

Durable Response to Crizotinib in a *MET*-Amplified, *KRAS*-Mutated Carcinoma of Unknown Primary

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Key Words

Carcinoma of unknown primary · *MET* amplification · *KRAS* mutation · Crizotinib · Next-generation sequencing

Abstract

Background: Carcinoma of unknown primary (CUP) accounts for 3–5% of all adult solid tumors. An extensive search for the anatomic site of origin is often undertaken in an attempt to tailor systemic treatment, but the latter often has limited efficacy – especially in the setting of an initial treatment failure. Molecularly targeted therapy is an emerging approach that may offer greater efficacy and less toxicity but is most likely to be effective when pairing a tumor harboring a sensitizing genomic alteration with an agent directed at the altered gene product. We report a patient with a CUP harboring a *MET* amplification with a complete metabolic response to crizotinib despite also harboring a *KRAS* mutation. **Methods:** Genomic profiling was performed using a clinical next-generation-sequencing-based assay, FoundationOne[®], in a CAP-accredited laboratory certified by Clinical Laboratory Improvement Amendments (Foundation Medicine, Cambridge, Mass., USA). **Results:** The CUP harbored both *MET* amplification (16 copies) and a *KRAS* G12V mutation. The patient was treated with crizotinib, a *MET* inhibitor, and has experienced a complete normalization of tumor metabolic activity for more than 19 months. **Conclusions:** Genomic profiling of CUP may reveal clinically meaningful genomic alterations that can guide targeted therapy decision-making. The use of this approach should be studied prospectively as a strategy for

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the effective treatment of CUP patients and for avoiding resource-intensive workups to identify the tumor site of origin.

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Introduction

Carcinoma of unknown primary (CUP) represents 3–5% of all malignancies, and carries a dismal prognosis. Median overall survival is less than a year (6–10 months) [1, 2], and cytotoxic therapy has limited efficacy. Molecularly targeted therapy can offer a treatment alternative that, when administered to a tumor with a sensitizing genomic alteration, is potentially more effective and less toxic than cytotoxic therapy. This is exemplified by the treatment paradigm of *ERBB2*-amplified breast cancer and anti-*ERBB2* (HER2)-targeted therapy [3]. We report a patient with an adenocarcinoma of unknown primary in whom a genomic profiling assay identified high-level *MET* amplification, and who is responding to crizotinib for more than 19 months.

Patients and Methods

We reviewed the medical records of a patient who presented with a cerebral metastasis of a CUP at the community practice Maryland Hematology Oncology.

Genomic Profiling

Next-generation sequencing (NGS) was performed using the Clinical Laboratory Improvement Amendments (CLIA)-certified FoundationOne® platform (version 2012; Foundation Medicine, Cambridge, Mass., USA). FoundationOne is a targeted assay utilizing NGS in routine cancer specimens [4]. The assay simultaneously sequences the entire coding sequence of 182 cancer-related genes plus 37 introns of 14 genes frequently rearranged in cancer to a minimum coverage depth of 250X [4]. The assay detects all classes of genomic alterations (including base substitutions, insertions and deletions, copy number alterations and rearrangements) and is performed on formalin-fixed, paraffin-embedded (FFPE) surgical specimens and needle biopsies that can be as small as 0.6 mm³ [4].

Case History

The patient is a 59-year-old white female with a 60-pack-year history of smoking who presented with new-onset seizures in May 2012. MRI of the brain with gadolinium revealed a solitary 2.6 × 1.9 cm mass in the right frontal lobe with surrounding edema. Further imaging with ¹⁸F-fluorodeoxyglucose positron emission tomography (¹⁸F-FDG-PET) and computed tomography (CT) revealed a left mid-abdominal mass of 2 × 4 cm with a standardized uptake value (SUV) of 6; no other lesions nor a definitive anatomic site of origin were identified.

