Infantile fibrosarcoma–like tumor driven by novel RBPMS-MET fusion consolidated with cabozantinib

Ajay Gupta,1 Jennifer A. Belsky,1 Kathleen M. Schieffer,2 Kristen Leraas,2 Elizabeth Varga,2 Sean D. McGrath,2 Selene C. Koo,3,4 Vincent Magrini,2,5 Richard K. Wilson,2,5 Peter White,2,5 Elaine R. Mardis,2,5 Kris R. Jatana,6,7 Catherine E. Cottrell,2,4,5 and Bhuvana A. Setty1,5

1Division of Hematology, Oncology, Blood and Marrow Transplant, 2The Steve and Cindy Rasmussen Institute for Genomic Medicine, 3Department of Pathology, Nationwide Children’s Hospital, Columbus, Ohio 43205, USA; 4Department of Pathology, 5Department of Pediatrics, The Ohio State University, Columbus, Ohio 43210, USA; 6Department of Otolaryngology, Nationwide Children’s Hospital, Columbus, Ohio 43205, USA; 7Department of Otolaryngology-Head and Neck Surgery, The Ohio State University, Columbus, Ohio 43210, USA

Abstract Infantile fibrosarcoma (IFS) is nearly universally driven by gene fusions involving the NTRK family. ETV6-NTRK3 fusions account for ~85% of alterations; the remainder are attributed to NTRK-variant fusions. Rarely, other genomic aberrations have been described in association with tumors identified as IFS or IFS-like. We describe the utility of genomic characterization of an IFS-like tumor. We also describe the successful treatment combination of VAC (vincristine, actinomycin, cyclophosphamide) with tyrosine kinase inhibitor (TKI) maintenance in this entity. This patient presented at birth with a right facial mass, enlarging at 1 mo to 4.9 × 4.5 × 6.3 cm. Biopsy demonstrated hypercellular fascicles of spindle cells with patchy positivity for smooth muscle actin (SMA) and negativity for S100, desmin, myogenin, and MyoD1. Targeted RNA sequencing identified a novel RBPMS-MET fusion with confirmed absence of ETV6-NTRK3, and the patient was diagnosed with an IFS-like tumor. A positron emission tomography (PET) scan was negative for metastatic disease. VAC was given for a duration of 10 mo. Resection at 13 mo of age demonstrated positive margins. Cabozantinib, a MET-targeting TKI, was initiated. The patient tolerated cabozantinib well and has no evidence of disease at 24 mo of age. We describe a novel RBPMS-MET driver fusion in association with a locally aggressive IFS-like tumor. MET functions as an oncogene and, when associated with the RNA binding protein RBPMS, forms an in-frame fusion product that retains the MET kinase domain. This fusion is associated with aberrant cell signaling pathway expression and subsequent malignancy. We describe treatment with cabozantinib in a patient with an IFS-like neoplasm.

[Supplemental material is available for this article.]

INTRODUCTION

Infantile fibrosarcoma (IFS) is the most common nonrhabdomyosarcoma soft-tissue sarcoma in infants (Bourgeois et al. 2000). Histologically, IFS consists of densely packed fascicles of spindle cells with frequently high mitotic activity and a nonspecific immunohistochemical staining pattern (Bourgeois et al. 2000). IFS is driven by gene fusions involving the NTRK (neurotrophic receptor tyrosine kinase) gene family and demonstrates favorable response
to Trk (tropomyosin receptor kinase) inhibition (Albert et al. 2019). ETV6-NTRK3 fusions (t(12;15)(p13;q25)) account for ~85% of alterations, with the remainder attributed to NTRK-variant fusions (Albert et al. 2019).

