Rationale for a Phase I Trial of Erlotinib and the Mammalian Target of Rapamycin Inhibitor Everolimus (RAD001) for Patients with Relapsed Non–Small Cell Lung Cancer

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Abstract

Background and Rationale: Only 10% of patients with relapsed non–small cell lung cancer (NSCLC) treated with chemotherapy or erlotinib have a partial response to treatment, and nearly all eventually recur and die from their NSCLC. Agents that can block other pathways in addition to the epidermal growth factor receptor signals may improve the therapeutic efficacy of erlotinib. Everolimus (RAD001) is an inhibitor of the mammalian target of rapamycin, which is downstream of initial epidermal growth factor receptor signaling. A trial combining erlotinib with everolimus has been undertaken for patients with relapsed NSCLC.

Materials and Methods: Subjects with previously treated NSCLC are treated with increasing doses of daily erlotinib and everolimus given either daily or once weekly. The study’s objectives in phase I are to assess the feasibility of combining daily erlotinib and either daily or weekly everolimus, to assess toxicity, and to determine the appropriate dose for subsequent trials.

Results: The protocol calls for patients to be treated with escalating daily or weekly everolimus in combination with erlotinib given at doses of 100 mg daily to escalate to 150 mg daily. The dose escalation with both daily and weekly everolimus and erlotinib is ongoing.

Conclusions: Everolimus has an appropriate rationale for therapeutic use in combination with erlotinib for patients with NSCLC. This manuscript will review the preclinical rationale for undertaking a study of erlotinib combined with everolimus for patients with relapsed NSCLC.

Erlotinib

The most extensively studied ErbB family member in non–small cell lung cancer (NSCLC) is the epidermal growth factor receptor (EGFR or ErbB1). The EGFR has been an attractive candidate for the development of antineoplastic agents because it is detected by immunohistochemistry in 50% to 80% of patients with NSCLC and is activated by increased copy number/amplification or mutations in a subset of these tumors (1–5). This biology prompted the development of small molecule tyrosine kinase inhibitors that act on the intracellular portion of the EGFR (i.e., gefitinib and erlotinib) and of monoclonal antibodies directed against the extracellular portion of the EGFR (e.g., cetuximab). The efficacy of erlotinib has now been shown in NSCLC. Phase II and phase III trials for erlotinib in patients with previously treated NSCLC showed that 9% to 12% of patients had a clinical response and a median survival duration of 7 to 8 months (6, 7). In addition, patients with previously treated NSCLC that were given erlotinib had a median survival prolongation of 2 months compared with those given placebo (7). This survival benefit has led to approval of erlotinib for patients with relapsed NSCLC by the U.S. Food and Drug Administration.

Phase II trials have also been done in patients with previously untreated advanced NSCLC given erlotinib as their initial treatment (8, 9). These trials have shown a clinical response rate of 10% to 23%, a median time to progression of 3 to 4 months, and a median survival of 11 to 13 months. Although treatment with erlotinib is associated with clinical responses and survival durations similar to those seen with chemotherapy (10, 11), the efficacy of treatment with erlotinib still needs to be improved. One approach is to combine an inhibitor of the mammalian target of rapamycin (mTOR) with erlotinib to increase the therapeutic efficacy.

Epidermal Growth Factor Receptor and the Akt Pathway

Previous work in NSCLC cell lines has shown that EGFR can signal through the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, which plays major roles in cell proliferation, survival,
and cellular transformation (12). Akt is a serine threonine kinase that ultimately can lead to up-regulation of mTOR (Fig. 1). The Akt pathway can be activated by the EGFR via multiple distinct mechanisms that have been described in NSCLC (13–16). One such mechanism is autocrine signaling by the ligands including the epidermal growth factor and transforming growth factor α, which binds to the EGFR and activates the receptor. The EGFR can be constitutively activated by somatic mutations involving exons 18 to 21 (2–4), deletion of a portion of the extracellular binding domain (EGFRvIII; ref. 17), or amplification and increased copy number of the receptor (5, 18). The activated EGFR signal can then stimulate the PI3K/Akt signaling pathway. In addition, Akt can be activated by mutations found less frequently in lung cancers. One of the activating mutations that can lead to malignant transformation and more rapid growth is the PIK3CA mutant (13). Another mechanism of activation of Akt is through loss of the phosphatase and tensin homologue PTEN. PTEN is frequently deleted in glioblastoma multiforme and rarely in lung cancer (19). PTEN negatively regulates PI3K; thus, when it loses function, PI3K/Akt is activated and sends survival and proliferation signals downstream. NSCLC cells that respond to erlotinib or gefitinib with growth arrest or apoptosis undergo a down-regulation of the PI3K/Akt (13, 14). However, there are a number of NSCLC cell lines that down-regulate phosphorylated EGFR but do not effectively down-regulate the PI3K/Akt signaling pathway (20). One potential way to improve the therapeutic efficacy of erlotinib is to combine erlotinib with an mTOR inhibitor, thus blocking multiple components of the same signaling pathway (Fig. 1).