The brain lesion was resected, and the pathologic examination of the specimen revealed a poorly differentiated metastatic carcinoma that had positive immunohistochemical staining for CK7 and epithelial membrane antigen, weak inconclusive staining for thyroid transcription factor-1 and negative staining for S100, p63 and CK20 (fig. 1). Real-time PCR assays for specific codons ('hotspot testing') demonstrated a *KRAS* G12V mutation and wild-type *EGFR*, while break-apart fluorescence in situ hybridization for *ALK* rearrangement was negative. The patient was diagnosed with adenocarcinoma of unknown primary and treated with 4 cycles of carboplatin and docetaxel. A subsequent ¹⁸F-FDG-PET/CT in September

2012 revealed no interval change on the left mid-abdominal mass but identified a new mass measuring 4.2 × 2.2 cm, which was inseparable from small bowel loops and adjacent to the original left mid-abdominal lesion as well as suggestive of disease progression (fig. 2, table 1).

Genomic Profiling

The FFPE-resected brain specimen was submitted to a CAP-accredited, CLIA-certified laboratory (Foundation Medicine) for genomic profiling by a clinical NGS-based assay [4]. Extracted tumor DNA underwent hybrid capture for the entire coding regions from 182 cancer-related genes plus 36 introns of 14 genes frequently rearranged in cancer and was sequenced to a median depth of 1,392X. The sequence was assessed for base substitutions, insertions and deletions, select rearrangements, and copy number changes. The tumor harbored amplification of *MET* (16 copies; fig. 3), *CCND1* (8 copies) and *MYC* (9 copies) as well as *KRAS* G12V, *TP53* R273L and *CARD11* N184S alterations with estimated mutant allele frequencies of 27, 46 and 71, respectively. A previous report described a *MET*-amplified non-small cell carcinoma (NSCLC) patient who responded to crizotinib, a commercially available inhibitor of *MET* [5]. On this basis, our patient began treatment with crizotinib (250 mg orally twice daily). Subsequent scans at 3-month intervals demonstrated an ongoing durable response exceeding 19 months with the fused PET-CT revealing complete resolution of the FDG-avid tumor in the abdominal mass (fig. 2, table 1).

Discussion

CUP is an advanced malignancy, which by definition presents with metastatic disease and accounts for 3–5% of all solid tumors [1]. As treatment, cytotoxic chemotherapy is administered based on a presumed or ‘most likely’ site of origin, which often follows a resource-intensive search for the anatomic site of origin. In our patient, a somatic *MET* amplification was identified by genomic profiling, and the patient experienced a durable response to crizotinib, all without knowledge of the site of tumor origin. Similarly, another CUP patient was identified as harboring a somatic *EGFR* L858R alteration via hotspot testing and responded to gefitinib, an *EGFR*-targeted therapy [4]. However, for our patient, broad-based genomic profiling, which analyzed more than 200 genes somatically altered in human cancer and identified all four classes of genomic alterations, was necessary to detect the clinically meaningful *MET* amplification. Such an unbiased approach of characterizing the same set of oncogenes and tumor suppressor genes in all solid tumors, given the unpredictable nature of genomic alterations that may be present in any given tumor, has been shown to identify genomic alterations associated with FDA-approved targeted therapies or clinical trials in more than 70–80% of the patients [6, 7].

Other groups have utilized molecular approaches to identify the site of origin and then selected tissue-appropriate chemotherapy regimens, which increased median survival to 12.5 months relative to a historical median survival of 6–10 months for CUP patients [5]. In contrast, the genomic profiling utilized in our case was intended to identify clinically meaningful somatic genomic alterations that suggest potential responsiveness to targeted therapy rather the site of origin. For this patient, inferring the site of origin from the *MET*-amplified genomic profile was unlikely to provide a definitive answer, as *MET* amplification has been reported in numerous tumor types, including glioblastoma multiforme, gastroesophageal carcinoma, lung adenocarcinomas, and lung squamous cell carcinomas, with all of these tumors responding to crizotinib [5, 8–10]. *KRAS* is also mutated in many tumor

types, including pancreatic ductal adenocarcinoma, NSCLC, and colorectal carcinoma, and thus this alteration does not suggest a site of origin [11].