IFS is usually localized, although metastases are more prevalent in patients with variant fusions, and overall prognosis is excellent for resectable tumors (Albert et al. 2019). In patients with unresectable disease, neoadjuvant therapy is standard. Prior to the era of highly selective Trk inhibitors, a rhabdomyosarcoma-like protocol was used. More recently, three patients with refractory IFS associated with LMNA–NTRK1 fusions were successfully treated with the oral MET/ALK/ROS1 tyrosine kinase inhibitor (TKI) crizotinib (Mody et al. 2015; Wong et al. 2016; Bender et al. 2019), buoyed by in vitro data of its action against TrkA (Vaishnavi et al. 2013). Additionally, an infant with ETV6-NTRK3 fusion–positive IFS responded to pazopanib suspension administered preoperatively (Yanagisawa et al. 2016).

Rarely, other genomic aberrations have been described in association with tumors identified as IFS. Based on the World Health Organization (WHO) 2013 definition of infantile fibrosarcoma, any histologically identical entity without a characteristic NTRK fusion could be considered IFS-like (Fletcher et al. 2013). Prior descriptions of IFS-like tumors revealed novel rearrangements involving the transmembrane receptor tyrosine kinase RET, including CLIP2-RET, SPECC1L-RET, MYH10-RET, and KHDRBS1-RET (Church et al. 2018; Antonescu et al. 2019; Davis et al. 2020). Furthermore, four patients between 0 and 3 yr old with IFS-like tumors were identified to have BRAF rearrangements, including a CUX1-BRAF fusion (Kao et al. 2018). Given the breadth of described alterations in IFS and IFS-like tumors, next-generation sequencing methodologies are well-suited for detection and identification of appropriate targeted therapies (Kao et al. 2018).

RESULTS

Clinical Presentation

Our patient was born with a large right facial mass measuring 4.0 × 4.1 × 5.4 cm on magnetic resonance imaging (MRI) (Fig. 1A). A bedside fine-needle aspiration of the mass on the fourth day of life demonstrated spindled cells, which were smooth muscle actin (SMA)-positive and S100-negative. Because of the patient’s age and tumor location, we opted for a short period of observation. At 1 month of age, repeat MRI showed enlargement of the mass to 4.9 × 4.5 × 6.3 cm. Open biopsy demonstrated variably cellular fascicles of spindle cells; tumor cells were positive for SMA (patchy) and CD163, and negative for S100, desmin, CD34, myogenin, and MyoD1 (Fig. 2). Reverse transcription polymerase chain reaction (RT-PCR) for ETV6-NTRK3 was negative. Next-generation sequencing identified an RBPMS-MET fusion suspected to be pathogenic, and the patient was diagnosed with IFS-like disease. A positron emission tomography (PET) scan was negative for metastatic disease. Because of continued tumor growth, neoadjuvant chemotherapy was started at 7 wk of age with vincristine, actinomycin D, and cyclophosphamide (VAC). The patient’s course was complicated by pneumatosis intestinalis, which resolved after a brief 50% dose reduction of vincristine and cyclophosphamide. She completed 10 mo of chemotherapy, at which point tumor burden decreased to 1.1 cm in its longest dimension in the right masticator space (Fig. 1B). At 13 mo of age, the residual mass was surgically resected en bloc, including a portion of the cheek skin and buccal mucosa, with meticulous dissection and preservation of the upper division of the facial nerve to maintain eye closure function. Final pathology demonstrated positive microscopic margins with no evidence of tumor necrosis. Postoperative imaging demonstrated postsurgical changes (Fig. 1C). Because of both the morbidity of additional surgery or radiation and the availability of tumor-specific genomic results, the decision was made to start cabozantinib, a MET-targeting TKI with liquid formulation, at 40 mg/m²/d.
Treatment has been tolerated well for 9 mo thus far without side effects, and our patient continues with no clinical or radiographic evidence of disease at 24 mo of age (Fig. 1D).

