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Initial Clinical Sensitivity and Acquired Resistance to MET Inhibition in *MET*-Mutated Papillary Renal Cell Carcinoma

Introduction

Papillary renal cell carcinoma (RCC) is the most prevalent nonclear cell histologic subtype of renal carcinoma and constitutes approximately 10% of renal cancers, affecting 5,400 patients per year in the United States.[1-3] Activating *MET* mutations are present in the majority of hereditary papillary RCCs and up to 13% of sporadic papillary RCCs.

Here we describe a patient with *MET*-mutated papillary RCC that responded to MET inhibition with a small molecule kinase inhibitor, PF-04217903, for 26 months. At the time of acquired resistance to treatment, we identified a genomic duplication that encompassed the mutated kinase domain of *MET*.

Case Report

A 58-year-old white man with no significant medical comorbidities presented with back and flank pain and was found to have a 3.8-cm right-sided kidney mass with tumor thrombus that extended from the right renal vein into the inferior vena cava and was associated with retroperitoneal lymphadenopathy and pulmonary nodules. Biopsy of the kidney mass revealed compact papillary structures forming solid islands that were negative by immunohistochemistry for thyroid transcription factor 1 and positive for cytokeratin 7 and pancytokeratin (Figs 1A and 1B). These findings were consistent with papillary RCC of the solid variant.

The patient was initially treated with a series of sequential systemic agents including sunitinib, temsirolimus, and ENMD-2076, an Aurora and angiogenic kinase inhibitor administered as part of a phase I clinical trial (An Open-Label, Dose-Escalation, Safety, and Pharmacokinetic Study of ENMD-2076 Administered Orally to Patients With Advanced Cancer). His best response to each of these therapies was disease progression after 2 months of treatment. Because of a marked resistance to systemic therapy and a good performance status, the patient underwent a palliative debulking surgery that included right-sided nephrectomy with cavotomy, removal of caval thrombus, and lymph node dissection. The pathology revealed multifocal papillary RCC.

Given the association between activating *MET* mutations and papillary RCC, the patient provided informed consent for *MET* mutational analysis, which was performed on archival tumor tissue that was obtained during his debulking surgery. Before these results were available, the patient began treatment with an experimental MET inhibitor, PF-04217903, as part of a phase I clinical study (Phase 1 Safety, Pharmacokinetic and Pharmacodynamic Study of PF-4217903 in Patients With Advanced Cancer). Soon after starting therapy, the patient was confirmed as having a heterozygous *MET* mutation at M1268T. The patient had a family history of kidney cancer, but no germline *MET* mutation was identified.

The patient had a reduction of 35% in the sum of one-dimensional measurements of target lesions after receiving treatment for 53 weeks, achieving a confirmed partial response by RECIST version 1.0[4] (Figs 1C and 1D, white arrows illustrating a decrease in bulky lymphoadenopathy). The patient continued to be treated as part of this study for 26 months, during which time he was asymptomatic from his cancer. Unfortunately, the patient subsequently had rapid disease progression with development of malignant ascites and carcinomatosis, which led to death as a result of his cancer.

Formalin-fixed, paraffin-embedded tumor tissue from the patient's debulking surgery was obtained. DNA isolation, polymerase chain reaction amplification, and sequencing of predefined regions of MET were performed as previously described.[5] DNA sequencing was performed on tumor tissue that was obtained before treatment with PF-04217903 (pretreatment sample) and using a cytospin preparation containing malignant cells from ascitic fluid that was obtained at the time of disease progression while the patient was receiving PF-04217903 (time-of-progression sample).

Dual-color fluorescent in situ hybridization (FISH) assays were performed on the pretreatment and time-of-progression tumor samples to check for a possible *MET* amplification. FISH was performed using a commercial *MET* probe (Abbott Molecular, Des Plaines, IL) and fosmid G248P87518A11 encompassing exons 12 through 21 of *MET* originating from the WIBR-2 Human Fosmid Library (BACPAC Resources, Oakland, CA) combined with alpha satellite probe CEP7 (Abbott Molecular), as previously described.[6]

The initial screening *MET*-mutation testing of the patient's pretreatment tumor showed a heterozygous M1268T mutation that resulted in a methionine to threonine change (ATG>ACG) (Fig 2). We believe that this represents a de novo somatic mutation in the patient's tumor, given that germline *MET* mutation testing was negative.

