



BRAF V600E-mutated metastatic pediatric Wilms tumor with complete response to targeted RAF/MEK inhibition

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Abstract Wilms tumor (WT) is the most common renal malignancy of childhood and accounts for 6% of all childhood malignancies. With current therapies, the 5-yr overall survival (OS) for children with unilateral favorable histology WT is greater than 85%. The prognosis is worse, however, for the roughly 15% of patients who relapse, with only 50%–80% OS reported in those with recurrence. Herein, we describe the extended and detailed clinical course of a rare case of a child with recurrent, pulmonary metastatic, favorable histology WT harboring a BRAF V600E mutation. The BRAF V600E mutation, commonly found in melanoma and other cancers, and previously undescribed in WT, has recently been reported by our group in a subset of epithelial-predominant WT. This patient, who was included in that series, presented with unilateral, stage 1, favorable histology WT and was treated with standard chemotherapy. Following the completion of therapy, the patient relapsed with pulmonary metastatic disease, that then again recurred despite an initial response to salvage chemotherapy and radiation. Next-generation sequencing (NGS) on the metastatic pulmonary nodule revealed a BRAF V600E mutation. After weighing the therapeutic options, a novel approach with dual BRAF/MEK inhibitor combination therapy was initiated. Complete radiographic response was observed following 4 months of therapy with dabrafenib and trametinib. At 12 months following the start of BRAF/MEK combination treatment, the patient continues with a complete response and has experienced minimal treatment-related side effects. This represents the first case, to our knowledge, of effective treatment with BRAF/MEK molecularly targeted therapy in a pediatric Wilms tumor patient.

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INTRODUCTION

Wilms tumor (WT) is the most common renal malignancy of childhood, occurring in 1 in 10,000 children (Doganis et al. 2019) and accounting for 6% of childhood malignancies (Davidoff 2012). Current strategies to treat WT include chemotherapy, surgery, and radiation therapy. This multimodal approach, along with advances in diagnostic evaluation and

optimal risk stratification, has led to an increase in the 5-year overall survival for children with unilateral favorable histology WT to greater than 85% (Dome et al. 2013).

Numerous somatic genetic alterations associated with WT have been identified, the most common of which involve *WT1*, *CTNNB1* (β -catenin), *DROSHA*, *AMER1* (*WTX*), and *TP53* (in anaplastic histology WT). Additionally, epigenetic alterations involving *IGF2* are commonly observed (Kalapurakal et al. 2004). Mutations in genes that encode elements of the RAS-BRAF-ERK signaling pathway, however, have not been previously associated with WT. BRAF V600E mutations, which are commonly found in melanoma, colon cancer, and other cancers, have been described in the majority of metanephric neoplasms, which morphologically overlap with differentiated forms of WT (Argani et al. 2016). The family of metanephric neoplasms of the kidney include metanephric adenoma (MA) and metanephric stromal tumor (MST), which harbor BRAF V600E mutations in 90% and 65% of cases, respectively (Chami et al. 2015; Udager et al. 2015). We recently described the novel finding of BRAF V600E mutations occurring in epithelial predominant WT with differentiated areas overlapping with MA in children and adults and noted briefly that one child had responded to BRAF-targeted therapy (Wobker et al. 2019). The efficacy of BRAF/MEK inhibition in BRAF V600E-mutated WT has not been reported previously. A basket trial of nonmelanoma cancers with BRAF V600E mutations demonstrated a modest response rate to inhibition of mutant BRAF in a wide range of tumors (Hyman et al. 2015). Herein, we describe the details of the clinical response to dual BRAF/MEK inhibition in a pediatric patient with metastatic epithelial-predominant WT with BRAF V600E mutation. The imaging, pathologic findings, molecular findings, and therapeutic approach are discussed.

RESULTS

Clinical Presentation

This case was previously included in our initial study of BRAF-mutated epithelial predominant WT with MA-like areas (Wobker et al. 2019; Case 8), but with only minimal clinical information. A 6-yr-old boy presented with right flank pain and hematuria. Abdominal computed tomography (CT) revealed a 7.4 × 8.4-cm mass in the right kidney (Fig. 1). No other sites of disease were identified. The patient underwent immediate right radical nephrectomy, as per North American practice, and histopathologic analysis of the specimen revealed a stage 1 WT of favorable histology. Of note, the tumor was triphasic but epithelial-predominant and contained differentiated areas that overlapped morphologically with MA (Fig. 2A–C). The patient received chemotherapy according to the National Wilms Tumor Study-5, Regimen EE-4A (vincristine, dactinomycin). Following an unremarkable 5-month treatment course, the patient was considered to have no evidence of disease. A surveillance chest CT scan performed 5 months post-completion of planned therapy revealed a solitary 3-mm left upper lobe (LUL) lung nodule of uncertain significance. Additional imaging obtained 3 months later showed an increase in size to 5 mm, without evidence of tumor recurrence in the abdomen or pelvis. Excisional biopsy of the pulmonary nodule was performed, and pathologic examination revealed metastatic WT (Fig. 2D). Next-generation sequencing (NGS), using the Johns Hopkins institutional Solid Tumor Panel version 3.0 (Dalton et al. 2017), on the pulmonary nodule demonstrated a BRAF V600E mutation, without other actionable mutations (Tables 1 and 2; Supplemental Table 1).