Genomic Analyses
Following consent on an IRB-approved protocol, the patient underwent comprehensive molecular profiling consisting of paired tumor/normal exome analysis and RNA sequencing. This included evaluation of germline and somatic single-nucleotide variation (SNV), small
indels, copy-number (CN) alterations, gene fusions, and aberrant gene expression. No pathogenic or likely pathogenic germline SNV or CN alterations were identified. Although no clearly medically meaningful somatic SNVs were identified (Supplemental Table 1), CN gain of Chromosomes 6, 8, 10, 11, 17, and 20 was found (Fig. 3A). Nonrandom gain among some of these chromosomes, in particular trisomy 11, is a cytogenetic abnormality commonly reported in IFS (Mandahl et al. 1989; Schofield et al. 1994). We identified the \textit{RBPMS-MET} fusion through RNA sequencing with confirmation by RT-PCR and subsequent Sanger sequencing to establish reproducibility and verify the inferred translational protein frame (Fig. 3B). Isoform structure was determined by long read sequencing of the tumor. The in-frame fusion event connected exon 5 of \textit{RBPMS} (NM_006867) and exon 15 of \textit{MET} (NM_000245) resulting in a 513-amino acid chimeric protein (Table 1; Fig. 3C). The fusion product resulted in juxtaposition of RBPMS, including the dimerization domain localized within the RNA recognition motif (RRM), to the intact MET protein kinase domain, a configuration predicted to result in kinase fusion gene activation (Stransky et al. 2014). Iso-Seq long read sequencing of the RNA identified a second smaller fusion transcript (409 amino acids in length; Fig. 3C). The shorter transcript begins with \textit{RBPMS} intron 4 sequence and encompasses the coding region of exon 5, with an identical breakpoint in MET to that

Figure 2. Histologic findings. The tumor is variably cellular, with (A) areas containing cellular fascicles of ovoid to spindled cells with vacuolated nuclei and (B) paucicellular areas consisting of cells with long tapered nuclei within fibrous stroma. Mild-to-moderate nuclear pleomorphism and hyperchromasia are present. Thin-walled variably ectatic blood vessels are seen throughout. Tumor cells are positive on immunohistochemical stains for smooth muscle actin (C) and CD163 (D) and negative for desmin (E), S100, CD34, myogenin, MyoD1, and nuclear β-catenin (not shown). Ki-67 proliferation index is focally up to 20% (F).
observed in the longer isoform. Analysis of gene expression data corresponded to aberrant signal transduction associated with the RBPMS-MET fusion. With the exception of rhabdomyosarcoma, MET gene expression was elevated relative to most of the soft-tissue tumors evaluated within a pediatric cohort comprised of institutional cases (n = 17) and those from the UCSC Treehouse Childhood Cancer Initiative (n = 270) (Fig. 3D), although this did not reach statistical significance (P = 0.68). We interpret elevated MET expression to be associated with the presence of the gene fusion, as the overexpression was skewed only toward those exons distal to the fusion breakpoint specifically exons 15-21 (Supplemental Fig. 1). Notably, the tumor demonstrated overexpression of PDGFRB (log2
| Fusion transcript | 5′ Gene (cytoband) | 5′ Gene RefSeq transcript | 5′ Gene genomic coordinates (hg38) | 5′ Gene exon | 3′ Gene (cytoband) | 3′ Gene RefSeq transcript | 3′ Gene genomic coordinates (hg38) | 3′ Gene exon | Fusion sequence |
|-------------------|-------------------|--------------------------|----------------------------------|-------------|-----------------|--------------------------|----------------------------------|-------------|----------------|
| Longer transcript | RBPMS (8p12)      | NM_006867.3              | 8:30504436                       | 5           | MET (7q31.2)    | NM_000245.2              | 7:11674881                      | 15          | CTCAGTTCATTGCCAGAGAGCCAT@ atcagtttcttaattctcagaaacggttcct |
| Shorter transcript| RBPMS (8p12)      | NM_006867.3              | 8:30503199-30504436*             | 5           | MET (7q31.2)    | NM_000245.2              | 7:11674881                      | 15          | CTCAGTTCATTGCCAGAGAGCCAT@ atcagtttcttaattctcagaaacggttcct |

*The shorter transcript reflects RBPMS sequence initiating within intron 4 and extending through exon 5 with the 3′ breakpoint of RBPMS and 5′ breakpoint of MET identical to those in the longer isoform.
fold change: 3.40, \( P = 0.003 \), which was comparable to two infantile myofibromatosis cases with activating alterations in \( PDGFRB \) (Fig. 3E).

