A phase II study of everolimus in patients with advanced solid malignancies with TSC1, TSC2, NF1, NF2 or STK11 mutations

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Background: Activation of the mTOR pathway has been implicated in the development of several malignancies and alterations in TSC1, TSC2, STK11 and NF1, can lead to the dysregulation of this pathway. Furthermore, mutations in TSC1 and NF2 are known to confer sensitivity to everolimus—an mTOR inhibitor. Based on these data, a single-arm, open label, single-institution phase II basket study was designed to assess the activity of everolimus in patients with solid malignancies whose tumors harbored mutations in TSC1, TSC2, NF1, NF2, or STK11.

Methods: A total of 12 patients with histologically confirmed diagnosis of advanced solid tumors (metastatic, recurrent, or unresectable) with mutations in TSC1, TSC2, NF1, NF2 or STK11 genes, who had failed at least one line of standard of care systemic therapy, were enrolled to this open label, single-arm study. Presence of mutations in TSC1, TSC2, NF1, NF2 or STK11 genes was assessed using targeted-next generation sequencing (NGS). All eligible patients were treated with everolimus at an initial dose of 10 mg orally once daily in cycles of 28 days. The primary endpoint of this study was overall response rate (ORR).

Results: Of 12 patients enrolled, 8 were evaluable for response at the end of 2 cycles. One complete response (CR) was observed (12.5%) and one patient (12.5%) had stable disease (SD), while six (75%) patients showed disease progression. Everolimus was overall well tolerated with anemia, decreased neutrophil and lymphocyte counts, peripheral edema and hyperglycemia representing the most common adverse events. One patient discontinued treatment due to a treatment related grade 4 pericardial effusion. Both patients with CR or SD had a diagnosis of lung adenocarcinoma with NF1 or STK11 mutations, respectively.

Conclusions: Although this study failed to meet its prespecified ORR threshold for success of 30% or higher, exploratory analyses suggest potential activity for everolimus in a subset of patients with lung adenocarcinomas with STK11 or NF1 mutations. Further studies are necessary to systematically explore the clinical activity of everolimus, potentially as a combination therapy, in these patients.

Keywords: Mammalian target of rapamycin (mTOR); everolimus; solid tumor; NF1; STK11

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Introduction

Mammalian target of rapamycin (mTOR) is a key protein kinase that regulates cell growth, proliferation and survival (1). Activation of the mTOR pathway has been implicated in the development of several malignancies (2,3). This protein kinase is mainly activated via the PI3K pathway through Akt and the tuberous sclerosis complex (TSC1/TSC2) (1). This pathway is also regulated by tumor suppressors such as STKI1 and NFI, which are frequently altered across different cancers (4,5). STKI1, also known as LKB1, activates mTOR through activation of AMPK and phosphorylation of TSC2, whereas NFI prevents the downstream activation of the mTOR pathway by terminating the active state of RAS proteins (4,5). Mutations in TSC1, TSC2, STK11 and NFI genes can lead to mTOR pathway dysregulation and promote tumor cell growth (6). Inhibiting mTOR could therefore represent an approach to treat solid tumors that harbor mutations in tumor suppressors such as STKI1, NFI, TSC1 and TSC2.

Inhibitors of the mTOR pathway have demonstrated activity in different human solid tumor cell lines and in mouse xenograft models (7-10). Everolimus is an mTOR inhibitor that was first approved in 2009 by the U.S. Food and Drug Administration (FDA) for the treatment of patients with advanced renal cell carcinoma (RCC) (11). Thereafter, approvals for everolimus for the treatment of different solid tumors were granted including subependymal giant cell astrocytoma, advanced hormone receptor positive HER-2 negative breast cancer, advanced pancreatic neuroendocrine and GI cancers and lung cancers, renal cell carcinoma, renal angiomyolipoma and tuberous sclerosis complex (12). Similarly other mTOR inhibitors such as temsirolimus, have also been studied in multiple cancers, and temsirolimus is currently approved in patients with renal cell carcinoma (13). Nevertheless, despite showing promising activity in preclinical studies across a variety of different cancers known to upregulate mTOR signaling, drugs such as everolimus and temsirolimus have failed to demonstrate significant efficacy in clinical trials—possibly owing to the lack of strong biomarkers that predict for responsiveness to mTOR inhibition (14).