Relapse therapy consisted of radiation therapy to the lungs (12 Gy) and chemotherapy per Children's Oncology Group protocol AREN0321 Regimen I (vincristine, doxorubicin, cyclophosphamide, etoposide). The patient completed 6 months of therapy and then was again considered to be without evidence of disease. Surveillance CT scan performed



Figure 1. (A) Coronal contrast-enhanced computed tomography (CECT) image of the abdomen demonstrates a heterogeneously enhancing mass measuring $7.3 \times 8.4 \times 8.4$ cm and arising from the right kidney. Calcific foci are present within the mass (not shown). (B) Origin of the mass from the right kidney, as indicated by the "claw sign" (arrows), is redemonstrated on the axial CT image.

3 months after completion of planned treatment, revealed at least ten 2- to 3-mm pulmonary nodules concerning for second metastatic relapse (Fig. 3A). Therapeutic options were considered, including conventional chemotherapy (including vincristine, irinotecan, carboplatin) followed by high-dose chemotherapy with autologous stem cell rescue (ASCR), as this approach has demonstrated efficacy in patients with WT in first relapse. Alternatively, a novel approach using molecularly targeted agents was considered, given the finding of BRAF V600E detected in the first metastatic tumor via NGS. The original kidney tumor and the lung metastasis were examined and confirmed to express BRAF V600E via immunohistochemistry (Fig. 2E,F). This finding lent support to the idea that BRAF V600E was an early event in the original tumor and not unique to the resected, first pulmonary metastasis. Given the expectation of a manageable toxicity profile, compared to chemotherapy followed by ASCR, combined BRAF and MEK inhibitor therapy was commenced. The patient was dosed according to recommended phase 2 doses (RP2D) determined in the phase 1 studies of these agents (McCowage et al. 2018; Kieran et al. 2019).

