Dual PI3K/mTOR Inhibitors: Does p53 modulate response?

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Running Title: Dual PI3K/mTOR Inhibitors

Summary: 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 in order to identify a subset of patients who can benefit from therapy, improving survival and decreasing toxicity.

In this issue of Clinical Cancer Research, Herzog and colleagues present data on testing novel dual PI3K/mTOR inhibitor PF-04691502 in preclinical models of head and neck squamous cell carcinoma (HNSCC) (1). Mutational analysis of head and neck cancer revealed a pattern of genes that are frequently altered in their expression. Inactivating mutations of p53, p16ink4a and
Notch and activating mutations of PI3K3CA and RAS oncogenes notably contribute to the tumorigenesis of HNSCC that has been studied extensively for the past twenty 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. Based on 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 demonstrated 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 demonstrated that PF-04691502 effectively inhibited recombinant PI3Kα, β, γ and δ isoforms with Ki’s of 1.2-2.2 nM and recombinant mTOR with a Ki of 9.1nM. PF-04691502 inhibited PI3K/mTOR signaling in SKOV3 ovarian cancer cells with PI3K mutations and in U87MG glioblastoma cells with altered PTEN expression. Xenograft studies with the aforementioned cell lines resulted in a dose dependent tumor growth inhibition up to 70%. PF-04691502 exhibited reduced phosphorylation of AKT and S6 ribosomal protein (S6RP) in NSCLC cell line harboring PI3KCA or EGFR mutations. Additionally the drug was efficient in exhibiting anti-proliferative activity of an Erlotinib resistant line NCI-H1975 and these results were reproducible in xenograft studies as well (5). Britten et al (6) evaluated the efficacy of PF-04691502 in a panel of twenty-two breast cancer cell lines harboring PIK3CA mutations. The cell lines achieved IC50 values of less than 100nM and exhibited inhibition of pAKT.

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 revealed a drastic reduction in glucose metabolism in addition to decreased expression of tissue biomarkers p-AKT and S6RP. On the contrary 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 the mitogen activated protein kinase
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 including colorectal cancer, NSCLC and breast cancer. Analysis of the tumor biopsies of the subjects exposed mutations in PKI3CA and for few patients PTEN loss were noticed. Patients were administered orally with 2, 4, 8 and 11 mg QD. PF-0491502 has been well tolerated in-spite of common treatment related adverse effects (AE). Preliminary data has showed no objective tumor response with this drug, but stable disease has been reported in 5 patients even after 16 weeks from the start of study (9). Severe unexpected AE were reported for one of the trials leading to the study discontinuation (10). Collectively, evidence indicates that PF-04691502 exhibits a profound inhibition of the PI3K/Akt/mTOR axis, demonstrates robust antitumor activity in a variety of cancer models and appears promising in 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 background. The authors found that PF-04691502 treatment resulted in profound up-regulation of p53 expression in the cell lines carrying wild-type TP53 (wtTP53), while 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 other reports indicating that not only does p53 regulate the IGF-1/PI3K/AKT/mTOR pathway, but p53 in turn is regulated by the mTOR pathway (11). Therefore up-regulation of the PI3K/AKT/mTOR pathway in cancer may be linked to repression of p53/p73 expression that can be reversed by inhibitors of PI3K-AKT-mTOR signaling. MDM2 is known to facilitate degradation of p53 as well as to regulate protein stability or activity of various proteins involved in cell proliferation and cell death, including p73 and p21. In the study by Du and colleagues MDM2 overexpression reduced p53 protein levels and its phosphorylation on Ser15, while inhibition of mTOR by rapamycin blocked IGF1- or Her-mediated up-regulation of MDM2 expression sensitizing cells to doxorubisin-induced apoptosis (11). Figure 1 depicts a simplified scheme of the coordinated regulation of 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 G0/G1 arrest and apoptotic SubG0 DNA after treatment with the drug as compared to mtTP53 cell lines. Nevertheless it is important to note that although wtTP53 cells were relatively more sensitive to PF-04691502 than mtTP53 cells there was a significant variation 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 cell lines, while one of the five wtTP53 cell lines was a resistant outlier with IC50 exceeding that of any of the other tested mtTP53 cell lines (see Figure 2C in (1)). This 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 to mtTP53 xenografts. The authors showed that combination of PF-04691502 and radiotherapy further augmented 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; it rather sensitizes cells to apoptotic stimuli suggesting that therapy targeting the PI3K/AKT/mTOR axis should have higher efficacy in combination with chemotherapeutic and/or radiotherapy. Furthermore rapamycin and its analogues are allosteric inhibitors of mTOR Complex 1, while 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.

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

The authors have no conflicts of interest to disclose.
References


Figure Legend

**Figure 1.** Simplified model of coordinated regulation of PI3K/AKT/mTOR pathway. mTOR signaling is activated due to mutation or overexpression of PI3K/AKT genes or loss of PTEN function. p53 negatively regulates PI3K/AKT/mTOR pathway via its up-regulation of PTEN, TSC2, AMPK β1 and other proteins. Emerging data indicate that PI3K/AKT/mTOR pathway has 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.
Figure 1:

Growth factors

PI3K

PTEN

PF-04691502

T308

S473

S2481

S65

S235/236

T389 and S371

Cell survival and growth

Stress

p53

MDM2

mTOR

Raptor

S6K1

S6

S163

T37/46/70

4EBP1

eIF4E

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CCR Translations
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Clin Cancer Res  Published OnlineFirst June 5, 2013.

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Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-13-1291

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