Growth Factor Receptor Fusions Predict Therapeutic Sensitivity

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**Running Title:** Fusions Genes Predict Therapeutic Sensitivity

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Summary

Dysregulated growth factor pathways promote tumor growth in many cancers, but receptor-targeting strategies frequently offer limited benefit despite activation by receptor overexpression or amplification. In contrast, tumors harboring growth factor receptor fusions display exquisite dependence on receptor activity, providing predictive markers for patient response to inform precise oncology treatment.
In this issue of *Clinical Cancer Research*, Di Stefano and colleagues discuss the use of RT-PCR-sequencing as a sensitive and specific method to identify FGFR-TACC fusion genes in patients with grade II–IV gliomas (1). In their study, FGFR3-TACC3-positive patients treated with a FGFR inhibitor derive clinical benefit.

Theodor Boveri proposed that acquired chromosomal abnormalities have an important role in the initiation of carcinogenesis a century ago (2). Growth factors promote a myriad of potentially oncogenic activities so it is not surprising that growth factor receptors and downstream effectors are commonly altered in cancers through transcriptional upregulation, gene amplification, activating mutation, and genomic fusion events. Fusion genes are generated when two previously separate genes rearrange and fuse together resulting in a hybrid gene. The first fusion gene described in cancer cells was the Philadelphia chromosome in chronic myeloid leukemia (CML) discovered in 1960 as a result of the work of Nowell and Hungerford (3). Four decades later, the extraordinary activity of imatinib against CML harboring the BCR–ABL1 fusion exemplified precision targeting of genetic targets as an effective therapeutic approach in oncology (4). In solid tumors, the EML4–ALK fusion reported in 4–7% of lung cancers has been successfully targeted by crizotinib and ceritinib (5).

The extension of imatinib activity to gastrointestinal stromal tumors, which harbor constitutive activation of the KIT receptor tyrosine kinase, appeared to confirm the broad potential for sustained tumor control with targeted therapeutics (6). Unfortunately, sustained responses to kinase inhibitors have often proved the exception, rather than the rule. Epidermal growth factor receptors (EGFR) are altered in the majority of glioblastoma, the most prevalent primary intrinsic brain tumor, but EGFR antagonists have repeatedly failed in clinical trials (7). Even when a promising mutational signature appeared to predict success (8), further studies
failed to validate EGFR inhibitor efficacy in glioblastoma (9). Failure of EGFR antagonists and numerous other kinase inhibitors in glioblastoma has tempered enthusiasm for receptor targeting approaches until a new wave of discoveries of novel fusion events.

Singh and colleagues first reported in-frame fusions of the tyrosine kinase coding domains of fibroblast growth factor receptor (FGFR) genes (FGFR1 or FGFR3) to transforming acidic coiled-coil (TACC) coding domains of TACC1 or TACC3 in 3% of glioblastoma (Fig. 1) (10). A flurry of reports has demonstrated FGFR family fusions in bladder, lung, breast, thyroid, oral, and prostate cancer. In in vivo model, oral administration of an FGFR inhibitor resulted in prolonged survival of mice harboring intracranial FGFR3-TACC3-initiated glioma. Hence, a subset of glioblastoma patients that harbor the FGFR-TACC fusions could derive benefit from targeted FGFR kinase inhibition. FGFR inhibitors for FGFR3–TACC3-positive glioblastomas may have large therapeutic indices due to the relatively low levels of wild-type FGFR3 within the brain. FGFR3 inhibitors would presumably target only the neoplastic compartment expressing FGFR3–TACC3, while sparing normal healthy tissues.