**DISCUSSION**

Herein, we report a unique case of an aggressive IFS-like tumor harboring a non-NTRK-variant fusion, \( RBPMS-MET \), and the common aneuploidy changes associated with IFS, including trisomies 8, 11, 17, and 20. An \( RBPMS-MET \) fusion has only been described in a single adult with cholangiocarcinoma with similar breakpoints as described in our tumor (The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium 2020). RNA-binding protein with multiple splicing (RBPMS) has been found to regulate cancer cell proliferation and migration (Fu et al. 2014) as well as smooth muscle plasticity (Sagnol et al. 2014). Under normal physiological conditions, the hepatocyte growth factor (HGF) ligand binds MET receptor and induces dimerization and autophosphorylation of the protein kinase domain, resulting in receptor signaling (Zhang et al. 2018). This activation induces cell proliferation, survival, growth, angiogenesis, and metastasis (Cui et al. 2011). In many receptor tyrosine kinase fusions, the 5’ gene partner contributes an oligomerization domain, such as a coiled-coil motif, which promote constitutively active and ligand-independent receptor activation (Du and Lovly 2018). The RRM domain of RBPMS is involved in both RNA recognition and homodimerization (Sagnol et al. 2014; Teplova et al. 2016). The in-frame fusion product retains the RRM and dimerization motifs of RBPMS and the protein kinase domain of MET, which is predicted to promote aberrant downstream signaling through Ras-MAPK and PI3K/AKT pathways. Activation of MET and downstream signaling through the PI3K/AKT and Ras-MAPK pathways occurs through tyrosine phosphorylation. Additionally, activation of other receptor tyrosine kinases, including PDGFRB, has been reported because of cross-talk of these receptors through trans-phosphorylation (Yeh et al. 2011; Lai et al. 2018). Using long read sequencing technologies, we also identified a shorter fusion isoform with a truncated RRM. The impact of this fusion on ligand-independent MET receptor activation is unknown, although given the likely impaired dimerization of RBPMS, this product may be hypothesized to have a lesser to noncontributory impact on downstream signaling and oncogenesis.

Genomic characterization and molecular diagnostics are expanding knowledge and treatment of IFS and IFS-like tumors. Classically, IFS is cytogenetically characterized by a translocation of Chromosomes 12p13 and 15q25, corresponding to an \( ETV6-NTRK3 \) gene fusion, and trisomy 11 (Sandberg and Bridge 2002), with occasional trisomies of 8, 17, and 20 (Sandberg and Bridge 2002). As mentioned previously, histologically identical entities without the classic gene fusion can be considered IFS-like and feature an assortment of novel rearrangements, now including an \( RBPMS-MET \) fusion with this report. Although sarcomas demonstrate the highest frequency of kinase fusions across 20 distinct solid tumor histologies (Stransky et al. 2014), MET fusions are infrequently reported as drivers. There is a single report of a \( TFG-MET \) fusion in an unusual S100-positive infantile spindle cell sarcoma (Flucke et al. 2017). In terms of \( RBPMS \) involvement, there are two prior reports of \( RBPMS-NTRK3 \) fusion positive sarcomas: an adult with a fibrosarcoma-like uterine sarcoma (Chiang et al. 2018) and a 20-mo-old with a fibroblastic central nervous system (CNS) tumor (Torre et al. 2018). Additionally, \( RBPMS-NRG1 \) fusions have been described in adult lung adenocarcinoma and renal cell carcinoma (Jonna et al. 2019).

