Original Study

Analysis of Molecular Cytogenetic Changes in Metastatic Renal Cell Carcinoma in the Setting of Everolimus Treatment: A Pilot Project

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Abstract

Currently there are no predictive biomarkers for preselecting patients with renal cancer who are more likely to benefit from mammalian target of rapamycin (mTOR) inhibitors. This study evaluated molecular cytogenetic changes in 10 patients with renal cancer treated with everolimus. Concomitant gain of platelet-derived growth factor-β (*PDGFβ*) and its receptor, *PDGFRβ*, was observed in 5 patients including 2 with a prolonged benefit. *PDGFβ* and *PDGFRβ* gene status might be of relevance to everolimus therapy.

Background: The mTOR inhibitors have improved outcomes for patients with metastatic renal cell carcinoma (mRCC) but the duration of benefit is variable. Currently there are no predictive biomarkers for preselecting patients who are more likely to benefit from these agents. We undertook an exploratory translational study evaluating molecular cytogenetic changes in the context of outcomes from treatment with everolimus. **Patients and Methods:** Ten patients with clear cell mRCC treated with everolimus were enrolled. Pretreatment tissue specimens were analyzed for molecular cytogenetic changes using fluorescence in situ hybridization and progression-free survival (PFS) data were obtained. Gene probes chosen for this analysis were: Von Hippel Lindau, fragile histidine triad, fibroblast growth factor receptor (*FGFR*) 1, *FGFR3*, *PDGFβ*, *PDGFRβ*, epidermal growth factor receptor, and myelocytomatosis viral oncogene. **Results:** Median PFS was 8.75 months. Two patients with the longest PFS (28 months and 23 months) had gain of *PDGFβ* and *PDGFRβ*. This was also observed in 3 other patients who had a PFS of 11.5 months, 8 months, and 5.5 months, respectively. Cytogenetic evolution was observed between primary and metastatic specimens. **Conclusion:** *PDGFβ* and *PDGFRβ* gene status might be of relevance to everolimus therapy. Further research evaluating the utility of these potential biomarkers is required.

Clinical Genitourinary Cancer, Vol. 12, No. 4, 256-61
Keywords: Biomarkers, mTOR inhibitor, Mammalian target of rapamycin inhibitor, PDGFβ, PDGFRβ

Introduction

There are 2 biologically and therapeutically relevant pathways in renal cell carcinoma (RCC): mammalian target of rapamycin (mTOR) and vascular endothelial growth factor (VEGF) pathways. Since the introduction of therapies targeting these 2 pathways, the prognosis for patients with metastatic RCC (mRCC) has substantially improved.[1] Despite these advances, durable complete responses are rarely achieved and only a subset of patients will derive a tumor response. Furthermore, for almost all patients, their disease will eventually acquire mechanisms of resistance to these therapies.[2]

Although mTOR inhibitors have established activity in RCC,[3,4] many patients have disease that is intrinsically resistant to mTOR inhibition and for those who do respond, the duration of benefit is variable.[2] It is therefore possible that there is a molecular phenotype that confers sensitivity to mTOR inhibition. To date, there are no predictive biomarkers to preselect patients for targeted therapy and therefore patients with advanced RCC are empirically given these therapies with variable outcomes.[1,2] Research into this area is thus essential so that clinicians have the ability to select suitable patients for this type of targeted therapy.

Everolimus is an oral mTOR inhibitor that has shown a progression-free survival (PFS) advantage over placebo in patients refractory

Preliminary results of this study were presented, in part, at the 2012 Genitourinary Cancers Symposium.

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Submitted: Oct 26, 2013; Revised: Jan 5, 2014; Accepted: Jan 6, 2014; Epub: Jan 9, 2014

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1558-7673/$ - see frontmatter
http://dx.doi.org/10.1016/j.clgc.2014.01.002

to the vascular endothelial growth factor receptor inhibitors, sunitinib, sorafenib, or both.[4] A recent phase II randomized trial was not able to demonstrate PFS noninferiority of first-line everolimus compared with first-line sunitinib.[5] This study affirms the place of everolimus in the second-line setting. The mTOR signaling pathway has been shown to be active in mRCC and plays a key role in cell growth, protein translation, and metabolism.[6,7] Upstream signaling and downstream effectors of the mTOR complex are dysregulated in many tumors[6] and it is hypothesized that varying dysregulation will affect a tumor's response to mTOR inhibitors (eg, everolimus, temsirolimus). Identifying tumors that are more dependent on the mTOR signaling pathway might help to predict longer time to progression when treated with mTOR inhibitors.

We undertook an exploratory translational research project in a small set of patients who had received or were receiving everolimus in an effort to identify potential biomarkers worthy of further investigation.

Patients and Methods

Patient Selection

Patients were identified through the genitourinary medical oncology clinics at Auckland City Hospital, New Zealand. The study was approved by the National Ethics Advisory Committee, New Zealand. Written informed consent was obtained for all patients and consent took place between March 2011 and November 2011. Eligible patients had to have either previously received everolimus or be receiving everolimus, as any line of therapy, for histologically confirmed mRCC. Patients were required to have available regular imaging for tumor response assessment. Patients also had to have archival tumor tissue available for analysis.

