Original Study

Comprehensive Genomic Profiling of Metastatic Tumors in a Phase 2 Biomarker Study of Everolimus in Advanced Renal Cell Carcinoma

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Abstract

Comprehensive genomic profiling of renal-cell carcinoma (RCC) metastases was successfully performed via a targeted next-generation sequencing panel on specimens derived from patients in a phase 2 biomarker study of the allosteric mTOR inhibitor everolimus. Alterations in the PI3K-AKT-mTOR pathway were common in metastatic RCC tumors. One partial response occurred in a patient with an mTOR mutation of unclear significance.

Introduction: Genomic events leading to activation of mechanistic target of rapamycin (mTOR) are common in renal cell carcinoma (RCC). Everolimus is an allosteric mTOR inhibitor with efficacy in metastatic RCC. We characterized the genomic profile of RCC tumors from metastatic sites and assessed whether particular alterations correlate with clinical response to everolimus. **Patients and Methods:** An open-label, single-arm phase 2 biomarker study of everolimus 10 mg daily was conducted in metastatic RCC patients. Needle biopsy or metastasectomy was performed on metastatic tumors before everolimus initiation. Next-generation sequencing was performed using a targeted hybrid capture panel detecting alterations within exons and key introns of $\geq$ 300 cancer-associated genes. Disease assessments were obtained every 8 weeks using standard radiographic modalities and evaluated by Response Evaluation Criteria in Solid Tumors criteria. **Results:** Objective response was seen in 1 (4.2%) of 24 patients. Two patients (8.3%) had stable disease lasting $>$ 6 months. Median (90% confidence interval) overall and progression-free survival were 20.1 (8.6, NA) and 3.8 (2.4, 5.4) months, respectively. Next-generation sequencing was successful on 18 pretreatment specimens and 3 on-treatment specimens. Alterations in the phosphatidylinositol 3-kinase–protein kinase B–mammalian target of rapamycin (PI3K-AKT-mTOR) pathway were identified in 8 (44%) of 18 pretreatment samples. An *mTOR* E2419D mutation was identified in the patient who experienced partial response. Alterations in *VHL*, *PBRM1*, *SETD2*, *KDM5C*, and *ATM* were common in the RCC metastases before initiation of everolimus. **Conclusion:** Nearly half of heavily pretreated RCC metastases may harbor mutations in components of the PI3K-AKT-mTOR pathway. Commonly mutated genes in primary RCC were also altered at a high frequency in RCC metastases.

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Introduction

Renal cell carcinoma (RCC) is the most common type of adult kidney cancer and has increased in incidence over the past several decades.[1] The treatment armamentarium against RCC has expanded greatly over the past decade, with approvals of multiple targeted therapies against the vascular endothelial growth factor (VEGF) and mechanistic target of rapamycin (mTOR) pathways as well as immune checkpoint inhibition targeting programmed cell death protein 1 (PD-1).[2] Therapeutic targeting of mTOR is particularly appealing, as components of the phosphatidylinositol 3-kinase–protein kinase B–mammalian target of rapamycin (PI3K-AKT-mTOR) signaling pathway are altered in approximately 28% of RCC samples.[3]

Everolimus is an allosteric mTOR inhibitor with proven clinical activity against metastatic RCC (mRCC). A randomized, double-blind, placebo-controlled trial of everolimus in mRCC patients with prior disease progression while receiving VEGF-targeted therapy showed improved progression-free survival (PFS) of 4.9 versus 1.9 months, leading to approval by the US Food and Drug Administration.[4] However, despite improvements in PFS and overall survival (OS) with allosteric mTOR inhibitors in randomized trials, clinical benefit is limited, with most patients displaying stable disease and less than 10% of patients experiencing partial response.[5,6]