There is substantial literature over the past 35 years to support the use of neoadjuvant chemotherapy during the infancy period for IFS (Grier et al. 1985; Ninane et al. 1991). Such regimens have included VAC, as in our patient, with variable use of doxorubicin, ifosfamide, and etoposide (Kynaston et al. 1993; Russell et al. 2009). However, the use of TKIs to treat sarcomas of infancy is rare. Imatinib has accrued the most experience, with
documented use in a mast cell sarcoma in an 11-mo-old (Bautista-Quach et al. 2013) and der- 
mematofibrosarcoma protuberans in 10- and 12-mo-old infants (Gooskens et al. 2010; Suzuki 
et al. 2011). Cabozantinib has been studied in COG phase I and II trials (ADVL1211 and 
ADVL1622) for children with recurrent or refractory solid tumors (Chuk et al. 2018), in which 
the youngest reported patient was 4 years old. Tolerability with extended disease control in 
children with neuroblastoma has been reported (Perisa et al. 2020). Although there were 
dose-limiting toxicities in the phase I trial at every dose level (including palmar-plantar eryth-
rodysesthesia syndrome and mucositis), the toxicity profile overall resembled that of adults: 
diarrhea, fatigue, hypothyroidism, hypertension, weight loss, anorexia, and nausea (Chuk 
et al. 2018). Lab abnormalities included elevations in alanine aminotransferase, lipase, bilir-
ubin, and proteinuria. Thus far, our patient has tolerated cabozantinib without significant 
clinical or laboratory toxicities.

In this patient’s tumor, this fusion was associated with aberrant cell signaling pathway ex-
pression. Based on the biological mechanism associated with MET fusion, we are success-
fully treating our patient with the tyrosine kinase inhibitor cabozantinib. Patient-centric 
studies can improve our understanding of the mechanisms of disease and describe novel 
variants, the knowledge of which can benefit both the individual, as well as the larger patient 
population.

METHODS

Patient Enrollment
The infant was enrolled as part of an Institutional Review Board (IRB)-approved study at the 
Institute for Genomic Medicine at Nationwide Children’s Hospital (NCH). Informed consent 
for comprehensive molecular analysis was provided by the parents. Peripheral blood (PB) 
was collected by routine venipuncture for genomic DNA extraction. Snap-frozen tumor tis-
sue from the initial surgery was obtained for tumor DNA and RNA extraction.

DNA Sequencing
Enhanced exome sequencing was performed on DNA extracted from peripheral 
blood (comparator sample) and disease-involved tissue (snap-frozen tumor). Pathology esti-
imated the sequenced section at 95% tumor cellularity. Libraries were prepared using 100 ng 
of input DNA beginning with enzymatic fragmentation, followed by end repair, 5’ phosphor-
ylation, A-tailing, and sequencing adapter ligation using NEB Ultra II FS (peripheral blood) 
and NEB Ultra II FS (snap-frozen) reagents (New England Biolabs). Target enrichment by 
hybrid capture was performed with IDT xGen Exome Research Panel v1.0 enhanced 
with the xGenCNV Backbone and Cancer-Enriched Panels-Tech Access (Integrated DNA 
Technologies). Paired-end 151-bp reads were generated on the Illumina HiSeq 4000. 
Secondary analysis was performed using Churchill, a comprehensive workflow for analysis 
of raw reads from genome alignment through to germline and somatic variant identification 
(Kelly et al. 2015). Reads were aligned to the human genome reference sequence (build 
GRCh37) using BWA (v0.7.15). Sequence alignments were refined according to communi-
ty-accepted guidelines for best practices (https://gatk.broadinstitute.org/hc/en-us). 
Duplicate sequence reads were removed using samblaster-v.0.1.22, and local realignment 
was performed on the aligned sequence data using the Genome Analysis Toolkit (v3.7–0). 
Churchill’s own deterministic implementation of base quality score recalibration was used. 
Germline variants were called using GATK’s HaplotypeCaller. Average sequencing coverage 
depth for the tumor sample was 240× and 265× for the comparator peripheral blood sample 
(Supplemental Table 2). Somatic SNV and indel detection was performed using MuTect-2
Somatic variants in cancer-associated genes were identified (Zhang et al. 2015). Copy-number variation (CNV) was assessed using VarScan2 (Koboldt et al. 2012).

