

# Activity of c-Met/ALK Inhibitor Crizotinib and Multi-Kinase VEGF Inhibitor Pazopanib in Metastatic Gastrointestinal Neuroectodermal Tumor Harboring EWSR1-CREB1 Fusion

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## Key Words

Case report · Crizotinib · Pazopanib · Gastrointestinal neuroectodermal tumor · Clear-cell sarcoma · EWSR1 · CREB1 · Comprehensive genomic profiling · Genomic profiling

## Abstract

Malignant gastrointestinal neuroectodermal tumor (GNET) is an aggressive rare tumor, primarily occurring in young adults with frequent local-regional metastases and recurrence after local control. The tumor is characterized by the presence of *EWSR1-ATF1* or *EWSR1-CREB1* and immunohistochemical positivity for S-100 protein without melanocytic marker positivity. Due to poor responses to standard sarcoma regimens, GNET has a poor prognosis, and development of effective systemic therapy is desperately needed to treat these patients. Herein, we present a patient with a small

bowel GNET who experienced recurrent hepatic and skeletal metastases after a primary resection. Comprehensive genomic profiling (CGP) in the course of clinical care with DNA and RNA sequencing demonstrated the presence of an exon 7 to exon 6 *EWSR1-CREB1* fusion in the context of a diploid genome with no other genomic alterations. In a clinical trial, the patient received a combination of 250 mg crizotinib with 600 mg pazopanib quaque die and achieved partial response and durable clinical benefit for over 2.8 years, and with minimal toxicity from therapy. Using a CGP database of over 50,000 samples, we identified 11 additional cases that harbor *EWSR1-CREB1* and report clinicopathologic characteristics, as these patients may also benefit from such a regimen.

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## Background

Malignant gastrointestinal neuroectodermal tumor (GNET), also known as clear-cell sarcoma (CCS)-like tumor of the gastrointestinal tract (CCSLGT), is a highly aggressive rare malignant tumor originating from the gastrointestinal tract that has a median survival of 18.5 months [1, 2]. Patients commonly present with regional lymph node and hepatic metastases and frequently recur [1–3]. GNET has features that occasionally overlap histologically with CCS of tendons and aponeuroses, including the immunohistochemical expression of S-100 protein [4]. However, CCS of tendons and aponeuroses often shows positivity for melanocytic markers (HMB45, melan-A, and tyrosinase), whereas GNET is often negative for melanocytic markers and is positive for synaptophysin [5]. These tumors share common genetic alterations: chromosomal translocation events t(12;22)(q13;q12) and t(2;22)(q34;q12), leading to chimeric fusion proteins *EWSR1-ATF1* and *EWSR1-CREB1*, respectively [3, 6].

Standard treatment regimens for CCS and CCSLGT include resection with regional lymph node dissection, radiotherapy, and chemotherapeutic regimens that include ifosfamide and doxorubicin but carry a poor prognosis. Herein, we present a case of metastatic GNET harboring *EWSR1-CREB1* with an unexpected partial response and durable clinical benefit to treatment with combined crizotinib and pazopanib exceeding 2 years.

## Methods

The original liver biopsy sample and post-relapse biopsy samples were submitted as FFPE blocks for comprehensive genomic profiling (CGP) [7]. Using FoundationOne Heme, DNA of 405 cancer-related genes plus select introns from 31 genes involved in rearrangements was sequenced to a median depth of coverage of 481×; 265 of these genes also went through RNA sequencing.

## Results

### Case Presentation

A 27-year-old female initially presented with symptoms of abdominal pain, thought to be due to cholecystitis. During cholecystectomy in October 2010, a liver tumor was observed, and intraoperative biopsies were performed. Pathology noted small round cell tumor morphology. She underwent an exploratory laparotomy with a right hepatectomy, placement of gold fiducials in