The durable response to crizotinib in this *MET*-amplified, *KRAS*-mutated patient is intriguing as the latter is a contraindication for EGFR-targeted therapy in colorectal carcinoma. Recent work has suggested that *KRAS*, *EGFR* and *ALK* alterations are largely mutually exclusive in NSCLC [12]. Moreover, no concurrent *KRAS* mutations are known to exist in *MET*-amplified patients who have responded to crizotinib [5, 9], but only limited molecular testing was performed on these tumors.

Dual *MET*-amplified, *KRAS*-mutated tumors have been identified at a low frequency in the TCGA database (February 2014) as 1 of 230 NSCLC harboring concurrent *MET* amplification and *KRAS* G12V and 1 of 219 stomach adenocarcinoma harboring *MET* amplification and *KRAS* G12D. Retrospective evaluation of *KRAS* alterations in responders to *MET* inhibitors may help understand whether co-existence of a *KRAS* alteration paradoxically enhances sensitivity to *MET* inhibitors as some evidence suggests [13].

Conclusion

Comprehensive genomic profiling led to the detection of an unexpected genomic alteration and successful institution of an available targeted therapy for our patient with previously treated CUP, while avoiding a resource-intensive investigation for the anatomic site of origin. Moreover, despite *KRAS* mutation being a contraindication for the treatment with RTK-targeted therapy in some carcinomas, our *MET*-amplified, *KRAS*-mutated CUP patient responded to crizotinib. Genomic profiling that identifies all four classes of genomic alterations may be of value in treatment decision-making for CUP patients and warrants further study.

Disclosure Statement

N.P., S.A., D.D., S.S., G.A.P., D.M., J.S.R., D.L. and V.A.M. report to be employed by and have equity interest in Foundation Medicine, Inc. J.O., K.W. P.J.S., M.P. and N.K. have nothing to disclose.

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Table 1. Imaging summary of CUP lesions before and after *MET*-targeted therapy

	September 2012	January 2013	May 2013	January 2014
Lesion 1	Mass in central mesentery level of the tip of the liver/right lower kidney pole 3.6 × 2.8 cm (max. SUV 5.5)	Mass has moved to the left side of midline due to peristaltic motion 1.3 × 2.4 cm (max. SUV 2); metabolic remission	No abnormal uptake in the region of the mesentery to indicate recurrent active tumor	Unchanged from previous imaging
Lesion 2	New additional mass inseparable from small bowel loops 4.2 × 2.2 cm (max. SUV 1.4)	Unchanged from previous imaging	–	–

Crizotinib treatment was initiated in October 2012.

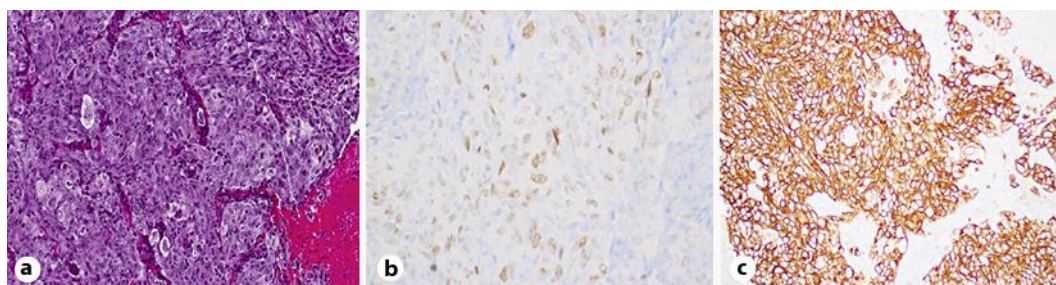


Fig. 1. Adenocarcinoma metastatic to the brain. Resection of a cerebral metastasis sample revealed a high-grade adenocarcinoma lacking tubular lumens but containing mucin and signet ring cells (a; ×20). Immunohistochemical workup demonstrated weak inconclusive thyroid transcription factor-1 staining (b; ×40), positive staining for CK7 (c; ×20) and negative staining for S100, p63 and CK20 (data not shown).