Our patient's clinical course was characterized by a prolonged period of response to therapy followed by rapid progression, which we suspected was a result of the tumor acquiring a secondary genetic defect that conferred resistance to PF-04217903. Massive parallel sequencing of the pretreatment sample and the time-of-progression sample revealed an increased representation of the M1268T mutated allele in the time-of-progression sample as compared with the pretreatment sample (Table 1). Additionally, other variant alleles in *MET* exon 21 were over-represented in the time-of-progression sample, which was consistent with a copy number gain. No additional therapy-driven *MET* mutations were identified.

To validate our primary sequencing data, which was suggestive of genomic amplification of the mutated MET allele in the time-of-progression biopsy, we performed FISH using both a commercial *MET* probe and a genomic probe that was created from a fosmid that spanned exons 12 to 21, and included exon 19, where the M1268T mutation resides. Amplification of *MET* as defined as clustered signals or a ratio of MET/CEP7 greater than 2 was not observed (Figs 3A and

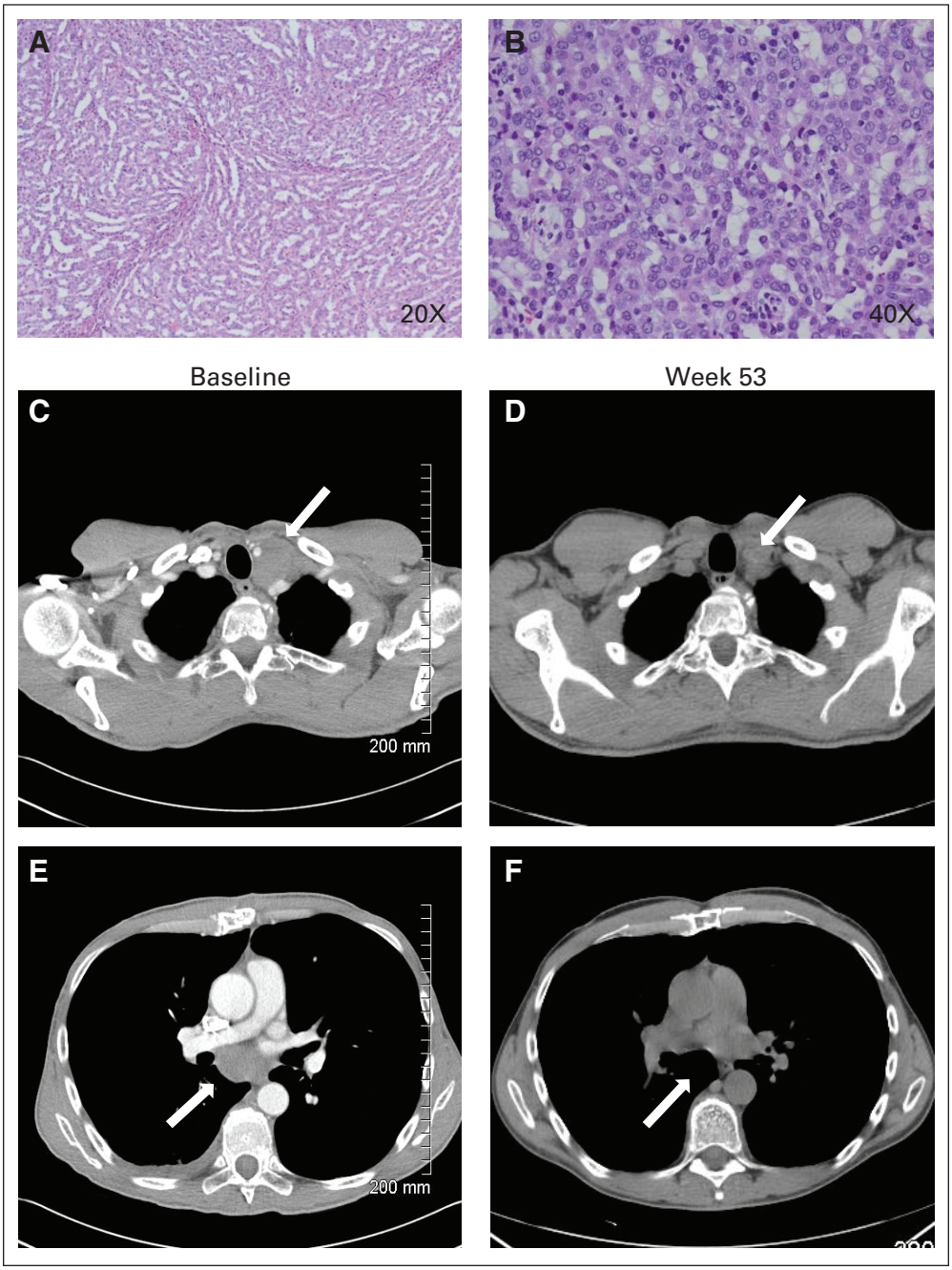

Fig 1.

3B); however, duplication of chromosome 7 was evident in the time-of-progression sample (Table 2). In addition, using fosmid-mediated FISH directed at MET exons 12 to 21, we observed split signals or doublets in approximately 50% of tumor cells in the time-of-progression sample that were not present in the pretreatment sample (Fig 3; arrows indicate doublets).

Discussion

This patient with metastatic papillary RCC had an aggressive disease course with early disease progression in response to treatment with both sunitinib and temsirolimus. This is consistent with retrospective reviews of patients with nonclear cell tumors who have inferior survival outcomes compared with patients with clear cell tumors.[1] Patients with nonclear cell RCC have traditionally been underrepresented in clinical studies of new agents for the treatment of RCC; however, this is changing, and many trials now select for specific subtypes of RCC.

