Dasatinib in recurrent glioblastoma: failure as a teacher

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The Radiation Therapy Oncology Group (RTOG) trial 0627 evaluated the efficacy of dasatinib in recurrent glioblastoma. Why was dasatinib, of unequivocal benefit in Philadelphia chromosome–positive leukemia and so promising in gastrointestinal stromal tumor, of interest in glioblastoma? And why did the drug fail?

Dasatinib is a potent oral multitargeted kinase inhibitor that inhibits all members of the Src family of kinases (SFK). At higher concentrations, dasatinib also inhibits BCR-Abl (breakpoint cluster region–Abelson murine leukemia), c-kit, platelet-derived growth factor receptor (PDGFR)–beta, and ephrin receptors. Among these other potential targets of dasatinib, negative clinical trials with imatinib have called into question the utility of attacking c-kit and PDGFR. In contrast, considerable evidence supports a central role of Src and other SFKs in glioblastoma.

As the cellular form of the Rous sarcoma virus, Src represents the first discovered human proto-oncogene. Ironically, Src is probably not a causative agent in human tumorigenesis; activating mutations in human tumors are extremely rare, and Src alone is insufficient to transform human cells. Rather, Src is thought to maintain the neoplastic phenotype and to play a role in tumor progression.

Src family members are non-receptor tyrosine kinases that function at the interface between extracellular signals and intracellular signaling pathways. Src is normally held in an inactive conformation through intramolecular interaction of the Src homology-2 (SH2) domain with phospho-Y527. Activation of Src occurs in conjunction with dephosphorylation of this residue and binding of the SH2 domain to alternate phospho-tyrosine residues on the cytoplasmic portion of activated receptor tyrosine kinases (including epidermal growth factor receptor [EGFR], PDGFR, insulin-like growth factor receptor, fibroblast growth factor receptor, c-met, and vascular endothelial growth factor receptor [VEGFR]) or other target phosphorylated transmembrane proteins such as integrins or ephrins. Auto-phosphorylation of Y416 within the kinase domain further contributes to kinase activation. Activated Src signaling has pleiotropic functions within the cell. Src functions in a complex with focal adhesion kinase (FAK) and proline-rich tyrosine kinase 2 to modulate cytoskeletal organization and remodeling accompanying adhesion, motility, invasion, and cell division.

By activating the pathways of mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase, Src and Lyn also promote cell proliferation and activate anti-apoptotic or prosurvival pathways. The relationship with receptor tyrosine kinases is bidirectional and synergistic; Src can phosphorylate EGFR and PDGFR, leading to increased MAPK pathway activity and enhanced mitogenesis and transformation. Under hypoxic conditions, Src activation promotes angiogenesis through stimulation of VEGF, matrix metalloproteinases, and interleukin-8 expression. Through phosphorylation of signal transducer and activator of transcription 3, Src activity induces VEGF expression. Downstream from the VEGF receptor, Src signaling is critical for FAK activation and VEGF-mediated disruption of tight junction and adherens junction integrity that contribute to a physical blood–brain barrier (BBB). Thus, SFKs play a central role in signaling downstream from various receptor tyrosine kinases and other signaling pathways that are critical both for normal cell and tumor cell growth, motility, and survival.

Preclinical and human tumor studies support a potentially important role of Src in human glioblastoma. Transgenic mice expressing v-Src develop glioblastomas. Bead technology assessing tyrosine kinase phosphorylation demonstrated Src kinase activation in glioblastoma cell lines as well as in primary patient samples. This latter result is consistent with common molecular alternations in glioblastoma multiforme (GBM) that drive Src activation, such as amplification of EGFR and PDGFR and upregulation of integrin receptors such as v3 and v5.

The Src family member Lyn kinase activity is very high in human GBM and is thought to promote migration. Based on the role for SFKs in glioma biology, there is a strong rationale to pursue SFK inhibitors such as dasatinib in GBM. Preclinical studies have demonstrated reproducible suppression of various phosphorylation targets. Dasatinib reduces autophosphorylation of Src and downstream signaling to Akt and S6 in GBM cell lines and reduces GBM cell growth and invasion. SFK inhibition by dasatinib also induces autophagic cell death in vitro in GBM. In U87 heterotopic flank tumors, dasatinib therapy was associated with a marked suppression of integrin membrane localization and suppression of tumor growth. Although no direct clinical data were available at the inception of RTOG...
RTOG 0627 targeted bevacizumab-naïve patients at first progression of glioblastoma following radiation therapy and temozolomide. The study attempted to enrich for sensitive tumors by requiring subjects’ initial glioblastoma specimen to stain strongly for at least 2 potential targets of dasatinib, including phospho-Src, PDGF-R, EphA2, and c-kit; 60% of enrolled patients had tumors that overexpressed activated Src. Moreover, the dasatinib dose for the first patient cohort was 200 mg daily, exceeding the FDA-approved dose of 100–140 mg daily. When unexpectedly little toxicity was seen, a second cohort starting at 200 mg daily allowed intra-patient dose escalation upon subsequent cycles. Despite these measures, dasatinib showed a resounding lack of activity against recurrent glioblastoma in this trial.

Several potential factors may account for this failure. Phase II single-agent studies of Src inhibitors in numerous solid tumors have yielded discouraging results, so Src may not be a critical signaling pathway, or there may be parallel signaling pathways that compensate for loss of Src activity. Alternatively, the enrichment strategy used was based on biomarker immuno-histochemistry assays that were selected somewhat empirically based on an understanding of Src signaling biology. However, given the complexity of the signaling networks governed by Src, this strategy may have failed to truly enrich for patient tumors that are highly dependent on SFK signaling. Moreover, the evaluation of tumor samples obtained at initial diagnosis may not adequately reflect the signaling biology in tumors that have been heavily irradiated and treated with temozolomide, both of which can be highly mutagenic. Thus, despite laudable attempts by the investigators to enrich for tumors most likely to respond to dasatinib, their ability to accurately define a relevant predictive biomarker may have been lacking.

Another important possibility to consider is that dasatinib is an active agent but was not adequately delivered to these recurrent brain tumors. In vitro and in vivo studies have demonstrated that dasatinib is a substrate for the efflux transporters p-glycoprotein and breast cancer resistance protein (BCRP), which are critical modulators of the biochemical BBB. In conjunction with a sleeping beauty transposon-generated, PDGF-driven genetically engineered mouse glioblastoma model, the area under the curve for exposure of dasatinib within the brain was increased 7-fold in knockout mice lacking both p-glycoprotein and BCRP expression. The increase in dasatinib exposure was particularly prominent in brain around the tumor, and dasatinib therapy was significantly more effective both in suppressing Src activation and in prolonging survival of those tumor-bearing mice with a defective BBB lacking the efflux transporters compared with wild-type mice. The design of RTOG 0627, and in fact all previous and ongoing studies of dasatinib in glioblastoma, did not provide for an assessment of whether dasatinib partitions across the BBB in glioblastoma at a sufficient concentration to inhibit target pathways. This systematic failure highlights the necessity to perform such “treat-biopsy-treat” or phase 0 studies before committing resources and patients in large studies.

As the study authors note, previous phase II studies in recurrent glioblastoma of dasatinib with erlotinib and with lomustine failed to show activity. The results of randomized phase II trials adding dasatinib to radiation plus temozolomide (Alliance N0877, NCT00869401) in newly diagnosed disease and to bevacizumab in recurrent disease (Alliance N0872, NCT00892177) are anticipated shortly. If these studies prove negative as well, they will likely represent the death knell of dasatinib in glioblastoma.

References

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