Response to Combined Molecular Targeting: Defining the Role of P-STAT3

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Abstract

Src family kinase (SFK)–targeting agents are currently undergoing clinical investigation for treatment of solid malignancies. Epidermal growth factor receptor (EGFR)–independent phosphorylation of STAT3 (P-STAT3) has been identified as a mechanism of tumor resistance to agents targeting SFK. Tumor P-STAT3 levels may be an important indicator of EGFR- and SFK-targeted antitumor treatment efficacy. Clin Cancer Res; 17(3); 1–3. ©2011 AACR.

In this issue of Clinical Cancer Research (CCR), Nagaraj and colleagues use preclinical models of pancreatic cancer to evaluate the antitumor efficacy of combining dasatinib with a U.S. Food and Drug Administration (FDA)–approved, targeted therapy treatment for unresectable or metastatic pancreatic cancer, erlotinib plus gemcitabine (1). Epidermal growth factor receptor (EGFR)–targeted therapies have been approved for the treatment of several cancers, including erlotinib (Genetech and OSI Pharmaceuticals), a small molecule tyrosine kinase inhibitor (TKI) of EGFR, as a monotherapy for non–small cell lung cancer (NSCLC) and in combination with gemcitabine (Eli Lilly and Company), a deoxycytidine analog, for pancreatic cancer. Gefinitib (AstraZeneca), also an EGFR TKI, was FDA-approved for treatment of NSCLC with revised labeling restricting indications for use. The EGFR-targeted chimeric monoclonal antibody cetuximab (Imclone and Bristol-Myers Squibb) has been FDA-approved for treatment of head and neck squamous cell carcinoma (HNSCC). Cetuximab and the fully human anti-EGFR antibody panitumumab (Amgen) are both FDA approved for the treatment of colorectal cancer xenograft models. From a panel of 9 pancreas cancer cell lines, the EGFR- and SFK-targeted therapies have been approved for the treatment of colorectal cancers. Except for the subpopulation of NSCLC tumors harboring EGFR-activating mutations in which tumor response to EGFR kinase inhibitors has been impressive, the clinical response rates to EGFR targeting have, in general, been modest.

Dasatinib (Bristol-Myers Squibb) is a spectrum kinase inhibitor of Src family kinases (SKF), EGFR, and several other kinases (2). SFKs are overexpressed and activated in many cancers, augment EGFR signaling, and mediate the activation of many pathways important in tumorogenesis and progression (Fig. 1). In addition to activation by EGFR, SFKs mediate signaling pathway activation by other receptor tyrosine kinases, G-protein–coupled receptors, and integrins. Because SFKs are central to cancer, and SFK inhibitors with acceptable toxicities are currently in clinical development, studies evaluating the antitumor efficacy of SFK inhibitors alone and in combination with other targeted therapies are ongoing. Of the SFK-targeting agents studied to date for treatment of solid malignancies, dasatinib, which has been FDA-approved for treatment of chronic myelogenous leukemia and acute lymphoblastic leukemia, is the most developed.

Sustained inhibition of SFK by dasatinib or specific knock-down of c-Src has been shown to result in Janus activated kinase (JAK)–dependent phosphorylation of STAT3 (P-STAT3) in NSCLC and HNSCC preclinical models (3, 4). In these cell lines, the EGFR- and SFK-independent, JAK-dependent phosphorylation of STAT3 provided a mechanism for the activation of signaling pathways important to cancer progression, even in the presence of EGFR and SFK blockade (Fig. 1). Similarly, Nagaraj and colleagues previously showed that STAT3 phosphorylation in the presence of dasatinib was associated with resistance to dasatinib in pancreatic cancer models, although the mechanism of sustained STAT3 phosphorylation was not defined (5). Importantly, in this issue of CCR, Nagaraj and colleagues show that the triple combination of dasatinib, erlotinib, and gemcitabine reduced levels of P-STAT3 in pancreatic cancer xenograft models. From a panel of 9 pancreas cancer cell lines, in vitro and in vivo data were presented primarily for 2 cell lines: the PANC1 cell line, which had the highest erlotinib and gemcitabine IC-50s (1,118 nM and 100.2 nM, respectively), the second highest dasatinib IC-50 (45.7 nM), and the BxPC3 cell line, which was most sensitive to erlotinib and dasatinib with IC-50 values of 99.7 nM and 2.8 nM, respectively, and intermediate sensitive to gemcitabine (IC-50 30.2 nM). Although these cell lines represented the extremes of the panel, both exhibited reduced in vitro cell migration in the presence of dasatinib, erlotinib, and gemcitabine compared with any single agent or
combination of 2 agents. Both cell lines also exhibited significant reduction in xenograft tumor growth with the triple agent combination compared with single or dual agent treatment and a corresponding reduction in P-STAT3 tumor levels. However, in vitro cell viability assays following single or combined agent treatments showed no statistically significant reduction in cell viability with the triple combination compared with dual agent treatment. These data are an example of the incompletely understood discordance between in vitro cell viability and in vivo xenograft tumor growth, suggesting the limitations of preclinical models.

