Mammalian Target of Rapamycin, Akt, and Phosphatidylinositol 3-Kinase Signaling

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The mammalian target of rapamycin (mTOR) protein complex belongs to the phosphatidylinositol 3-kinase (PI3K)-related family of kinases. Activation occurs through a complex signaling network in which activation of a transmembrane receptor leads to activation of PI3K, subsequently stimulating Akt phosphorylation and signaling through mTOR to initiate protein synthesis (Figure 1). Signaling through mTOR can have multiple functions, in that mTOR complex 1 (mTORC1) is responsive to rapalogs, whereas mTOR complex 2 (mTORC2) is relatively insensitive to rapalogs, and both are involved in negative feedback loops which modulate signaling. The mTOR pathway is constitutively activated in non-small cell lung cancer (NSCLC) as evidenced by phosphorylation of mTOR (69%), p70 s6K (81%), and p4EBP-1 (79%) in tumor tissue. In addition, activation of Akt occurs frequently in NSCLC and has been associated with tobacco carcinogen-induced cellular transformation, promotion of tumor invasion, angiogenesis, and resistance to therapy.1,2 More than 70% of NSCLC tumors demonstrate activation of Akt at both the ser473 and thr308 phosphorylation sites, which is associated with a shorter survival.3 Furthermore, phosphorylation of Akt can be inhibited by the phosphatase and tensin homolog gene (PTEN), and loss of PTEN is also associated with poor prognosis in NSCLC.4 Therapy with rapalogs as single agents inhibited by the phosphatase and tensin homolog gene (PTEN), and loss of PTEN is also associated with poor prognosis in NSCLC.4 Therapy with rapalogs as single agents inhibited by the phosphatase and tensin homolog gene (PTEN), and loss of PTEN is also associated with poor prognosis in NSCLC.4 Therapy with rapalogs as single agents inhibited by the phosphatase and tensin homolog gene (PTEN), and loss of PTEN is also associated with poor prognosis in NSCLC.4 Therapy with rapalogs as single agents can result in limited tumor responses in lung cancer, and prolonged treatment induces resistance, which seems to be mediated by Akt signaling.5 Blocking PI3K may decrease the up-regulation of Akt signaling induced by mTOR inhibition. Thus, combined blockade of PI3K/Akt and mTOR may result in enhanced antitumor activity.

mTOR Inhibition

Sirolimus (rapamycin) is an oral rapalog that has demonstrated synergism in combination with pemetrexed in vitro and in vivo in NSCLC models. Pemetrexed is an antifolate drug that blocks multiple pathways in folate metabolism. Recently, a downstream target has been described, aminomimidazolecarboxamide ribonucleotide formyltransferase, which results in inhibition of mTOR through increased cellular ZMP.6 Accumulation of ZMP activates AMP-activated protein kinase, which in turn, blocks mTOR and subsequent protein synthesis and cell growth. Therefore, the combination of pemetrexed and mTOR inhibition may further decrease signaling through the mTOR pathway in NSCLC. A phase I/II trial evaluating pemetrexed and sirolimus in patients with advanced NSCLC with tumors that demonstrate activation of mTOR is ongoing. A phase I dose escalation will be followed by a phase II portion that requires a biopsy sample to establish mTOR activation before drug administration and after cycle 2 of therapy. The end points include determination of dose-limiting toxicities and maximum tolerated dose in the phase I portion; and response rate, PFS, and modulation of mTOR activity in the phase II portion. Twelve patients are evaluable to date, with three partial responses.

Everolimus has been studied extensively in NSCLC as monotherapy and in combination with chemotherapy and epidermal growth factor receptor (EGFR) tyrosine kinase inhibition (TKI). A phase I study assessed the combination of gefitinib and everolimus in former smokers, which resulted in two partial responses in eight evaluable patients.7 This led to a phase II trial that enrolled patients who were current or former smokers into two cohorts, untreated versus prior chemotherapy, and the primary end point was objective response rate. Sixty-two patients were enrolled, and eight (13%) patients had partial or complete response, five untreated, and three previously treated. Two responders in the untreated cohort harbored KRAS mutations (both G12F), two carried EGFR mutations, and one had neither. In the previously treated cohort, one patient harbored an EGFR mutation, and two were wild type for both EGFR and KRAS. The most common drug-related toxicities included rash, diarrhea, oral ulcerations, and fatigue. Two patients were removed from study for pulmonary toxicity. The role of mTOR inhibition in G12F KRAS mutated NSCLC is under investigation. Additional studies of everolimus have attempted to define molecular end points through preoperative evaluation in NSCLC tumors. A study evaluating everolimus given for 3 weeks preoperatively has enrolled 12 patients to date and has found a reduction in pS6 with up-regulation of pAkt after therapy.