In an attempt to identify predictors of response to everolimus, Iyer et al. performed whole genome sequencing (WGS) on tumoral DNA from a patient with metastatic bladder cancer that achieved complete response while on everolimus, and identified a two-base-pair deletion in the TSC1 gene and a nonsense mutation in the NF2 gene (15). Additional analyses by Iyer and colleagues also found a longer duration of response with everolimus in patients whose tumors harbored TSC1 mutations, compared to patients with wild type (WT) (15). Additionally, findings from other groups suggest a role for mTOR inhibition in malignant peripheral nerve sheath tumors (MPNSTs) with NFI mutation, and breast cancer with STKI1 mutation (16,17). Based on these observations, we designed a phase II basket study to examine the activity of mTOR inhibition in patients with solid tumors containing TSC1, TSC2, NFI, NF2, or STKI1 mutations. Everolimus was selected as the mTOR inhibitor of choice for this study given its relatively well tolerated toxicity profile and ease of oral administration.

We present the following article in accordance with the TREND reporting checklist (available at https://dx.doi.org/10.21037/jtd-21-195).

Methods

Study design

This was a single-arm, open label, single-institution phase II basket study of everolimus in patients with advanced solid tumors (ClinicalTrials.gov identifier NCT02352844) conducted at the outpatient oncology clinics at Washington University School of Medicine and Alvin J. Siteman Cancer Center in St. Louis, MO. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was reviewed and approved by the Washington University Human Research Protection Office institutional review board (IRB ID # 201502067). All patients provided written informed consent for participation in this study.

Eligibility

Adults (age ≥18 years) with histologically confirmed diagnosis of advanced solid tumor (metastatic, recurrent or unresectable) with mutations in TSC1, TSC2, NFI, NF2 or STKI1 genes, who had failed at least one line of standard of care systemic therapy were eligible. Patients needed to have a 2 week washout between the last line of antineoplastic therapy and initiation of everolimus. There was no limit to the number of prior treatments patients could have received prior to enrollment. Further eligibility criteria included adequate bone marrow function (leukocytes >3,000/mcl, ANC >1,500/mcL, platelets >100,000 and hemoglobin >9.0 g/dL), hepatic function (total serum bilirubin ≥2.0×
normal limit and AST/ALT ≤2.5× normal limit), and renal function (serum creatinine ≤1.5× normal limit or creatinine clearance 45mL/min), fasting cholesterol ≤300 mg/dL and fasting triglycerides ≤2.5× normal limit. Patients were required to have an ECOG performance status of 0–2. Patients with symptomatic brain metastases and those on chronic treatment with corticosteroids, or other immunosuppressive agents, were excluded.

Mutations in TSC1, TSC2, NF1, NF2 or STK11 genes were assessed using in-house and/or commercially available targeted-next generation sequencing (NGS) assays that utilized DNA extracted from formalin fixed paraffin embedded (FFPE) tissue blocks as part of their standard of care treatment (18). Patients with circulating tumor DNA (ctDNA) test results demonstrating mutations in these genes were also eligible for enrollment (19).

Treatment procedures

All eligible patients were treated with everolimus at an initial dose of 10 mg orally daily as a take-home medication in cycles of 28 days. Treatment with everolimus was continued until disease progression or unacceptable toxicity, whichever occurred first. Everolimus-related toxicities were managed with temporary dose delays or subsequent dose reductions. Dose reductions of 50% were implemented for recurrent grade 2 stomatitis, grade 3 hyperlipidemia or grade 3 hyperglycemia, and for grade 2 non-infectious pneumonitis. The study drug was discontinued if more than two dose reductions (50% and then 75%) were required due to toxicities. The use of live vaccinations was not permitted within a week of starting study treatment and during the study.

