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Dual PI3K/mTOR Inhibitors: Does p53 Modulate Response?

Oleksandr Ekshyyan, Arunkumar Anandharaj, and Cherie-Ann O. Nathan

Head and neck squamous cell carcinomas have multiple genetic alterations that can influence clinical response to treatment. It is important to evaluate how distinct alterations affect response to targeted agents to identify a subset of patients who can benefit from therapy, improving survival and decreasing toxicity. *Clin Cancer Res; 19(14); 3719–21. ©2013 AACR.*

In this issue of *Clinical Cancer Research*, Herzog and colleagues present data on testing novel dual phosphoinositide 3-kinase (PI3K)/mTOR inhibitor PF-04691502 in preclinical models of head and neck squamous cell carcinoma (HNSCC; ref. 1). Mutational analysis of head and neck cancer revealed a pattern of genes that are frequently altered in their expression. Inactivating mutations of *TP53*, *CDKN2A*, and *NOTCH1* and activating mutations of *PIK3CA* and *HRAS* oncogenes notably contribute to the tumorigenesis of HNSCC that has been studied extensively for the past 20 years (2). Approximately 30% of HNSCC exhibit reduced PTEN expression, which then additionally promotes AKT activation. Thus, PI3K and its downstream proteins, AKT and mTOR, are attractive targets for small-molecule drug discovery. On the basis of the type of regulation, structure, and substrate specificity, PI3K is classified into four different subfamilies: class I, II, III, and IV. PI3K and mTOR fall under class IV (3). Lead optimization strategies like structure-based drug design and physical properties–based optimization paved the way for the discovery of 2-amino-8-[*trans*-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxypyridin-3-yl)-4-methylpyrido[2,3-d]pyrimidin-7(8H)-one(PF-04691502) from Pfizer (4). It showed a profound inhibitory effect on the kinase activity of both PI3K and mTOR *in vitro* as well as in mouse tumor xenograft studies *in vivo*.

In vitro biochemical assays showed that PF-04691502 effectively inhibited recombinant PI3Kα, β, γ, and δ isoforms with a K_i of 1.2 to 2.2 nmol/L and recombinant mTOR with a K_i of 9.1 nmol/L. PF-04691502 inhibited PI3K/mTOR signaling in SKOV3 ovarian cancer cells with *PIK3CA* mutations and in U87MG glioblastoma cells with altered PTEN expression. Xenograft studies with the aforementioned cell lines resulted in dose-dependent tumor growth inhibition up to 70%. PF-04691502 exhibited reduced phosphorylation of AKT and S6 ribosomal protein (S6RP) in a non–small cell lung cancer (NSCLC) cell line harboring *PIK3CA* or *EGFR* mutations. In addition, the drug was efficient in exhibiting antiproliferative activity of an erlotinib-resistant line, NCI-H1975, and these results were reproducible in xenograft studies as well (5). Britten and colleagues (6) evaluated the efficacy of PF-04691502 in a panel of 22 breast cancer cell lines harboring *PIK3CA* mutations. The cell lines achieved IC_{50} values of less than 100 nmol/L and exhibited inhibition of p-AKT.

The therapeutic response to PF-04691502 using an orthotopic primary transplant model in mice showed 72% inhibition of tumor growth. Animal imaging studies using FDG–PET (2[18F]fluoro-2-deoxy-D-glucose–positron emission tomography) revealed a drastic reduction in glucose metabolism in addition to decreased expression of tissue biomarkers p-AKT and S6RP. On the other hand, as a single agent, PF-04691502 did not induce tumor regression in ovarian cancer harboring *KRAS* mutations. However, tumor regression was achieved after combining PF-04691502 with a MEK inhibitor, suggesting that abrogation of PI3K/mTOR signaling may be overcome by upregulation of the mitogen-activated protein kinase pathway downstream of Kras (7). PF-04691502 treatment of MDA-MB-231 cells resulted in reduced levels of p27-kip1 protein. Pretreatment with the drug in an *in vivo* model significantly impaired tumor metastasis (8). Given its effectiveness in preclinical models, the first-in-patient study of PF-04691502 recruited 30 patients with diagnoses including colorectal cancer, NSCLC, and breast cancer. Analysis of the tumor biopsies of the subjects exposed mutations in *PIK3CA*, and for a few patients, PTEN loss was noticed. Patients were administered orally with 2, 4, 8, and 11 mg of PF-0491501 every day. PF-0491502 has been well tolerated in spite of common treatment-related adverse effects. Preliminary data have shown no objective tumor response with this drug, but stable disease has been reported in 5 patients even after 16 weeks from the start of the study (9). Severe unexpected adverse effects were reported for one of the trials, leading to study discontinuation (10). Collectively,

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evidence indicates that PF-04691502 exhibits a profound inhibition of the PI3K/AKT/mTOR axis, shows robust antitumor activity in a variety of cancer models, and seems promising based on the results of the first clinical trial.

In the current report, the authors addressed the differential responses of tumors with distinct genetic alterations to targeted therapy. The therapeutic potential of the dual PI3K/mTOR inhibitor PF-04691502 was evaluated in HNSCC models with different genetic backgrounds. The authors found that PF-04691502 treatment resulted in profound upregulation of p53 expression in the cell lines carrying wild-type *TP53* (*wtTP53*), whereas the cell lines with mutant *TP53* (*mtTP53*) responded to the treatment with less of an increase in p53 expression. A moderate increase in p73 expression was also observed in HNSCC cells after drug treatment (1). These findings are in agreement with those from other reports indicating that not only does p53 regulate the insulin-like growth factor I/PI3K/AKT/mTOR pathway but also p53 in turn is regulated by the mTOR pathway (11). Therefore upregulation of the PI3K/AKT/mTOR pathway in cancer may be linked to repression of p53/p73 expression that can be reversed by inhibitors of the PI3K/AKT/mTOR signaling. MDM2 is known to facilitate degradation of p53 as well as to regulate protein stability or activity of variousproteins involved in cell proliferation and cell death, including p73 and p21. In a study by Du and colleagues, MDM2 overexpression reduced p53 protein levels and its phosphorylation on Ser15, whereas inhibition of mTOR by rapamycin blocked IGF-I- or Her-mediated upregulation of MDM2 expression, sensitizing cells to doxorubicin-induced apoptosis (11). Figure 1 depicts a simplified scheme of the coordinated regulation of the PI3K/AKT/mTOR pathway.