RNA Sequencing

In parallel, 500 ng of snap-frozen tumor RNA was subjected to DNase treatment and ribodepletion prior to using Illumina’s TruSeq Stranded Total RNA Sample prep (performed with 2-min chemical fragmentation). An independent RNA-seq library was constructed for whole-transcriptome sequencing. Paired-end 151-bp reads were generated on the Illumina HiSeq 4000, and reads were aligned to the human genome reference sequence (GRCh38) with the resultant output representing 278,462,016 uniquely mapped reads (Supplemental Table 2). RNA sequence data were processed using an ensemble approach of seven fusion callers (STAR-Fusion [v.1.2.0] [Haas et al. 2019], MapSplice [v.2.2.1] [Wang et al. 2010], TopHat-Fusion [v.2.1.0] [Kim and Salzberg 2011], FusionCatcher [v.0.99.7c] [Nicolici et al. 2014], FusionMap [v.mono-2.10.9] [Ge et al. 2011], JAFFA [v.1.09] [Davidson et al. 2015], and SOAPfuse [v.1.27] [Jia et al. 2013]). Fusions were assessed for biological significance if identified by at least three tools and the fusion was rare (<10% frequency) within our internal cancer cohort. Fusion of genes located within 200 kb on the same chromosome strand were considered readthrough events and not evaluated further. A comprehensive literature review of the fusion genes was performed to determine the putative mechanism (i.e., activation due to fusion of an oncogene or loss of function of a tumor suppressor) and to identify previous reports of similar fusion events. Transcripts per million (TPM) values were generated from paired-end RNA sequence data using Salmon with bootstrapping set to 100 (Patro et al. 2017). Gene expression data were compared to publicly available RNA sequence data from the University of California Santa Cruz (UCSC) Treehouse Childhood Cancer Initiative (https://treehousegenomics.soe.ucsc.edu/). Gene coverage was evaluated at the exon level. Briefly, a BED file was generated of exon coordinates using UCSC Genome Browser. Coverage was obtained from sorted BAM files using BEDtools (v.2.29.0) multiBamCov (Quinlan and Hall 2010). GenVisR was used to visualize coverage depth across RBPMS and MET (Skidmore et al. 2016).

Sanger Sequencing of RBPMS-MET Fusion

We used 500 ng of RNA with MultiScribe reverse transcriptase (ThermoFisher) and random hexamers (Applied Biosystems) for RT-PCR. PCR of cDNA was performed with the forward primer in RBPMS exon 5 (5’ATCCGCTTCGATCCTGAAAT 3’) and reverse primer in MET exon 15 (5’TGACTTCATTGAAATGCACAA 3’). PCR product was purified using the QIAquick purification kit (QIAGEN). Forward and reverse Sanger sequencing reactions were performed with the Big Dye v3.1 terminator mix (ThermoFisher). Sequencing was performed on an Applied Biosystems 3130 instrument.

SMARTer cDNA Synthesis

First-strand cDNA was prepared from 1 µg tumor total RNA in a single SMARTer (SMARTer PCR cDNA Synthesis Kit, Takara Bio USA) according to the company protocol. First-strand cDNA was diluted with 90 µL Buffer EB (QIAGEN). Second-strand synthesis was performed in a large-scale PCR reaction with a universal PCR primer added during first-strand cDNA synthesis. Second-strand synthesis reactions were prepared using PrimeSTAR GXL DNA polymerase in the following reaction: 10 µL diluted first-strand cDNA, 0.2 mM dNTPs, 0.24 µM 5’ PCR Primer IIA, 1.25 units PrimeSTAR GXL DNA Polymerase in 1× PrimeSTAR GXL Buffer with PCR cycling conditions of 30 sec at 98°C, 12× (10 sec at 98°C, 15 sec at 65°C, 10 min at 68°C) and 5 min at 68°C. Large-scale PCR reactions were combined after
amplification and concentrated with 1× Pacific Biosciences AMPure. Concentrated second-strand cDNA was eluted in 50 µL Buffer EB. The concentrated second-strand cDNA concentration was determined with Qubit 1× dsDNA HS Working Solution kit and the Qubit 3.0 fluorometer (Thermo Fisher Scientific Inc.).

