown. Patient 2 (E–G). E, two different MRI slice levels of superior and middle part of the lesion are presented. F, analysis of SPD before and during the anti-FGFR treatment. During the anti-FGFR treatment, a reduction of 22% of tumor size was observed. G, volumetric evaluation showed a 28% tumor reduction. Vertical red arrow indicates the start of anti-FGFR treatment (baseline).

rare rearrangements occur in about 3% of human GBM but also revealed that FGFR–TACC fusions are present in the subgroup of IDH wild-type lower grade glioma (grades II–III) with prevalence similar to that of GBM. IDH wild-type grade II and III glioma have a significantly worse clinical outcome than the IDH-mutant glioma and manifests molecular and clinical features that resemble GBM (5). Our finding that FGFR–TACC fusions occur in IDH wild-type but not in IDH-mutant glioma provides an important clue for the molecular characterization of this glioma subtype. Furthermore, the clustering of such potent oncogenic events in IDH wild-type glioma underscores the particularly aggressive nature of this group of glioma. While we showed that FGFR–TACC fusions cluster within the poor clinical outcome subgroup of IDH wild-type glioma, these translocations do not seem to carry prognostic value within the IDH wild-type subgroup of patients with glioma. However, the sample size of patients harboring FGFR–TACC fusions is too small to draw definitive conclusions with respect to the impact on survival, and larger studies will be necessary to clarify the prognostic role of FGFR–TACC fusions in IDH wild-type glioma.

Besides mutual exclusivity between IDH1 mutations and FGFR–TACC fusions, our results showed that patients with FGFR3–TACC3 rearrangements lack EGF amplification and EGFRvIII but are significantly enriched for amplification of CDK4 (and MDM2 to a lesser extent). Knowledge of these molecular characteristics will help select those patients who most likely harbor FGFR–TACC rearrangements and design combinatorial targeted therapies that might be more effective in the FGFR–TACC-positive glioma subgroup.

The molecular screen uncovered 6 new FGFR3–TACC3 fusion events. Together with the previously identified variants, others and we have reported 12 distinct isoforms of FGFR3–TACC3, thus revealing a remarkable variability of FGFR3–TACC3 transcripts in human cancer (see Supplementary Table S1 summarizing the structure of all the FGFR–TACC variants identified to date). The structural heterogeneity of FGFR3–TACC3 fusions is yet more pronounced at the genomic level, whereby each fusion event harbors distinct genomic breakpoints, even for identical fusion transcripts. This finding underscores the notion that targeted genomic analyses are unlikely to be suitable approaches for the molecular diagnosis of FGFR3–TACC3 positivity. Conversely, the unbiased identification of FGFR3–TACC3-positive tumors with the RT-PCR sequencing assay reported here overcomes the limitations of screening only for previously identified FGFR3–TACC3 fusions and provides a simple molecular diagnostic assay.

Rather than displaying uniform amplifications of the FGFR3 and TACC3 genomic loci, FGFR3–TACC3–positive samples harbor small, intragenic microamplifications events typically encompassing only the exons of the FGFR3 and TACC3 genes included in the breakpoint (6). This finding is consistent with the notion that a "fusion breakpoint principle" sustains the CNVs of driver gene fusions such as FGFR3–TACC3 in which local CNVs target exclusively the breakpoint region (38). We note that such small and irregular CNVs may easily go undetected from CNV analyses performed using platforms less-sensitive than the high-density SNP6.0 genomic arrays. Furthermore, the notion that FGFR3–TACC3-negative GBM may harbor uniform amplifications across
the FGFR3 and TACC3 loci argues against the standard analysis of FGFR3 and/or TACC3 CNVs as a method for the selection of FGFR3–TACC3–positive tumors.

There is a growing body of evidence supporting the notion that GBM is a markedly heterogeneous tumor. The formidable degree of intratumor heterogeneity of GBM is a potential cause of failure of targeted therapies in these tumors. In particular, the intratumor heterogeneity of GBM has previously been recognized in light of the mosaic expression of the RTK genes FGFR, PDGFR, and MET by neighboring cells (16–19). Thus, in the majority of GBM, amplification or overexpression of individual RTK genes are present in a subclonal fraction of tumor cells and co-exist with amplification/expression of other RTK-coding genes within the tumor mass. Therefore, it was essential to determine whether such heterogeneity was also present in gliomas harboring FGFR–TACC translocations. The immunostaining of FGFR3–TACC3–positive tumors revealed that positive specimens manifest strong and uniform expression of the fusion protein, which is also retained after recurrence. This behavior is reminiscent of other driver chromosome translocations (BCR-ABL, EMLA-ALK) and is compatible with the glioma-initiating functions of FGFR–TACC fusions (6). It is also the scenario expected for a driver oncogene whose activity remains essential for tumor maintenance regardless of secondary genetic alterations that occur during tumor progression.

The strong antitumor effects obtained with INI-42756493 in glioma cells harboring FGFR3–TACC3 fusions have built a compelling rationale for the treatment of patients with glioma positive for FGFR–TACC rearrangements. INI-42756493 is an oral ATP-competitive pan-FGFR selective inhibitor that inhibits tyrosine phosphorylation of activated FGFR at nanomolar concentrations (34, 35). The enrollment of 2 patients with recurrent FGFR3–TACC3–positive GBM in a phase I trial with INI-42756493 showed that this treatment has tolerable toxicity and clear antitumor activity, thus validating FGFR–TACC as a therapeutic target. Therefore, targeted inhibition of FGFR-TK in preselected IDH wild-type FGFR–TACC–positive glioma may provide clinical benefits for patients with recurrent glioma who currently lack valuable therapeutic options. In conclusion, we have shown the importance and feasibility of prospective genotyping for FGFR–TACC fusions in patients with glioma and provided a preliminary evidence of clinical response that warrants the investigation of the sensitivity of gliomas harboring FGFR–TACC rearrangements to FGFR kinase inhibition in clinical trials.

Disclosure of Potential Conflicts of Interest

J. Savatovsky reports receiving speakers bureau honoraria from Bayer and Philips Healthcare. J.-C. Soria and J. Tabernero are consultant/advisory board members for Johnson & Johnson. No potential conflicts of interest were disclosed by the other authors.

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Writing, review, and/or revision of the manuscript: A.L. Di Stefano, A. Fucci, P. Zoppoli, M. Touat, F.R. Luo, J.-C. Soria, J. Tabernero, G. Finocchiaro, A. Lasorella, M. Sanson, A. Iavarone

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.L. Di Stefano, M. Labussiere, M. Gay, R. Patiera, A. Lasorella

Study supervision: A. Lasorella, M. Sanson, A. Iavarone

Other (histologic diagnosis and immunohistochemical analysis of the tumoral samples): K. Mokhtari

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References


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