In the study by Herzog and colleagues (1), five HNSCC cell lines carrying *wtTP53* were determined to be significantly more sensitive to growth-inhibitory effects of PF-04691502 than six *mtTP53* HNSCC cell lines. Similarly, *wtTP53* HNSCC cell lines exhibited increased G_0–G_1 arrest and apoptotic sub-G_0 DNA after treatment with the drug as compared with *mtTP53* cell lines. Nevertheless, it is important to note that although *wtTP53* cells were relatively more sensitive to PF-04691502 than were *mtTP53* cells, a significant variation was observed in the sensitivity to the drug within each group. Two out of six *mtTP53* cell lines were as sensitive to growth-inhibitory effects of PF-04691502 as *wtTP53* were cell lines, whereas one of the five *wtTP53* cell lines was a resistant outlier with IC_{50} exceeding that of any of the other tested mtTP53 cell lines (see Fig. 2C in ref. 1). This finding suggests that other factors can modulate the cell's sensitivity to PF-04691502 besides *TP53* status. The results in the xenograft model corroborated the *in vitro* data. *WtTP53* xenografts exhibited greater induction of p53 and p73 as well as tumor growth arrest after treatment compared with *mtTP53* xenografts. The authors showed that combination of PF-04691502 and radiotherapy further augmented

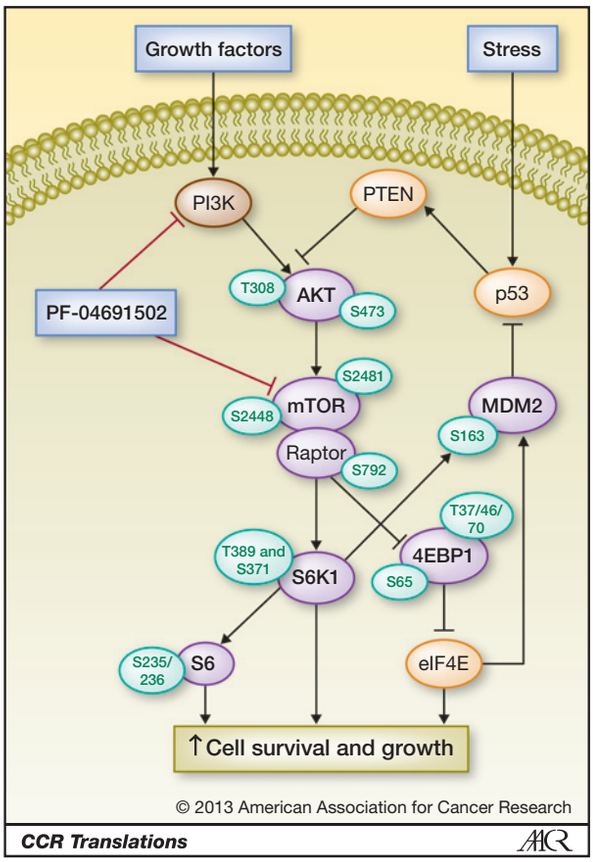

Figure 1. Simplified model of coordinated regulation of the PI3K/AKT/mTOR pathway. mTOR signaling is activated because of mutation or overexpression of *PIK3CA/AKT* genes or loss of PTEN function. p53 negatively regulates the PI3K/AKT/mTOR pathway via its upregulation of PTEN, TSC2, AMPK β1, and other proteins. Emerging data indicate that the PI3K/AKT/mTOR pathway has a negative effect on p53 expression through upregulation of MDM2 that facilitates degradation of p53. mTOR inhibitors, including PF-04691502 can relieve this inhibitory effect on p53, restoring p53-dependent tumor suppression and heightening cancer cell sensitivity to cytotoxic stimuli.

tumor growth-inhibitory effects of monotherapies. It is important to note that many studies indicated mTOR inhibition alone usually is not effective in inducing cancer cell death; rather, it sensitizes cells to apoptotic stimuli, suggesting that therapy targeting the PI3K/AKT/mTOR axis should have higher efficacy in combination with chemo- and/or radiotherapy. Furthermore, rapamycin and its analogues are allosteric inhibitors of mTOR complex 1, whereas second-generation mTOR inhibitors, such as PF-04691502, target the catalytic activity of mTOR, inhibiting both mTOR complexes, and many of these second-generation mTOR inhibitors have added benefit by also targeting PI3K catalytic activity. These properties of second-generation mTOR inhibitors result in a more profound inhibition of mTOR activity and prevention of the negative feedback activation of PI3K/AKT signaling often observed with the first-generation mTOR inhibitors.

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In conclusion, Herzog and colleagues identified *TP53* status and other markers as likely modifiers of response to PI3K/mTOR inhibitors that merit further studies and evaluation as potential biomarkers in future clinical trials.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: O. Ekshyyan, A. Anandharaj
Development of methodology: A. Anandharaj
Writing, review, and/or revision of the manuscript: O. Ekshyyan, A. Anandharaj, C.O. Nathan
Study supervision: C.O. Nathan

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