Efficacy and safety

Patients were evaluated clinically and by laboratory assessment with every cycle of everolimus in the outpatient clinic (every 4 weeks). Baseline scans were followed by repeat imaging with computed tomography (CT) scans obtained every 8 weeks (2 cycles) to evaluate response to treatment. Tumor response and progression were defined by the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (20). The duration of overall response was defined as the time from when the measurement criteria for complete response (CR) or partial response (PR), were first met, until the time at which recurrent or progressive disease was objectively documented. Duration of stable disease (SD) was similarly defined. Patients who discontinued study therapy prior to the first radiological exam were not considered evaluable for response, but were still considered evaluable for toxicity. Adverse events (AEs) were recorded and graded according to the National Cancer Institute Common Terminology Criteria version 4.0 (21).

Outcomes

The primary end point of this study was overall response rate (ORR). Secondary end points included an examination of the correlation between presence of different mutations in the mTOR pathway and response to everolimus, and investigation of the genetic changes associated with disease progression following initial response to everolimus.

Statistical analysis

The study was designed to assess the response rate of everolimus in solid tumors that harbor TSC1, TSC2, NF1, NF2 and STK11 mutations. Since this was a study in patients with specifically targeted mutations, a substantial treatment effect size was expected. Based on historical data, we assumed a null hypothesis of an ORR of <1% in this setting. We hypothesized that an overall response rate of 30% or higher would warrant further investigation. Assuming a dropout rate near to zero, a target accrual of 12 patients was planned for this study, based on the exact single-stage design with 85% power at a 1-sided 0.05 significance level (using ph2single function from library clinfun in the R package) (22,23).

All data including demographic and clinical characteristics of enrolled patients, and response and toxicity by grade were summarized using descriptive statistics, i.e., statistics for continuous variables included medians and ranges, and statistics for qualitative variables included counts and percentages. Waterfall plot was graphed to visualize tumor responses in terms of percent change of tumor size from baseline.

Results

Patient characteristics

Between February 2016 and June 2017, 12 patients with advanced solid malignancies were enrolled to this study. Patient characteristics are listed in Table 1. The majority of patients had a diagnosis of non-small cell lung cancer.
(NSCLC) (6/12; 50%), followed by carcinoma of unknown primary site (2/12; 17%), small cell lung cancer (1/12; 8.3%), gall bladder adenocarcinoma (1/12; 8.3%), pancreatic adenocarcinoma (1/12; 8.3%) and cervical cancer (1/12; 8.3%). Seven patients (58%) were female, and eleven (92%) were Caucasian. The median age of patients enrolled to the trial was 66 years (range, 40–86 years). With regard to evaluable patients (8/12; 75%), four patients had STK11 mutations and four harbored mutations in NF1.

Antitumor activity

Four patients were not evaluable for response due to study withdrawal prior to first evaluation. Three of these four patients were lost to follow-up and one patient withdrew from study after 2 weeks at the discretion of the treating provider. The median number of cycles administered was 2 across the cohort of evaluable patients. Among the eight evaluable patients, one complete response (CR) was observed in a patient with non-small cell lung cancer representing a response rate of 12.5%. This patient only received treatment with everolimus for 2 weeks, before discontinuation due to treatment-related toxicities including grade 3 hyponatremia and a grade 4 pericardial effusion. However, follow-up assessment at 10 week mark showed a complete response that was durable and lasted more than 10 months. One patient with metastatic NSCLC (12.5%) demonstrated SD and 6 (75%) had disease progression on the first post-treatment scan. The patient with SD was on therapy for 6 cycles before discontinuing treatment for progression. Notably, in this small cohort, disease control (CR, PR and SD) was observed in 2 of the 5 patients with lung adenocarcinoma.