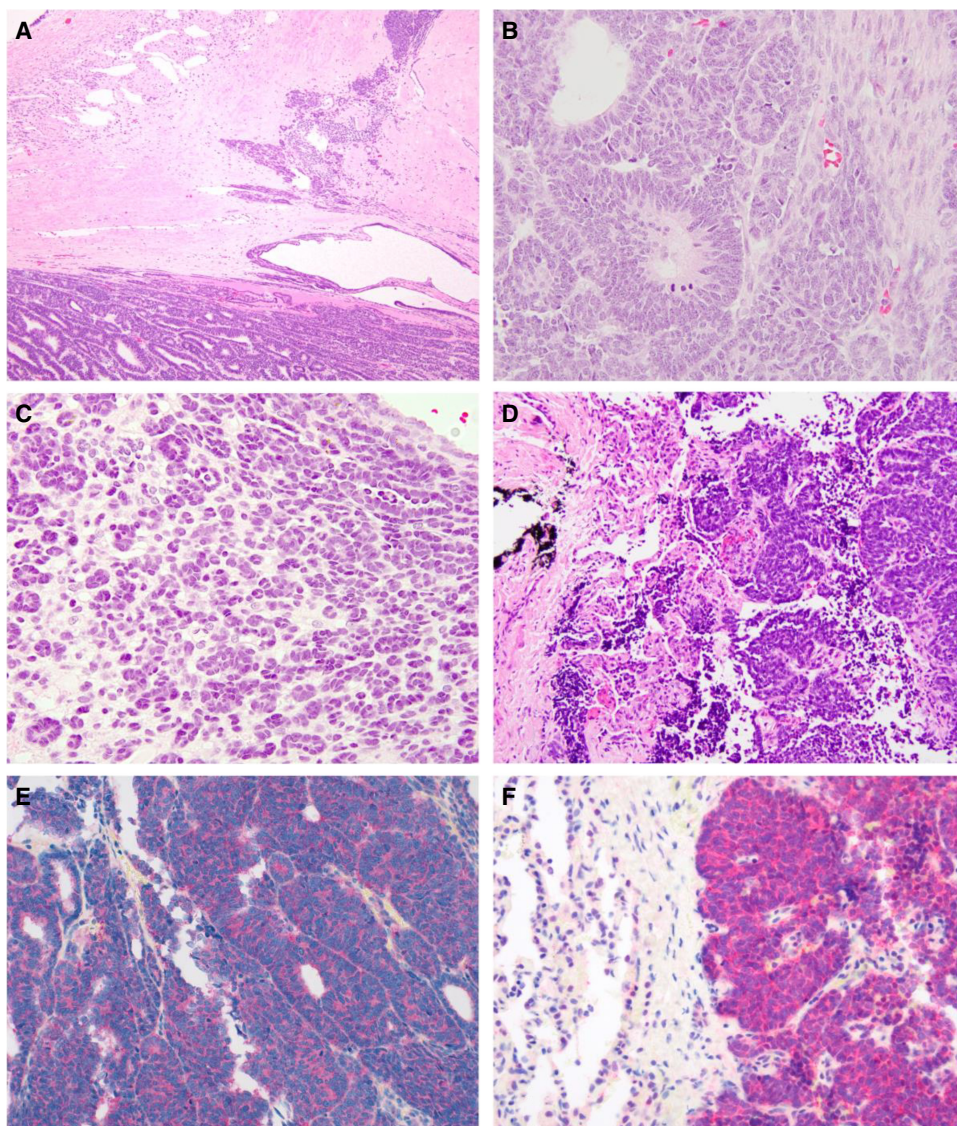


Figure 2. (A) The nephrectomy revealed an epithelial predominant Wilms tumor (WT). However, there was a differentiated area resembling metanephric adenoma (MA) associated with sclerosis (*upper right*). Normal kidney adjacent to tumor is at the *upper left*. (B) WT, triphasic area. Note the mitotic figures. (C) WT, differentiated area mimicking MA. Note the absence of mitotic figures and minimal cytoplasm of the neoplastic cells, which form tubules. (D) Lung metastasis of WT. (E) Cytoplasmic BRAF V600E protein immunoreactivity in the primary renal WT. (F) Cytoplasmic BRAF V600E protein immunoreactivity in the metastatic WT in the lung.

Table 1. Genomic variants identified using the Johns Hopkins Solid Tumor Panel v. 3.0: Genomic analysis

Chr:Pos	Base change	Reference database ID	Gene	AA_change	VAF (%)
Chr 7:140453136	A > T	COSM476	BRAF	p.V600E	45.14

(VAF) Variant allele frequency.

Table 2. Variant table

Gene	Chromosome	HGVS DNA reference	HGVS protein reference	Variant type (substitution, deletion, etc.)	Predicted effect	dbSNP/dbVar ID	Genotype (heterozygous/homozygous)
ATR	Chr 3:142178067	NM_001184.3: c.7349 + 2T > C	NA (splicing variant)	Substitution (splicing)	VUS	rs200556378; not reported in ClinVar	Heterozygous
BRAF	Chr 7:140453136	NM_004333.4: c.1799T > A	p.V600E	Substitution (missense)	Activation	rs113488022; ClinVar (29000)	Likely heterozygous
CDH1	Chr 16:68835713	NM_004360.4: c.304G > A	p.A102T	Substitution (missense)	Benign/Likely benign/VUS	rs368492235; ClinVar (152292)	Heterozygous
DICER1	Chr 14:95574707	NM_177438.2: c.2390A > G	p.D797G	Substitution (missense)	VUS	rs755375348; ClinVar (463780)	Heterozygous
DUSP9	Chr X:152914768	NM_001395.3: c.455C > T	p.P152L	Substitution (missense)	VUS	rs782504547; not reported in ClinVar	NA (X chromosome in male)
EP300	Chr 22:41574829	NM_001429.3: c.7114A > G	p.M2372V	Substitution (missense)	VUS	rs768061933; not reported in ClinVar	Likely heterozygous
FAS	Chr 10:90762940	NM_000043.5: c.185C > G	p.P62R	Substitution (missense)	VUS	rs757780022; not reported in ClinVar	Heterozygous
GPR124	Chr 8:37697730	NM_032777.9: c.2603C > T	p.A868V	Substitution (missense)	VUS	rs1443346346; not reported in ClinVar	Heterozygous
MAFB	Chr 20:39316965	NM_005461.4: c.526G > A	p.A176T	Substitution (missense)	VUS	rs750186410; not reported in ClinVar	Heterozygous
MYO18A	Chr 17:27417892	NM_078471.3: c.5240G > A	p.R1747Q	Substitution (missense)	VUS	rs767048813; not reported in ClinVar	Heterozygous
PASK	Chr 2:242066165	NM_015148.3: c.2165T > C	p.L722P	Substitution (missense)	VUS	rs201982321; not reported in ClinVar	Heterozygous
PCLO	Chr 7:82474598	NM_033026.5: c.14035G > C	p.G4679R	Substitution (missense)	VUS	Not reported in ClinVar	Heterozygous
SYNE1	Chr 6:152631852	NM_182961.3: c.16867C > T	p.R5623C	Substitution (missense)	VUS	rs570556738; not reported in ClinVar	Heterozygous
TCF3	Chr 19:1615488	NM_003200.3: c.1618C > G	p.P540A	Substitution (missense)	VUS	rs778885981; not reported in ClinVar	Heterozygous