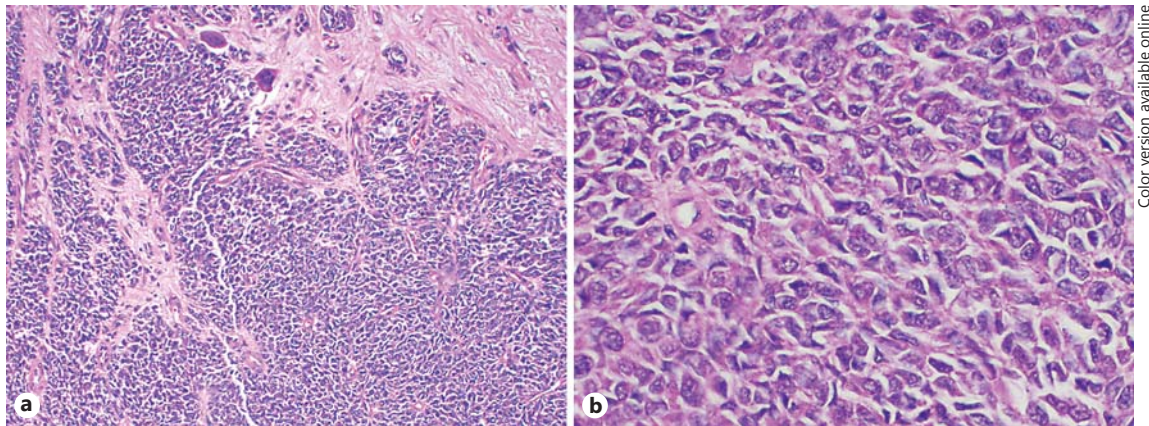
the left lobe of the liver, small bowel resection, abdominal lymphadenectomy, and placement of an On-Q pain pump. Pathology from the surgical specimen revealed multifocal metastatic sarcoma to the liver. Her small bowel specimen was diagnosed as CCS of the bowel with transmural extension. She had micrometastatic sarcoma (less than 1 mm) in 1/21 mesenteric lymph nodes (see fig. 1 for pathology reviewed at MD Anderson).

Final pathology revealed a diagnosis of GNET with metastatic disease in both the liver and 1/21 mesenteric lymph nodes. Two months later, the left liver was noted to be free of disease after a follow-up MRI. A year later, additional whole body imaging showed the presence of a left hepatic lobe hypermetabolic lesion, which was biopsied 6 months later; pathology showed this to be a hematoma. A year later, a PET scan revealed metastatic lesions in the lung, liver, and ilium. Subsequent palliative radiotherapy with 30 Gy in 10 fractions was completed in November 2013.

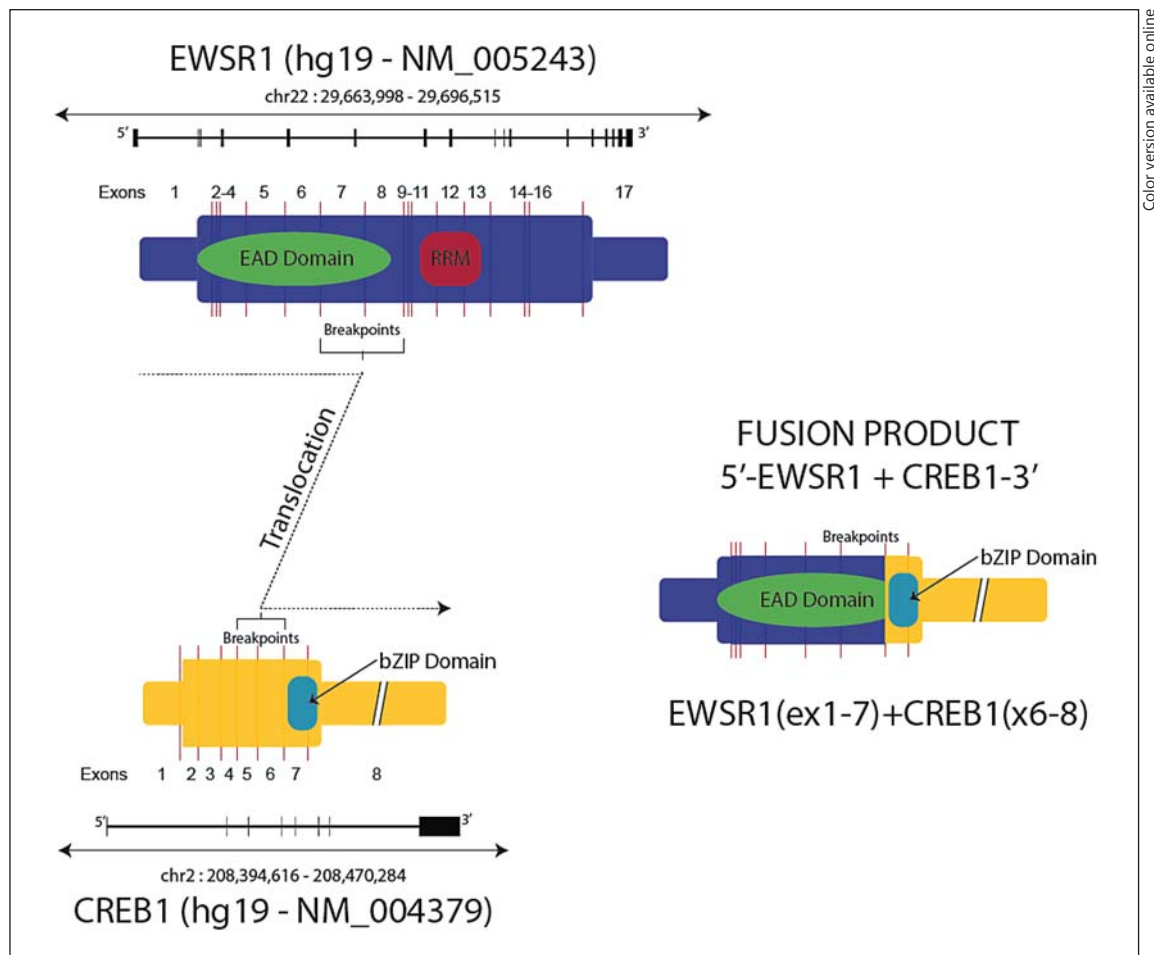
A rearrangement between chromosomes 22 and 2 was found giving rise to *EWSR1-CREB1* comprised of *EWSR1* exons 1–7 and *CREB1* exons 6–8 (see fig. 2). In addition, three variants of unknown significance were identified; CTCF P315S, NSD1 V2584L and RARA I258fs\*13. The tumoral genome was otherwise assessed as diploid, and no other genomic alterations were identified in the sample.