Our patient with somatic papillary RCC was found to have a heterozygous M1268T mutation in *MET* that has previously been identified in both somatic and hereditary forms of this disease.[7,8] This mutation results in an amino acid substitution in the $P + 1$ loop of the MET kinase domain, which is integral to substrate recognition. This

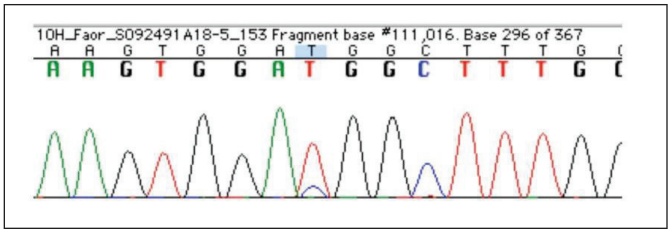

Fig 2.

mutation is one of the most effective in inducing MET phosphorylation, leading to downstream signal transduction.[9,10] The patient case we report here serves as the first clinical proof of principle for the role of MET inhibition in a patient with papillary RCC harboring an activating *MET* mutation.

There are a number of MET inhibitors in various stages of preclinical and clinical development (Table 3). PF-04217903 is a highly selective MET inhibitor, whereas crizotinib (PF-02341066) is a potent inhibitor of both MET and ALK. On the basis of remarkable activity in ALK-translocated non–small-cell lung cancer, crizotinib received US Food and Drug Administration approval for use in the United States and represents the first commercially available MET inhibitor in the United States, even if it is technically licensed for its anti-ALK activity.[11,12] The ongoing development of crizotinib includes an exploration of its activity in patients who are prescreened for evidence of *MET* mutations in papillary RCC (in the Phase 1 Safety, Pharmacokinetic and Pharmacodynamic Study of PF-02341066, a c-Met/HGFR Selective Tyrosine Kinase Inhibitor, Administered Orally to Patients With Advanced Cancer).

This report adds to an increasing body of evidence that supports MET as an attractive target for blockade in cancers that are dependent on its constitutive activation for growth and metastasis and proves that blockade of this single kinase has potential for antitumor activity.[23] Because it is assumed that it is the molecular biology, rather than the histology per se, that is driving the benefit from the MET inhibitor, we propose that selection that is based solely on papillary renal histology will be insufficient. Instead, screening papillary renal cancers for *MET* mutations in advance to most appropriately direct them to MET inhibition within clinical trials should be considered.