cDNA Size Fractionation
Concentrated second-strand cDNA was size-fractionated with a double-sided SPRIsel ect reaction. Initially, 50 µL second-strand cDNA was combined with 20 µL SPRIsel ect reagent (0.4×). The supernatant of this reaction was transferred to a clean tube and combined with 30 µL SPRIsel ect (0.4/1×). This sequential reaction results in cDNA sized at >2 kb and 0.2–2 kb, respectively. The size-fractionated cDNA concentration was determined with Qubit 1× dsDNA HS Working Solution kit and the Qubit 3.0 fluorometer and the average library size for the cDNA size fractions was determined with the Agilent High Sensitivity DNA Kit on the Bioanalyzer 2100.

SMRTbell Template Prep
Molar concentrations for each cDNA fraction were calculated using the average cDNA size determined by the 2100 Bioanalyzer and the mass as determined by Qubit. Prior to PacBio library preparation, an equal molar dilution was prepared from the 0.4× SPRIsel ect and 0.4/1× SPRIsel ect fractions. A SMRTbell Template Prep Kit 1.0 library was prepared from 1 µg size-fractionated cDNA according to the PacBio Iso-Seq protocol (PN 101-070-200 Version 05 [November 2017]). The final library was diluted 1:5 with Buffer EB and assessed for DNA concentration by Qubit dsDNA HS and size by 2100 Bioanalyzer High Sensitivity DNA kit.

Pacific Biosciences Sequel Iso-Seq Sequencing
Library metrics metadata values including available volume, concentration, and average insert size were entered into the SMRT Link Sample Setup worksheet. The worksheet calculates the required sequencing primer v3 annealing and polymerase binding conditions for each SMRTbell library. The SMRTbell library was complexed with the Sequel Binding Kit 2.1, purified and concentrated according to the AMPPure PB protocol loaded at 8 pM with the diffusion protocol. Sequencing was performed with two v2 LR SMRT Cells, v2.1 Sequencing Reagents, and 4-h preextension followed by 20-h movie collection. Coverage metrics are provided in Supplemental Table 2.

Iso-Seq Analysis in SMRT Link
Both SMRT Cells were analyzed with the Iso-Seq application in SMRT Link version 8.0.0.79519 with the default parameters (Maximum CCS Read Length = 15,000, Minimum CCS Read Length = 50, Polish CCS = false, Filters to add to the DataSet = none, Minimum Accuracy for High-Quality Isoforms = 0.99, Maximum Fuzzy Junction Difference (bp) = 5, Ignore BioSamples records from Run Design = false, Minimum Mapped Concordance (%) = 95, Minimum Mapped Coverage (%) = 99, Minimum Mapped Length (bp) = 50, Require and Trim Poly(A) Tail = true, Run Clustering = true). The resulting BAM file of Mapped High-Quality Isoforms was visualized in the Integrated Genomics Viewer version 2.8.0 to identify RBPMS-MET fusion transcripts.
Data Deposition and Access
Molecular data from the diagnostic case have been deposited to dbGAP accession phs001820.v1.p1., under submitter: Institute for Genomic Medicine (IGM) Clinical Laboratory, Nationwide Children’s Hospital. Details are provided in the Methods. The RBPMS-MET fusion has been submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) under accession number SCV001433870.

Ethics Statement
All patients or their guardians provided written informed consent for genomic sequencing. This research is under a protocol approved by the Institutional Review Board at Nationwide Children’s Hospital (IRB17-00206).

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Author Contributions
A.G. wrote the first manuscript. A.G., J.A.B., K.M.S., C.E.C., S.C.K., K.R.J., and B.A.S. wrote, reviewed, discussed, edited, and revised the manuscript. V.M., S.D.M., K.L., E.V., R.K.W., P.W., E.R.M., C.E.C., and K.M.S. performed data analysis and interpretation.

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