The patient was enrolled in a trial of daily 250 mg crizotinib plus 600 mg pazopanib (clinicaltrials.gov No. NCT01548144). After the first cycle of treatment, the patient showed an early response, and therapy continued to display clinical benefit of partial response per RECIST for 1.5 years (see fig. 3). At that time, a lump that was thought to be progressive hematoma was noted and was completely excised to reveal CCS. CGP was performed on the recurrent CCSLGT and harbored the same *EWSR1-CREB1* fusion with low-level amplification (3×) of several genes, but no additional known driver or resistance mutations were identified. The patient continues on therapy for a total of 2.8 years from start of therapy.

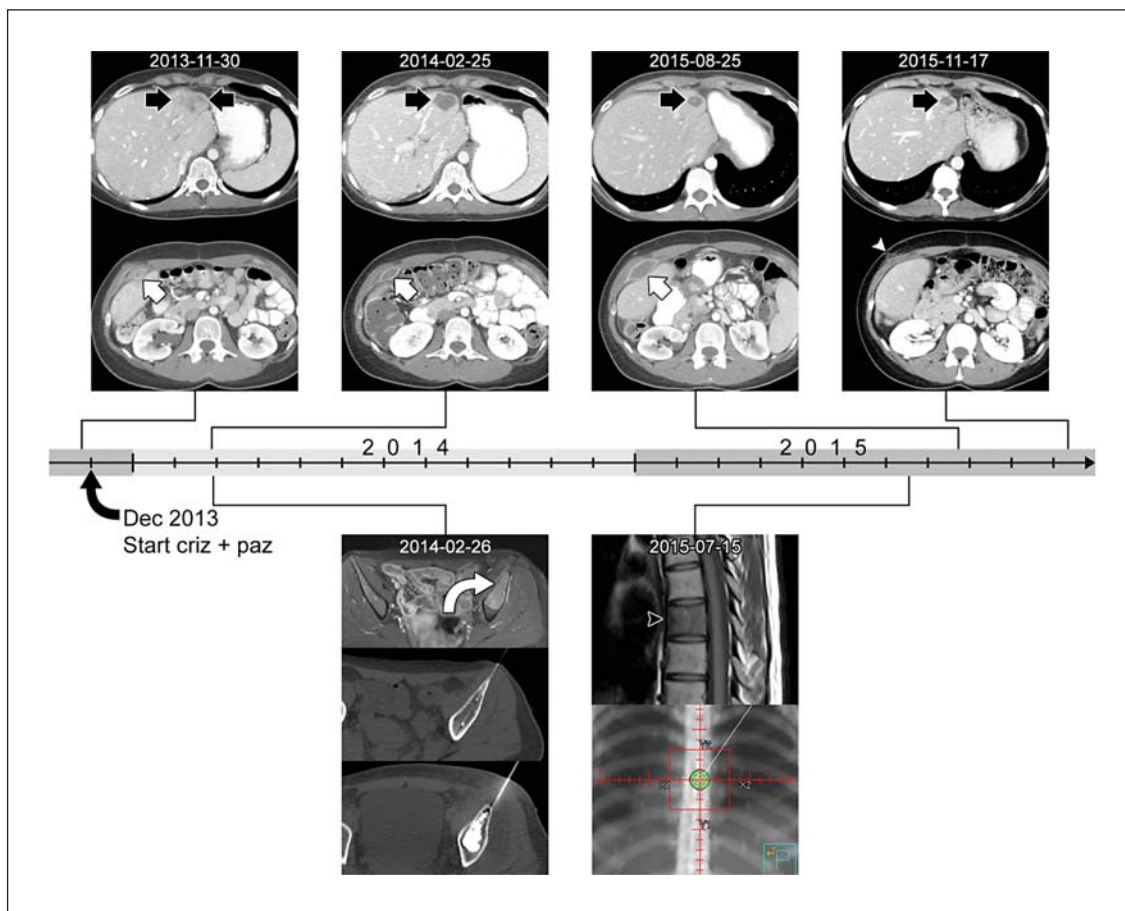
In reviewing 45,632 carcinomas, 3,803 sarcomas, and 1,526 melanomas assayed with CGP in the course of clinical care, we identified 11 additional cases that harbor *EWSR1-CREB1* fusions. These patients were 5 males and 6 females with a mean age of 34 (range 13–51). The tumors carried the diagnoses as follows: 5 sarcomas not otherwise specified (2 of which originated from the small intestine), 2 GNETs, 2 malignant neoplasms of