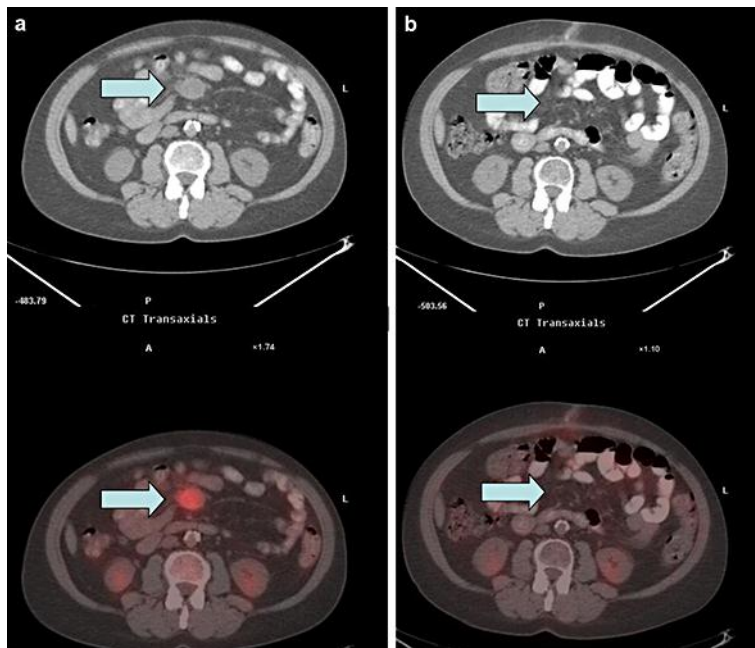


Fig. 2. Metabolic imaging of tumor response. Low-dose CT (top) and ^{18}F -FDG PET/CT (bottom) fusion of the transaxial left mid-abdominal mesenteric mass. **a** One month prior to starting treatment with crizotinib (max. SUV = 5.5; mass measures 3.6 cm by 2.8 cm AP). **b** Three months after starting treatment with crizotinib (max. SUV = 2.0; indicates that the minimal residual mass is metabolically inactive).

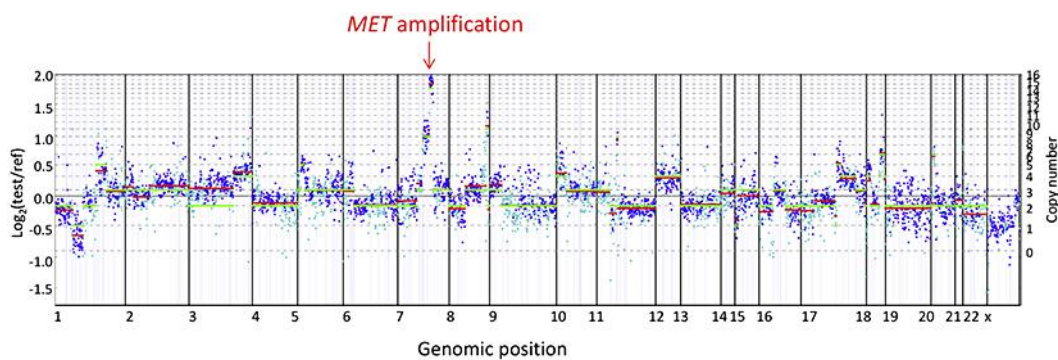


Fig. 3. Genomic profiling identified amplification of *MET*. Genomic profiling by a NGS-based assay was performed on the resected brain specimen (FFPE tissue) to a coverage depth of 1,392X, revealing 6 distinct genomic alterations including *MET* amplification. *MET* copy number (16 copies) was determined by modeling copy variation and aneuploidy across the genome and was compared to process-matched normal controls.