**mTOR**

The mTOR is an intracellular serine threonine kinase involved in the control of translational initiation. PI3K/Akt–dependent phosphorylation signals through tuberin, the protein product of TSC1/TSC2 complex, which leads to activation of mTOR (Fig. 1). mTOR phosphorylates S6 kinase, which phosphorylates the ribosomal protein S6. The phosphorylation of S6 leads to initiation of protein translation and plays a role in tumorigenesis (21, 22). mTOR also phosphorylates eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1). This causes 4E-BP1 to dissociate from the eukaryotic translation initiation factor 4E, allowing activation of protein translation. Rapamycin and its related compounds inhibit the function of mTOR, leading to down-regulation of S6 kinase, decrease in phosphorylation of S6, and maintenance of the 4E-BP1 eukaryotic translation initiation factor 4E complex. Three different agents are being clinically developed as anticancer agents: CCI-779/temsirolimus (Wyeth Pharmaceuticals), AP23573 (Ariad Pharmaceuticals), and RAD001/everolimus (Novartis Pharmaceuticals). Here, we focus on everolimus, which is being studied in combination with the EGFR inhibitor erlotinib in patients with relapsed NSCLC. Everolimus is an orally available macroclide that is in current use as a posttransplant immunosuppressive agent.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Depiction of the potential biomarkers of activity or predisposition to response to EGFR and mTOR inhibition. Phosphorylated (activated) form of EGFR (top two structures). The activated EGFR signals through the PI3K complex and activates Akt. Activated pAkt signals through the TSC1/TSC2 complex and increases mTOR. This results in up-regulation of S6 kinase and phosphorylation of 4E-BP1. These changes lead to increased protein production and ultimately gene transcription, cell growth, and cell proliferation. RAD001 can down-regulate mTOR (dashed line from RAD001 to the right of the mTOR circle). This down-regulation of pS6K and p4E-BP1 can paradoxically lead to an up-regulation of Akt phosphorylation. This is likely caused by RAD001 inhibiting the normally occurring negative feedback loop (line going from mTOR to insulin receptor substrate 1 (IRS1) on the cell surface which signals through PI3K and up-regulates Akt). The normally occurring negative feedback loop is represented by the solid line, whereas the signal which up-regulates PI3K through insulin receptor substrate 1 is represented by a dashed line.
**In vitro and In vivo Studies of Rapamycin and Everolimus**

*In vitro* and *in vivo* studies with rapamycin and everolimus have been done in NSCLC cell lines and murine lung cancer models that have helped assess their effect on cell signaling pathways and growth. Rapamycin and everolimus can cause a down-regulation in pS6K and p4E-BP1 (12, 20, 23, 24). Rapamycin can inhibit growth in some lung cancer cell lines and produce cell cycle arrest (23, 24). Rapamycin can also inhibit growth and prevent metastases in a murine lung cancer model, KLN-0205.

Studies have shown that everolimus and rapamycin alone can have an effect on PI3K/Akt signaling. The treatment of multiple NSCLC cell lines with the mTOR inhibitors everolimus and rapamycin leads to the down-regulation of pS6K and p4E-BP1 but paradoxically can lead to an up-regulation of Akt phosphorylation (20, 23, 24). This is likely due to an inhibition of a normally occurring negative feedback loop. An increase in Akt phosphorylation is likely to promote tumor survival and clearly limits the potential clinical efficacy of single-agent rapamycin. Therefore, treatment with everolimus and rapamycin may lead to increased survival or proliferation rather than growth inhibition and has prompted additional combination studies with receptor tyrosine kinase inhibitors. The rapamycin-induced increase in Akt phosphorylation can be suppressed by PI3K inhibitors (24, 25). Pretreatment of tumor cell lines with the insulin-like growth factor IR inhibitor NVP-AE541 blocked the rapamycin-induced increase in Akt phosphorylation (25). Combined use of insulin-like growth factor IR and rapamycin led to additive growth inhibition, compared with either agent alone, in prostate and breast cancer cell lines (DU-145, MCF-7, and MDA-MB-468), wherein rapamycin treatment alone resulted in an increase in Akt phosphorylation (25). Findings from these studies suggest that a combined approach using an mTOR inhibitor and an agent that prevents Akt activation, such as a receptor tyrosine kinase inhibitor, may be an effective therapeutic approach.

**Phase I/Phase II Trial of Everolimus**

A phase I/phase II trial of everolimus (5 to 10 mg/day) has been completed and reported in 27 patients with hematologic malignancies (26). No dose-limiting toxicities were observed in the trial. Pharmacodynamic studies showed that the downstream targets of mTOR, p4E-BP1, and/or p70 S6 kinase were down-regulated in six of nine patient samples. Multiple phase I and phase II single-agent studies with everolimus are completed or under way. One of these phase I trials studied the pharmacodynamic marker pAkt in tumor specimens from patients with solid tumors (eight patients with breast and colorectal carcinoma) to see if there is an up-regulation of PI3K/Akt with everolimus similar to the effect observed in lung cancer cell lines (25). Immunohistochemical analysis showed a significant up-regulation of pAkt after 4 weeks of everolimus therapy compared with the pretreatment biopsy specimen (25). There have also been pharmacodynamic studies on peripheral blood mononuclear cells and skin from normal human volunteers to determine if there is a similar effect seen in the *in vitro* studies with the NSCLC cell lines (27). Two patients' blood samples were treated with 20 nmol/L everolimus. This led to a >95% decrease in the S6 kinase activity in the peripheral blood mononuclear cells, supporting its use as a pharmacodynamic biomarker.