**Fig. 1.** **a** Cellular tumor composed of epithelioid cells, rare giant cells, arranged in variably sized nests and separated by fibrous septae (H&E, 10× magnification). **b** High power reveals the tumor is composed of epithelioid cells with oval nuclei and vesicular chromatin (H&E, 40× magnification). Tumor was positive for S-100 protein while being negative for pan-melanocytic cocktail.



**Fig. 2.** Cartoon showing the EWSR1, CREB1 and the EWSR1-CREB1 fusion product.



**Fig. 3.** Time course of imaging response following initiation of crizotinib and pazopanib in early December 2013. The CT images above the timeline show the response of a liver lesion (black arrow) and an abdominal wall lesion (white arrow) to therapy. The liver lesion had heterogeneous enhancement at the baseline CT from November 2013. On follow-up imaging, it decreased in size and degree of enhancement, developing central low attenuation representing necrosis. The abdominal wall lesion also had heterogeneous enhancement at baseline and decreased in degree of enhancement on subsequent CTs. In contrast to the liver lesion, it enlarged and became symptomatic, and was finally resected in

September 2015. Postoperative CT from November 2015 shows the surgical scar (white arrowhead). The images below the timeline show other lesions that underwent local control. In February 2014 contrast-enhanced T1-weighted MR image shows a bone metastasis in the left ilium (curved arrow, top row). This subsequently underwent cryoablation (middle row) followed by cement augmentation (bottom row) to reduce risk of pathological fracture. In July 2015, a metastasis was detected on MRI at T7 (black arrowhead on T1-weighted image). This lesion underwent palliative radiation to a dose of 2,000 cGy in 5 fractions (bottom row, radiation therapy planning image).

unknown primary, 1 melanoma, and 1 head and neck mucoepidermoid carcinoma. All but one case harbored fusions between *EWSR1* exons 1–7/8 and *CREB1* exons 6/7–8. The melanoma case fused *EWSR1* exons 1–7 with *CREB1* exons 5–8. With the exception of one sarcoma, which harbored *CDKN2A/B* homozygous deletion, there was a very low prevalence of other known mutations, none of which were in drivers of oncogenesis, implicating *EWSR1-CREB1* fusion as the main driver in these cases.

### Conclusions

Herein, we present the first case of a GNET patient harboring *EWSR1-CREB1* who exhibited a durable near-complete response to crizotinib and pazopanib for 1.5 years. The durable response to pazopanib combined with crizotinib presented in this case study was unanticipated. The current paradigm of advanced cancer benefitting from targeted therapy relies on the presence of genomic alterations that predict response, such as in the case of

non-small cell lung cancer that harbors oncogenic drivers such as *ALK* fusions and *EGFR* mutations [8, 9]. Inherent proof of the specificity of the pairing of therapy and genomic alteration is manifested in very specific mechanisms of acquired resistance, such as base substitutions in *ALK* fusions and the emergence of T790M or *MET* amplification in *EGFR* mutant lung cancer [10–13].