At the time of disease progression, the M1268T mutation remained, with no evidence of additional mutations; however, the patient's tumor acquired tandem duplication of the mutated *MET* allele in approximately 50% of tumor cells. In our patient, we speculate that this cytogenetic change in mutated gene copy number was selected at the time of resistance to treatment because of the mutated gene's ability to overcome inhibition by PF-04217903.

Copy number gain has been observed as a mechanism of resistance to crizotinib in *ALK*-rearranged non–small-cell lung cancer, both in preclinical models and in patients with an initial response to crizotinib treatment that is followed by disease progression.[24,25] The significance of tandem duplication of M1268T *MET* will need to be explored in additional models of *MET*-mutated papillary RCC and in additional acquired resistance specimens, but may be useful in the future as a marker of resistance to small-molecule MET inhibitors. This patient case illustrates the importance of performing tumor tissue sampling when it is safe and feasible at the time of acquired resistance to therapy to understand mechanisms of resistance, with a goal of developing new treatments or combinations of treatments to overcome resistance.

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Table 1. Next-Generation Sequencing of Pretreatment and Time-of-Progression Samples

Variant Allele		Pretreatment Variant Allele		Time-of-Progression Variant Allele	
Position on Chromosome 7	Exon	Frequency (%)	Reads (No.)	Frequency (%)	Reads (No.)
*116423474	19	15.07	425 of 2,820	32.97	4,001 of 12,137
116436022	21	52.46	6,703 of 12,777	73.37	19,787 of 26,968
116436097	21	58.38	11,583 of 19,841	72.07	27,180 of 37,711

*Increased representation of the M1268T mutated allele.

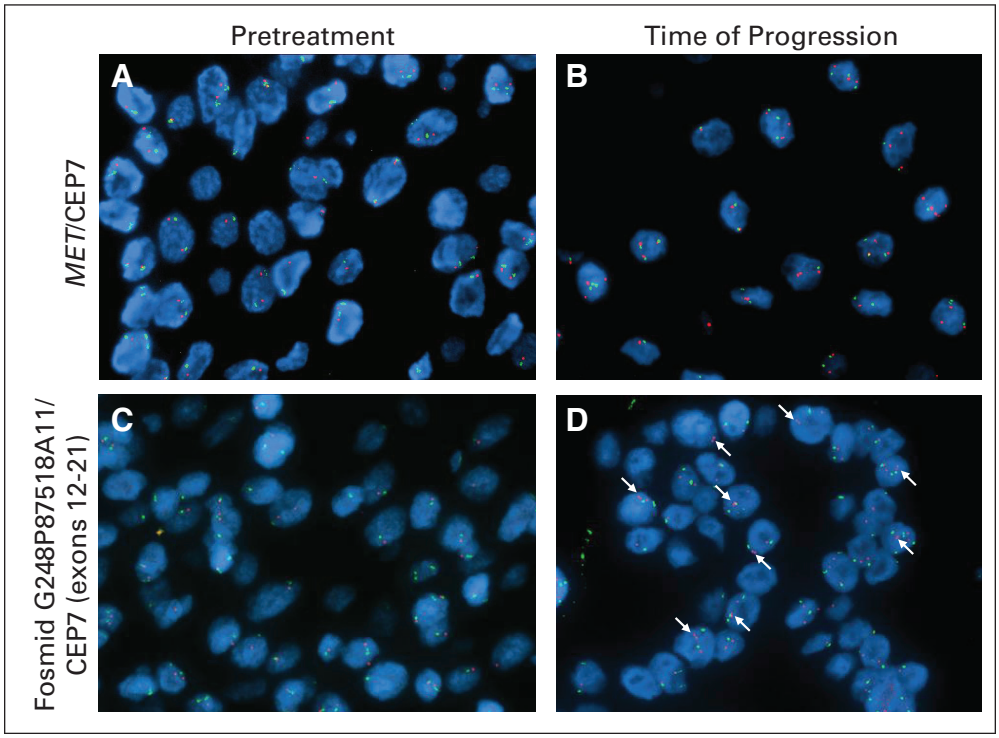

Fig 3.