Clinical data including age, sex, ethnicity, and Eastern Cooperative Oncology Group performance status were collected. Pathological data included Tumor, Node, Metastases stage, histological subtype, and Fuhrman grade.

Fluorescence In Situ Hybridization Probe Selection

We chose a number of gene probes empirically based on their relevance to RCC biology. Gene probes used for fluorescence in situ hybridization (FISH) analysis of tumor specimens and their control samples are shown in Table 1.

Von Hippel Lindau/Fragile Histidine Triad. Clear-cell RCC is characterized cytogenetically by biallelic gene inactivation of the von Hippel Lindau (*VHL*) gene located on the small arm of chromosome 3 (3p).[1,8] This occurs by a deletion of 3p and the concomitant loss/malfunction of the remaining *VHL* gene located at 3p25.[9] *VHL* is a tumor suppressor gene and its loss is responsible for the von Hippel-Lindau disease.[10] In normal conditions, *VHL* encodes the VHL protein which targets hypoxia-inducible factor (HIF) for proteolysis. In clear-cell RCC, inactivation of *VHL* results in a defective VHL protein being produced and therefore the hypoxia response pathway is activated, promoting angiogenesis and tumorigenesis. Evidence exists in cell lines that loss of *VHL* sensitizes renal carcinoma cells to mTOR inhibitors.[11] In addition, the fragile histidine triad (*FHIT*) gene, located in the proximal part of chromosome 3 (3p14), has been associated with clear-cell RCC.[12] RCC tumors that acquire malignant potential via loss of *FHIT* as an early tumorigenic event might behave more aggressively.[13] We therefore wanted to establish if there is any correlation between loss of this gene and sensitivity/resistance to mTOR inhibitors such as everolimus. *VHL* and *FHIT* gene probes can be used to detect differing breakpoint loss of 3p in RCC.

Fibroblast Growth Factor Receptor 1 and 3. Studies have demonstrated that there is upregulation of the fibroblast growth factor receptor (FGFR) 1 pathway in metastatic RCC. Tsimafeyeu et al observed expression of *FGFR1* in 98% of primary tumors and in 82.5% of lymph node metastases in RCC. In contrast, expression of *FGFR1* was significantly lower in normal kidney tissue (2.5%; $P < .01$).[14] We used *FGFR1* and *FGFR3* probes to check for gain/amplification of these genes.

Platelet-Derived Growth Factor-β and Its Receptor. Platelet-derived growth factor-β (PDGFβ) and its receptor, PDGFRβ, are known to play a role in RCC tumorigenesis. It has been postulated that

Table 1 Gene Probes Used for FISH Analysis of Tumor Specimens

Gene	Control	Manufacturer and Probe Target
Von Hippel-Lindau (*VHL*)	3 Centromere, D3Z1 (Zytovision)	Zytovision ZytoLight SPEC VHL/CEN 3 Dual Color targeting the 3p25 *VHL* locus
Fragile Histidine Triad (*FHIT*)	3 Centromere, D3Z1 (Zytovision)	Zytovision Zytolight SPEC FHIT/CEN 3 Dual Color Probe targeting the 3p14.2 *FHIT* locus
Fibroblast Growth Factor Receptor 1 (*FGFR1*)	8 Centromere, D872 (Zytovision)	Zytovision ZytoLight SPEC FGFR1/CEN 8 Dual Color targeting the 8p11 *FGFR1* locus
Fibroblast Growth Factor Receptor 3 (*FGFR3*)	14q32, IGH@ (Abbott Molecular)	Abbott Molecular LSI IGH/FGFR3 Dual Color/Dual Fusion targeting the 4p16.3 *FGFR3* locus
Platelet-Derived Growth Factor Receptor (*PDGFRβ*)/ Colony Stimulating Factor Receptor Type 1 (*CSF-1R*)	5p15.2, D5S23, D5S721 (Abbott Molecular)	Abbott Molecular LSI CSF1R SpectrumOrange/LSI D5S23:D5S721; SpectrumGreen targeting the 5q33-q34 CSF1R-*PDGFRβ* locus
Platelet-Derived Growth Factor β (*PDGFβ*)	22q13, no control (Zytovision)	ZytoLight SPEC PDGFB Dual Color Break Apart targeting the 22q13 *PDGFRβ* locus
Epidermal Growth Factor Receptor (*EGFR*)	7 Centromere, D7Z1 (Abbott Molecular)	Vysis LSI EGFR/CEP 7 Dual Color targeting the 7p12 *EGFR* locus
Myelocytomatosis Viral Oncogene (*MYC*)	8 Centromere, D872 (Zytovision)	Abbott Molecular LSI IGH/MYC/CEP 8 Dual Fusion targeting the 8q24 *MYC* locus

Abbreviation: CEP = chromosome enumeration probe; FISH = fluorescence in situ hybridization; IGH = immunoglobulin heavy; LSI = locus-specific identifier probe.