This case may exist outside that paradigm, as *EWSR1* fusions are currently not strongly predicted to be sensitizing to any targeted therapies. However, one group of studies reported durable, partial responses to sunitinib in extra-skeletal myxoid chondrosarcoma patients harboring the characteristic *EWSR1-NRF4A3* but not *TAF1-NRFA3* [14]. The initial observation was of the partial response of two patients, and then repeated in 8 more patients, with 6 having partial response, and 2 having stable disease. These paradigm-setting observations suggest that promiscuous multikinase inhibitors can be an effective treatment for a tumor driven by a specific *EWSR1* fusion, although the mechanism of action remains unclear.

The case reported here raises questions of mechanism of action, applicability of this finding to other GNETs, and more broadly applicability to other tumors harboring *EWSR1* fusions. These questions can only be answered by further investigation, but some implications do arise from this single patient narrative.

How does the combination of pazopanib with crizotinib, a known *MET* inhibitor, produce a response in this GNET patient? *EWSR1-ATF* and *EWSR1-CREB1* are both thought to upregulate the *MET* pathway via *MITF* and, thus, may confer a vulnerability to a *MET* inhibitor [15]. A recent report of interim results of a phase 2 EORTC study of crizotinib monotherapy in patients with *MET*-driven (likely assessed by IHC) CCS demonstrated stable disease in 7/12 cases lasting approximately 5 months [16]. Although the predetermined response rate endpoint was not met in this trial, for the case at hand, the addition of pazopanib, a promiscuous multikinase inhibitor, to crizotinib, which may already confer some clinical benefit, could further inhibit downstream signaling. An additive, possibly synergistic effect, could explain the greater response here, both in terms of the magnitude of the response and the duration of 1.5 years. A similar effect was observed in a recent case study that reported a prolonged response (>6 months) to the same therapeutic combination of crizotinib and pazopanib in a patient with an inflammatory myofibroblastic tumor harboring a *DCTN1-ALK* fusion, which was in contrast to other *ALK+* inflammatory myofibroblastic tumors that have often not responded as well to crizotinib monotherapy [17]. It is

unclear what the low-level amplification across multiple genes means as a mechanism of acquired resistance, but this relatively focal change is in keeping with resistance mechanisms observed with targeted therapy.

We additionally report other patients that harbor the same *EWSR1-CREB1* fusion, with the thought that such patients may also benefit from the combination of crizotinib and pazopanib. This fusion was found in cases carrying a range of diagnoses, including 5 sarcomas (not otherwise specified), which inherently pose a diagnostic challenge as highlighted in a recent study [18]. The cases diagnosed as melanoma, with positivity for melanocytic markers, and mucoepidermoid carcinoma, with a head and neck anatomic location, could be reconceived as CCS and an *EWSR1* rearranged tumor of the salivary gland, such as clear-cell carcinoma [19]. Regardless of diagnosis, such patients are likely eventually refractory to standard therapy and thus could benefit from an empiric challenge with the regimen described here.

More broadly, over 10 distinct *EWSR1* fusions have been reported across a large range of tumor types, predominantly in sarcomas. Could these cases also respond to the combination regimen listed here? This question warrants investigation, as does assessment of monotherapy with tyrosine kinase inhibitors, such as sunitinib. Given that the length of time likely needed to definitively understand a possible mechanism of action is unclear, empiric investigation of treatment may be warranted, as these patients typically progress through standard of care regimens. However, it may be just as likely that there are fusion-specific therapeutic sensitivities, both depending on the partner to *EWSR1*.

We have reported a case of GNET harboring an *EWSR1-CREB1* fusion in a patient who showed a partial response to combination therapy with the receptor tyrosine kinase inhibitors crizotinib and pazopanib, with clinical benefit for over 2.8 years. To our knowledge, this represents the first report of a significant clinical response to combination targeted therapy in a tumor harboring an *EWSR1-CREB1* fusion, and suggests possible benefit for other oncology patients harboring the same fusion, and potentially *EWSR1* fusions generally.

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## Statement of Ethics

Treatment and consent on the investigational trial and data collection were performed in accordance with the guidelines of The University of Texas MD Anderson Cancer Center Institutional Review Board. Clinical evaluation and assessments were performed per protocol. Written informed consent was obtained from the patient for enrolling on the clinical trial and for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

## Disclosure Statement

O.H., K.G., D.S., J.A.E., R.L.E., J.-A.V., J.H.S., P.J.S., V.M., J.S.R. and S.M.A. are employees of and have equity interest in Foundation Medicine, Inc.

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