Temsirolimus is an ester of sirolimus and has shown minimal activity as monotherapy in lung cancer. Combination therapy with EGFR TKI, chemotherapy, vascular endothelial growth factor (VEGF) inhibitors, and VEGF receptor inhibitors have demonstrated the potential for augmented...
tumor responses in a variety of tumor types, although combination trials in NSCLC remain in early phases.

**TORC1 and TORC2 Inhibition**

OSI-027 attenuates Akt activation through inhibition of both mTORC1 and mTORC2. The compound has been shown to induce apoptosis in multiple solid tumor and hematologic malignancy models, including those resistant to rapamycin. It has been shown to potentiate chemotherapy-induced apoptosis and to decrease VEGF production and blood vessel formation. A phase I trial is ongoing evaluating weekly, intermittent, and continuous dosing of OSI-027, and the recommended phase 2 dose has been determined for all cohorts. Pharmacokinetics indicate a dose response for increasing concentrations, and pharmacodynamic data evaluating p4EBP-1 levels in peripheral blood mononuclear cells demonstrate inhibition of mTOR signaling.

AZD8055 inhibits both mTORC1 and mTORC2, resulting in increased tumor apoptosis and decreased cell proliferation. It has been shown to induce dose-dependent antitumor activity and to modulate pS6 and pAkt. A phase I trial is ongoing evaluating pS6 and pAkt. The drug demonstrated dose-dependent PI3K inhibition as measured by elevation of plasma C-peptide levels.

PX-866 is a PI3K inhibitor, which induces antitumor effects through the inhibition of pAkt. Evaluation of markers to determine response demonstrates resistance in tumors with mutant KRAS, whereas loss of PTEN and alterations in PI3K predicts for sensitivity.

**PI3K Inhibition**

PI3K pathway inhibition may inhibit tumor growth and proliferation and sensitize cancer cells to programmed cell death. BEZ235 is an imidazo[4,5-c]quinoline derivative and acts as a dual PI3K and mTORC1 and mTORC2 inhibitor. The compound binds the ATP-binding cleft of the p110α subunit of PI3K and mTORC1 and mTORC2. It induces G1 cell cycle arrest and apoptosis and inhibits downstream effector activation in multiple malignancies. It has demonstrated antitumor activity in xenograft models harboring PI3K alterations. It has been shown to inhibit VEGF-induced angiogenesis and microvessel permeability with blockade of endothelial nitric oxide synthase. A phase I trial has been completed with 59 subjects and two partial responses. The drug demonstrated dose-dependent PI3K inhibition as measured by elevation of plasma C-peptide levels.

**Akt Inhibition**

Combinations of Akt and EGFR inhibition may prove beneficial in EGFR TKI-resistant NSCLC. PHT427 is an Akt inhibitor, which binds to the phosphorylation domains of Akt and phosphoinositide-dependent kinase 1 (PDK1), inhibits phosphorylation at ser473, and decreases tumor growth. Its effects have been most pronounced in KRAS-mutant NSCLC when used in combination with erlotinib.

Hepatocyte growth factor can induce EGFR TKI resistance through c-met, and coexpression of c-met and EGFR can stimulate synergistic tumor cell growth. Erlotinib and MK2206 have been shown to augment antitumor effects in both KRAS/EGFR wild-type and EGFR-mutant NSCLC models. Furthermore, MK2206 can reverse hepatocyte growth factor-induced resistance to erlotinib. A phase II trial is planned to evaluate patients with advanced NSCLC who have progressed on erlotinib after prior response to EGFR TKI therapy. Patients will be stratified by EGFR mutation status.
and will receive erlotinib daily and MK2206 on an every other day schedule. Tumor tissue will be evaluated for Akt pathway inhibition.

**FUTURE DIRECTIONS**

Selection of patients who are responsive to the mTOR/PI3K/Akt pathway is essential as these agents are developed for NSCLC therapy. Studies are underway to identify key molecular and genetic changes, which may be important in regulating this pathway. Two tumor suppressor genes have been linked to promoting NSCLC and mTOR signaling. \textit{LKB1} has serine threonine kinase activity and controls cell differentiation, cell polarity, and energy control, and alterations occur in approximately 39\% of NSCLC. \textit{BRG1} is important in ATP-dependent chromatin remodeling, cell cycle arrest, DNA repair, cell differentiation and regulation of apoptosis, and 33\% of NSCLC harbor mutations. \textit{LKB1} and \textit{BRG1} inactivating mutations correlate with a unique gene expression profile in NSCLC that affects many crucial signaling pathways. Importantly, \textit{BRG1} plays an essential role in peripheral airway development, whereas \textit{LKB1} inhibits proliferation and invasion through the mTOR pathway. Further delineation of these genes and others involved in signaling through mTOR will enhance our understanding of lung cancer pathogenesis. Opportunities for targeting this axis require an improved understanding of the biology and signaling within the individual tumors, and combination treatments with rapalogs and PI3K inhibitors or novel agents may provide new approaches.

**REFERENCES**