(J.R.D.). We thank Pamela Vizcarra for preparing the tumor slides for analysis.

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Table 2. FISH Analysis With *MET*/CEP7 Probes

		MET				CEP7				
				% Cells With				% Cells With		
Tumor Sample	Probes	Mean	SD	≤ 2 Signals	≥ 3 Signals	Mean	SD	≤ 2 Signals	≥ 3 Signals	*MET*/CEP7
Pretreatment	*MET*/CEP7	1.64	0.56	96	4	1.58	0.54	98	2	1.04
	Fosmid G248P87518A11/CEP7 (exons 12 to 21)	1.98	0.89	80	20	1.78	0.74	90	10	1.11
	Fosmid G248P88585E3/CEP7 (exons 4 to 15)	1.84	0.77	86	14	1.82	0.63	92	8	1.01
Time of Progression	*MET*/CEP7	2.56	0.79	42	58	2.22	0.91	72	28	1.15
	Fosmid G248P87518A11/CEP7 (exons 12 to 21)	2.42	0.91	52	48	2.28	0.86	64	36	1.06
	Fosmid G248P88585E3/CEP7 (exons 4 to 15)	2.26	0.78	58	42	2.04	0.75	80	20	1.11

Abbreviations: FISH, fluorescent in situ hybridization; SD, standard deviation.

Table 3. HGF/MET-Targeted Agents in Clinical Development

Compound	Targets	Study Phase	Treatment Combinations and Disease Sites of Ongoing Studies	Reference
Crizotinib (PF-02341066)	MET, ALK TKI	Phase I/III	NSCLC with *ALK* translocation or inversion; *ALK*-positive NSCLC; crizotinib *v* pemetrexed and cisplatin; anaplastic large cell lymphoma; erlotinib for NSCLC; PF-00299804 for NSCLC; pharmacokinetic and bioavailability studies in advanced solid tumors	11,12
Cabozantinib (XL184)	MET, RET, VEGFR2 TKI	Phase I/III	Advanced solid tumors; temozolomide and radiation for malignant glioma; erlotinib for NSCLC; rosiglitazone for RCC, thyroid cancer; medullary thyroid cancer, lymphoma, malignant glioma	13
Foretinib (XL880)	MET, VEGFR2 TKI	Phase II	Erlotinib for NSCLC; lapatinib/breast cancer; papillary RCC, HCC, breast cancer	14
MetMAb (PRO143966)	Anti-MET Ab	Phase II	Paclitaxel, bevacizumab for breast cancer; erlotinib for NSCLC	15
Rilotumumab (AMG 102)	HGF Ab	Phase II	Platinum chemotherapy for SCLC; panitumumab for wild-type *KRAS* mCRC; erlotinib for advanced NSCLC; mitoxantrone and prednisone for CRPC; bevacizumab for malignant glioma; epirubicin, cisplatin, and capecitabine for gastric or esophagogastric junction cancer; pemetrexed and cisplatin/mesothelioma for RCC, ovarian cancer	16-19
AMG 208	MET TKI	Phase I	Advanced solid tumors	20
ARQ 197	MET TKI	Phase II	Irinotecan and cetuximab for mCRC; gemcitabine for advanced solid tumors; sorafenib for advanced solid tumors; erlotinib for NSCLC; RCC, alveolar soft tissue sarcoma, clear cell sarcoma, gastric cancer, HCC, GCT	21
AV-299	HGF Ab	Phase I/II	Advanced solid tumors, lymphomas, myeloma; gefitinib for NSCLC	20
E7050		Phase I/II	Sorafenib for HCC; advanced solid tumors	20
MGCD265	MET, VEGFR1-3, Ron, Tie2 TKI	Phase I/II	Erlotinib or docetaxel for advanced solid tumors or NSCLC; advanced solid tumors	22

Abbreviations: ALK, anaplastic lymphoma kinase; CRPC, castration-resistant prostate cancer; GCT, germ cell tumor; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; HGF Ab, neutralizing antibody against human HGF; mCRC, metastatic colorectal carcinoma; NSCLC, non–small-cell lung cancer; RCC, renal cell carcinoma; SCLC, small-cell lung cancer; SD, standard deviation; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.

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