Although the combination of erlotinib, dasatinib, and gemcitabine resulted in reduced xenograft tumor volumes for both the sensitive and insensitive cell lines, the mechanism of action of was largely undefined. Image analysis of the xenograft tumors indicated that pAKT, pSFK, Ki67, and caspase 3 levels in both xenograft tumor types were not altered from dasatinib-erlotinib treatment levels by the addition of gemcitabine, yet P-STAT3 levels in both tumor types were markedly reduced following the triple agent treatment compared with single or any dual agent treatment. Given the pleiotropic actions of gemcitabine, it is difficult to speculate about the precise mechanism of P-STAT3 abrogation. However, STAT3 is activated and, hence, tyrosine phosphorylated downstream of both EGFR and SFKs. Thus, dual inhibition of EGFR and SFK provides more potent blockade of STAT3 activation. In HNSCC, the activation of STAT3 following knockdown of c-Src with small interfering RNA (siRNA) has been reported to be JAK dependent and result from reduced suppressor of cytokine signaling 2 (SOCS2) expression (6). Whether the activation of STAT3 following dasatinib treatment in pancreatic cancer cells results from the same alterations in JAK and SOCS2 activities is unknown. Like many malignancies, pancreatic cancers have been reported to be genetically heterogeneous (7). The mechanisms contributing to STAT3 activation and the ability of combined treatment with erlotinib, dasatinib, and gemcitabine to reduce P-STAT3 may contribute to the success of this therapeutic strategy across heterogeneous pancreatic cancers. It is possible that the addition of gemcitabine to EGFR and SFK cotargeting may have utility for treatment of other cancers in which treatments combining EGFR- and SFK-targeting agents are being clinically evaluated, including NSCLC, HNSCC, and colorectal cancers. More generally, STAT3 phosphorylation has been identified as a mechanism of resistance to SFK-targeted therapies, but the precise contribution of STAT3 phosphorylation and/or activation to cancer therapy resistance remains unknown.

As has often been the case with targeted therapies, heterogeneity of sensitivities to the 3 agents was observed among the panel of cell lines evaluated by Nagaraj and colleagues, but no identified correlation with baseline levels of c-Src, EGFR, or their phosphorylated forms was observed. In clinical trials to date combining erlotinib with gemcitabine for the treatment of pancreatic cancer, neither tumor EGFR gene amplification nor KRAS mutation status was found to be associated with treatment response to combined erlotinib-gemcitabine treatment (8, 9), and pancreatic cancers have been reported to very rarely harbor erlotinib-sensitizing EGFR-activating mutations (8). Although tumor biomarkers associated with response and/or resistance to EGFR-targeted therapies have been identified for NSCLC and colorectal cancers (10), no biomarker has yet been identified to be associated with response to SFK-targeting agents in patients with any solid tumor (11).

Clinical trials evaluating combined EGFR- and SFK-targeting agents are currently in phase I-II. One phase I-II clinical study evaluating dasatinib with erlotinib for treatment of 34 NSCLC patients reported 2 partial responses in which this regimen was tolerated (12). Two phase I-II trials combining SFK- and EGFR-targeting agents are currently
ongoing (1): combining dasatinib with erlotinib for NSCLC and (2) combining dasatinib with cetuximab and radiation with or without cisplatin for patients with locally advanced HNSCC. However, there is a need to prospectively define responsive patient subpopulations. P-STAT3 levels in early post-treatment tumor biopsies have potential as a predictive biomarker, but these correlative studies may be difficult even for accessible tumors given the short biological half-life of dasatinib. More importantly, the response rates with combined EGFR- and SFK-targeted treatment in the NSCLC phase I-II study were modest. Identifying agents that abrogate P-STAT3 following combined EGFR- and SFK-targeted agents, such as gemcitabine in pancreatic preclinical models, may improve upon these response rates.

Disclosure of Potential Conflicts of Interest

A.M. Egloff and J.R. Grandis: commercial research grant, Bristol Myers-Squibb.

Received November 23, 2010; accepted December 7, 2010; published OnlineFirst January 25, 2011.

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Clin Cancer Res  Published OnlineFirst January 25, 2011.

Updated version  Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-10-2925

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