Several studies have attempted to identify biomarkers that may predict the subset of patients who may respond well to mTOR inhibition. In particular, genomic alterations along the PI3K-AKT-mTOR pathway have been an area of focus as mutations in *mTOR*, *TSC1*, and *TSC2* have been found in case reports and small case series to be associated with improved response to mTOR inhibition in RCC and other tumor types.[7-12] However, the largest series in RCC patients found that while somatic mutations in mTOR pathway genes were more common in patients whose disease responded to mTOR inhibitors than those whose disease did not, a large proportion of those with responsive disease had no alterations in mTOR pathway genes, and several patients with mTOR pathway mutations had disease that was nonresponsive to mTOR inhibitor therapy.[12] Other studies in RCC have examined the correlation between common RCC mutations in *PBRM1*, *BAP1*, *SETD2*, and *KDM5C* and response to everolimus in the first-line setting.[13] These studies investigated genomic alterations predominantly in primary tumor samples. Given the documented intratumoral heterogeneity of RCC, metastatic tumors may harbor a very different genomic profile not previously seen in studies using nephrectomy specimens.

We report here the results of comprehensive genomic profiling via a targeted next-generation sequencing (NGS) panel of cancer-associated genes performed on the metastatic tumors of patients enrolled onto a phase 2 trial of everolimus for advanced RCC. We aimed to characterize the genomic profile of mRCC lesions and evaluate whether alterations in critical genes in the PI3K-AKT-mTOR pathway or other common alterations in RCC may be associated with clinical benefit from everolimus.

Patients and Methods

Patients and Study Design

An open-label, single-arm phase 2 trial of everolimus with pretreatment biopsy of a metastatic lesion or metastasectomy was conducted in patients with advanced RCC (NCT00827359). Key eligibility criteria included Eastern Cooperative Oncology Group performance status of ≤ 1; no prior treatment with mTOR inhibitors; age 18 years or older; and adequate kidney, liver, and bone marrow function. Patients were also required to have a safely accessible site of metastatic disease for biopsy and at least one additional site of measurable disease. A subset of patients was asked to undergo an on-treatment biopsy of the same metastatic lesion between days 7 to 14 after initiation of everolimus. The on-treatment biopsy was not a requirement for participation in the trial. All patients received everolimus 10 mg by mouth daily for each 28-day cycle and continued treatment until disease progression, intolerable toxicity, or withdrawal of consent. The trial accrued patients at 3 major academic cancer centers—Beth Israel Deaconess Medical Center (Boston, MA), Dana-Farber Cancer Institute (Boston, MA), and Duke University Cancer Center (Durham, NC)—and was approved by their respective institutional review boards.

The original primary objective of the trial was to prospectively validate the expression of phospho-Akt and phospho-S6 as predictive biomarkers of response to everolimus by measure of PFS. However, phospho-Akt and phospho-S6 immunohistochemistry was unsuccessful as a result of the lack of adequate tissue samples and was not pursued further. One secondary end point was to develop a genomic predictor of response to everolimus that was based on microarray analysis of pretreatment biopsy samples. The secondary end point was subsequently modified to use comprehensive genomic profiling of metastatic tumors via a targeted NGS panel to evaluate whether PI3K-AKT-mTOR pathway alterations in mRCC tumors may predict for response to everolimus.

Twenty-five patients enrolled onto the study between April 2009 and November 2012. One patient became ineligible because of the inability to obtain a pretreatment biopsy sample. A total of 27 tissue samples were successfully obtained from 24 patients, with 3 sets of paired tissues obtained before treatment and while receiving treatment. Targeted NGS was successful in 21 samples from 19 patients, including 2 pairs of pretreatment and on-treatment tissues and 1 on-treatment sample from a patient whose pretreatment sequencing was unsuccessful. Of the 18 patients who had sequencing from pretreatment tissue available, 4 discontinued treatment because of unacceptable toxicity. Therefore, the analyses of genomic predictors of response to everolimus include 14 patients who had pretreatment NGS of metastatic tumor and for whom response data were available.

Tumor Assessments

Objective tumor response was determined every 8 ± 1 weeks on study by either computed tomography or magnetic resonance imaging according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria. Patients who left the study had evaluations performed at the time of everolimus discontinuation and before initiation of other therapies. All patients, including those who discontinued therapy early, were followed for response until progression and for survival for 2 years from the date of registration. Following off-study visit, the study team updated survival data every 6 months for up to 3 years. Best overall response was determined by