Safety

Table 2 summarizes grade 2 and higher treatment-related toxicities. Twelve patients that received everolimus were evaluable for toxicity. Overall, treatment with everolimus was well tolerated and no new toxicities were observed.
Grade 2 toxicities observed in greater than 10% patients included anemia in 5 patients (42%), peripheral edema in 2 patients (17%), neutrophil decrease in 2 patients (17%), and pleural effusion in 2 patients (17%). Grade 3 toxicities included lymphopenia in 3 patients (25%), hyperglycemia in 2 patients (17%), followed by fatigue, increased blood bilirubin, hypertension, hyponatremia, oral mucositis, nausea, and low white blood cell count in 1 patient each (8% each). Grade 4 pericardial effusions were observed in 2 patients on study. However, only one of these events was assessed as being related to everolimus and led to treatment discontinuation.

**Molecular characteristics**

Of the patients who were evaluable for a response, 50% (n=4) of the patients had tumors with NF1 mutations and 50% (n=4) had tumors with STK11 mutations (Table 3). None of the alterations in NF1 or STK11 were recurrent. Mutations observed in NF1 were missense (n=3) and nonsense (n=1), and STK11 mutations were mostly nonsense (n=2). The single patient with a CR had two
noncoding variants in cis in the STK11 gene, with one of the alterations occurring at a canonical splice acceptor site of the second intron, and the second occurring upstream of this splice site acceptor site. Tumor from the patient with SD demonstrated a missense mutation in NF1.

Discussion

This phase II study explored the response rate to everolimus in a heavily pre-treated population of patients with solid tumors with mutations in tumor suppressor genes that regulate the mTOR pathway. Overall, this study failed to demonstrate significant single agent activity with everolimus in solid tumors with mutations in NF1, NF2, TSC1, TSC2, and STK11. Nevertheless, our findings suggest that everolimus may be active in a subset of patients including those with adenocarcinoma of the lung. Two of four evaluable patients with lung adenocarcinoma demonstrated disease control with everolimus in our cohort. It is possible that the lack of activity with everolimus in other tumor types included in our study could be a result of differences in tumor biology associated with site of origin. Such differential responses to targeted therapies based on tissue of origin have previously been reported in the context of BRAF and KRAS inhibitors (24-26). For instance, while inhibitors targeting the BRAF V600E mutation or KRAS G12C mutation have demonstrated considerable activity in melanoma (24) and lung cancers (25), respectively, these drugs have failed to demonstrate significant single agent activity in patients with colorectal cancer (26).

Alternatively, the lack of efficacy with everolimus in this study, could also be related to the effect of co-occurring genomic alterations in tumor samples. Patients that derived benefit from everolimus in our study had lung adenocarcinomas that contained NF1 and STK11 mutations, while lacking mutations in the RAS pathway (such as KRAS and HRAS). On the contrary, the lung adenocarcinoma patients whose disease failed to respond to treatment, demonstrated combined alteration of KRAS and STK11 or NF1 and HRAS. Furthermore, two other patients with non-lung malignancies who failed to respond to treatment, also had tumors with co-existing KRAS mutations, raising the possibility that co-occurring KRAS mutations could predict for resistance to everolimus in this patient population (Table 3). Such a hypothesis would be supported by observations from pre-clinical studies in which