**Rationale for Erlotinib plus Everolimus in Lung Cancer**

Erlotinib has been tested *in vitro* and *in vivo* in combination with rapamycin or everolimus. Rapamycin plus erlotinib can decrease the production of EGFR and the signaling molecules downstream from mTOR, pS6K, and p4E-BP1 in NCI-H460 cells (20). *In vitro* studies of six NSCLC cell lines with wild-type EGFR show either additive or synergistic effects when erlotinib is combined with rapamycin (20). Notably in erlotinib-sensitive cell lines (H292 and BxPC3), in which erlotinib leads to down-regulation of Akt and S6 phosphorylation, erlotinib and rapamycin caused an additive inhibition in growth. In contrast, in cell lines (Calu-6, H460, and HCT-116) wherein erlotinib alone had minimal effects on growth and Akt and S6 phosphorylation, the combination of erlotinib and rapamycin led to synergistic growth inhibition (20). Notably, in four of the five cell lines (Calu6, H460, HCT-116, and H292), rapamycin alone lead to an increase in Akt phosphorylation. This has also been shown in human trials where eight patients with solid tumors given daily or weekly everolimus for 4 weeks had up-regulation of pAkt in their tumor biopsy (25). Analogous to the studies by O’Reilly and colleagues, erlotinib could block the rapamycin-induced increase in Akt phosphorylation in lung cancer cell lines. In addition, *in vivo* xenograft studies of the NSCLC cell line Calu 6 show a synergistic effect for the combination of rapamycin and erlotinib (20). Therefore, one can attempt to block the mechanism of Akt activation in human lung cancers by combining erlotinib and mTOR inhibition. These observations prompted an ongoing phase I dose escalation trial to assess the feasibility and tolerability of combining daily erlotinib with either daily or weekly everolimus.

**Summary**

Erlotinib is an effective agent for patients with relapsed NSCLC, but its therapeutic efficacy needs to be improved. An mTOR inhibitor, everolimus, can block mTOR signaling downstream from the EGFR and other receptor tyrosine kinases. The combination of erlotinib plus everolimus or rapamycin has been studied in lung cancer cell lines and xenografts. These agents can have additive or synergistic interactions against some NSCLC cell lines and xenografts. The combination is now being tested in a phase I trial to determine the safety and tolerability of the regimen and to select doses for further phase II combination trials (28).

**Open Discussion**

**Dr. Thomas Lynch:** What do you think you will get out of this phase I; and then what would your next step be in terms of designing trials for this compound?

**Dr. Johnson:** When you combine these different agents that have overlapping toxicities of mucositis, diarrhea, skin rash, you run into problems. In the limited experience that we have had, we have seen durable responses on this oral regimen. Once we get the phase I doses, we will make a decision about the trial
design for the phase II. There has not been much research effort in taking people who are progressing on erlotinib and adding a second drug to it to see if the drug resistance can be reversed. I know some innovative trialists have thought of ways of potentially doing that for future studies.

Dr. Gregory Riely: We added RAD001 to patients with acquired resistance to erlotinib and had a 0 out of 10 response rate. You are right about the toxicity profile. It is a difficult combination with erlotinib or gefitinib, particularly if started upfront. It was easier to add RAD001 with the acquired resistance patients who had been on erlotinib or gefitinib for a long time because they had acclimated to the side effects of the EGFR TKI.

Dr. Lynch: I understand why RAD001 might be helpful when added to erlotinib. But what is the advantage of adding erlotinib to RAD001 if erlotinib’s target is downstream?

Dr. Jeffrey Engelman: EGFR is not just regulating PI3 kinase signaling, but also erbB and STAT signaling. When you give gefitinib, you will block MAP kinase and STAT signaling, but that will not produce a significant apoptotic effect if you haven’t inhibited the PI3 kinase signaling pathway. By combining with an mTOR inhibitor, you can inhibit part of the PI3 kinase signaling pathway and gefitinib is blocking other parallel pathways. So, it is not only how they are both intersecting on this specific pathway.

Dr. Philip Bonomi: Would PTEN be important with this too?

Dr. Engelman: Yes. If you have a PTEN-deficient cancer cell line where the EGFR is controlling those other pathways, when you give it gefitinib, you can’t downregulate PI3 kinase signaling. That would be the situation where you would give an mTOR inhibitor in combination.

Dr. Johnson: There are good data in brain tumors to show that if they are PTEN-mutated, they are not sensitive to erlotinib.

References
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