The precise application of FGFR targeting for patients with the relevant fusions will require rapid and accurate detection of these fusion events. Selection of methods utilized to diagnose the fusion genes is informed by the type of fusion. Fusion genes resulting from translocations are usually diagnosed by fluorescence in situ hybridization (FISH), detecting abnormal chromosomes within the cell, e.g. BCR–ABL1 fusions in chronic CML. Microarray techniques are also used to diagnose fusion genes that arise as a result in overexpression of one of the fusion partners for e.g. TMPRSS2–ERG fusions in prostate cancer (11). Next-generation whole transcriptome sequencing aids in diagnosis of fusions genes by analyzing the different reads from fusion gene as compared with normal tissue. Polymerase chain reaction (PCR) with
primers flanking the fusion junction followed by sequencing informs the diagnosis of fusion gene if both fusion partner genes are present on analysis. In the current issue, Stefano and colleagues describe the detection, characterization and inhibition of FGFR-TACC fusions in glioma with wild type isocitrate dehydrogenase 1 (IDH1). Due to the close proximity of FGFR3 and TACC3 on chromosome 4p16.3, FISH detection of FGFR3-TACC3 rearrangements is not optimal using currently available methods. The authors developed an RT-PCR assay to identify the known and possibly novel 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. Critically, confirmation of the inframe breakpoint was performed by Sanger sequencing.

Three of 85 wild type IDH1/2 grade II-III gliomas (3.5%) harbored FGFR3-TACC3 fusions; however, none of 126 IDH1/2 mutant tumor samples demonstrated evidence of FGFR3-TACC3 fusions. Seventeen of the 584 glioblastoma (2.9%) harbored FGFR-TACC rearrangements. These findings are consistent with the 3% incidence of FGFR-TACC rearrangements in glioblastoma described earlier (10). IDH wild type grade II-III glioma (diffuse glioma and anaplastic glioma) have similar prevalence of FGFR-TACC fusions to that of glioblastoma, suggesting an early mutation consistent with a tumor driver. The authors report considerable structural variability among FGFR3-TACC3 fusion isoforms, with five of the identified variants occurred only in individual cases. In addition, the investigators identified 6 new fusion transcripts that have not been reported before. Collectively, these results suggest that FGFR fusions may serve as early tumor drivers in the absence of IDH1 mutations and that the direct contribution TACC to oncogenic function may be modest.
Recent work from the comprehensive analysis of 293 grades II and III gliomas using multiple genomic and proteomic platforms from the Cancer Genome Atlas (TCGA) reported three super clusters of these tumors (12). Group 1 tumors are wild type for IDH1/IDH2; group two tumors are IDH1/IDH2 mutant with chromosome 1p/19q intact; and group three harbors IDH1/IDH2 mutations with co-deletion of chromosome 1p/19q. The IDH wild type group of grade II or II gliomas have a glioblastoma-like phenotype: focal gains of EGFR, CDK4 and MDM4, mutations in NF1, EGFR and PTEN, and a poor median survival, compared to those who harbor IDH1/IDH2 mutations. Stefano and colleagues also report that FGFR-TACC rearrangements are mutually exclusive with IDH1/2 mutations and EGFR amplification whereas co-occur with CDK4 amplification and MDM2 to a lesser extent. In this era of precision medicine and targeted therapy, knowledge of these molecular characteristics will help enrich the future trials with patients who harbor appropriate mutations and that are likely to derive benefit from agents targeting the mutation.

In the current study, the authors further describe clinical experience of targeting two patients with fusion gene positive patients with JNJ-42756493, a FGFR1, 2, 3 and 4 inhibitor that demonstrated clinical benefit. This preliminary clinical activity supports further evaluation of FGFR inhibition in FGFR-TACC-positive patients. Previous trials of FGFR inhibitors such as ninetanib (13) or dovitinib (M.S. Ahluwalia; unpublished data) in unselected recurrent glioblastoma did not demonstrate meaningful clinical activity. However, an ongoing Phase 2 Study of BGJ398 in recurrent glioblastoma enriches patients that harbor amplification, translocation, or activating mutation in FGFR1, 2, 3 or 4 (NCT01975701) and may address the clinical utility of these agents in this patient population. Whether the success in targeting BCR–ABL1 in leukemia and in the EML4–ALK fusion in lung cancer will be replicated for the FGFR
inhibitors in gliomas with FGFR fusions remains to be determined, but new hope for targeted therapies against growth factor pathways is emerging for these tumors.

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**References**


Figure 1. Downstream impact of FGFR-TACC fusion oncogene
Coiled coil

Downstream altered regulation of ERK and STAT3 pathway

Loss of microRNA regulation

Constitutive FGFR kinase leading to chromosomal segregation errors such as chromosomal instability or aneuploidy

Cell proliferation and prevention of apoptosis

miR99a fails to regulate expression
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