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Duration of treatment (cycles)</th>
<th>Best response</th>
<th>Reason for discontinuation</th>
<th>Mutation of interest</th>
<th>RAS pathway mutation</th>
<th>Other mutated genes</th>
</tr>
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<tr>
<td>Non-small cell lung cancer (NOS)</td>
<td>2</td>
<td>PD</td>
<td>Disease progression</td>
<td>NF1 p. E8*</td>
<td>N/A</td>
<td>BRCA1, CDH1</td>
</tr>
<tr>
<td>Adenocarcinoma of the gallbladder</td>
<td>2</td>
<td>PD</td>
<td>Disease progression</td>
<td>STK11 p.E199*</td>
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<td>ATM, ERCC2, GNAS,</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MAP3KX1, UGT1A7</td>
</tr>
<tr>
<td>Adenocarcinoma of the lung</td>
<td>2</td>
<td>PD</td>
<td>Disease progression</td>
<td>STK11 c.375-2del</td>
<td>N/A</td>
<td>SMAD4, BRCA2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c.375-G&gt;C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma of the pancreas</td>
<td>1</td>
<td>CR</td>
<td>Pericardial effusion</td>
<td>STK11 c.7190C&gt;T</td>
<td>N/A</td>
<td>CDKN2A, TP53,</td>
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<td></td>
<td></td>
<td></td>
<td>(related to treatment)</td>
<td>and c.7253C&gt;T</td>
<td></td>
<td>SMAD4, CSF1R</td>
</tr>
<tr>
<td>Adenocarcinoma of the lung</td>
<td>6</td>
<td>SD</td>
<td>Disease progression</td>
<td>NF1 p. Y489C</td>
<td>N/A</td>
<td>MAP2K2, TP53</td>
</tr>
<tr>
<td>Adenocarcinoma of the lung</td>
<td>2</td>
<td>PD</td>
<td>Disease progression</td>
<td>STK11 Exon 1 E33X</td>
<td>KRAS G12C</td>
<td>TP53</td>
</tr>
<tr>
<td>Adenocarcinoma of the endocervix</td>
<td>2</td>
<td>PD</td>
<td>Disease progression</td>
<td>STK11 L282fs*5</td>
<td>KRAS G12C</td>
<td>CDKN2A</td>
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</table>

Table 3: Patient disease molecular characteristics

1, identified through plasma genotyping (circulating tumor DNA). NOS, not otherwise specified; SD, stable disease; CR, complete response; PD, progression of disease; N/A, not applicable.
the presence of \textit{KRAS} mutations has been shown to predict for resistance to mTOR inhibition in PIK3CA mutated cancer cell lines and xenografts (27). In this study, Di Nicolantonio \textit{et al.} observed that colorectal cancer cell lines carrying PIK3CA mutations were responsive to everolimus, except when KRAS mutations occurred concomitantly (27). Furthermore, when they performed genetic analysis of tumors from patients with different solid tumors (mostly colorectal and breast cancer) that were treated with everolimus, patients whose tumors carried PIK3CA and KRAS mutations displayed lack of clinical benefit from everolimus (27). Finally, the functional significance of the specific mutations affecting genes of interest in our study was not always clear—particularly in samples that contained missense mutations. It is possible that some of the variants observed in this study were passenger mutations that did not lead to activation of mTOR signaling.

Overall, our findings suggest that patients with adenocarcinoma of the lung with \textit{NF1} or STK11 mutations without co-occurring mutations in \textit{KRAS} or \textit{HRAS} genes may derive some benefit from everolimus (Figure 2). STK11 and \textit{NF1} mutations are frequently seen in lung cancer (17.4\% and 11.7\%, respectively) (28,29). Nevertheless, these observations should be viewed as hypothesis generating and will require further validation in larger studies. Additional pre-clinical studies exploring genomic features that predict for response to everolimus in lung adenocarcinoma will also be necessary to inform the design of future studies.

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\textbf{Footnote}

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\textbf{Data Sharing Statement:} Available at https://dx.doi.org/10.21037/jtd-21-195

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Millenium, CRAB, Bristol Myers Squibb, and Alliance for Clinical Trials in Oncology, outside the submitted work. She also is on the Board of Directors for Lung Cancer Connection and serves on the American Society for Clinical Oncology e-Learning Editorial Board. Dr. SNW reports that the trial is supported by Novartis Pharmaceuticals. She reports funding from the SWOG-Clinical Trials Partnership, honoraria from the American Society for Clinical Oncology (ASCO) and serves as Chair of Data Safety Monitoring Board for a study through the Hoosier Oncology Group outside this submitted work. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was reviewed and approved by the Washington University Human Research Protection Office institutional review board (IRB ID # 201502067). All patients provided written informed consent for participation in this study.

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