(HGVS) Human Genome Variation Society, (dbSNP) Single Nucleotide Polymorphism Database, (dbVar) Database of Genomic Structural Variation, (VUS) variant of uncertain significance, (NA) not applicable.

Treatment Outcome

The patient began treatment with dabrafenib, a selective inhibitor of mutant BRAF, and trametinib, a selective allosteric inhibitor of MEK1/2. Chest CT obtained 8 weeks after start of therapy demonstrated resolution of several previously identified pulmonary nodules, and

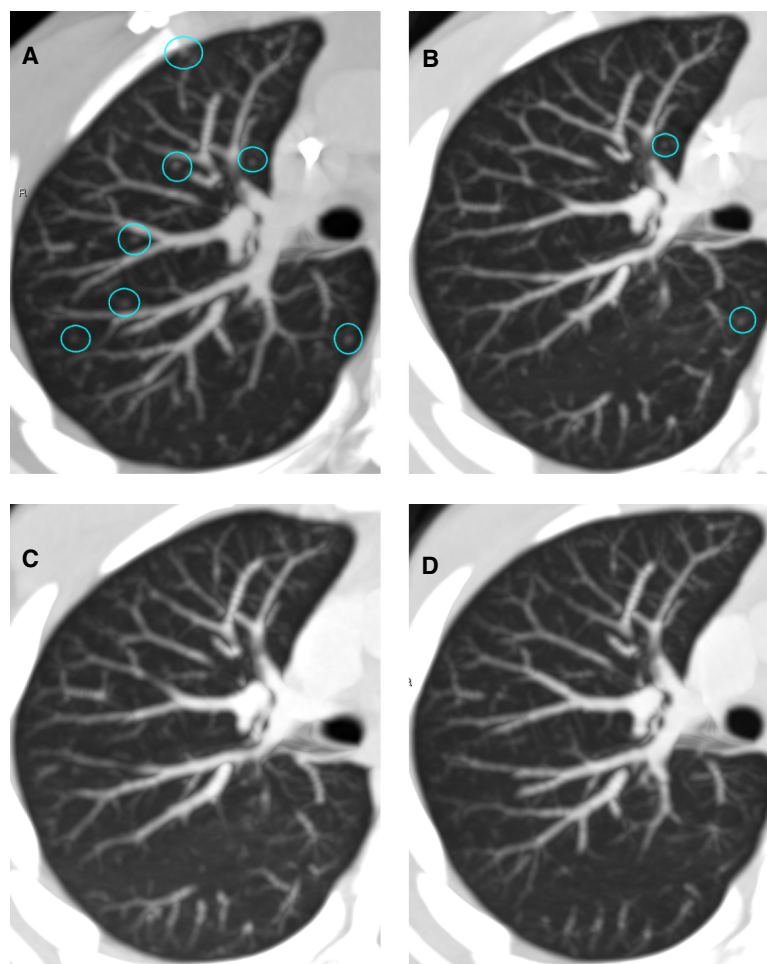


Figure 3. Axial nonenhanced chest CT (NECT) images demonstrating metastatic pulmonary nodules in the same region of the right upper lobe over a period of time on treatment, including (A) baseline (week 0), with multiple (>10) 2- to 3-mm nodules, (B) after 8 weeks of treatment, with a decrease in size of several nodules and resolution of others, (C) after 16 weeks of treatment, with complete resolution of all previously visualized nodules, and (D) 6 months after initiation of treatment on combined dabrafenib/trametinib, with continued complete imaging response to treatment.

decrease in size of the remaining nodules (Fig. 3A,B). Additional imaging obtained at 16 weeks on therapy revealed complete radiographic resolution of all previously identified pulmonary nodules (Fig. 3C), and no new evidence of tumor recurrence. The patient remains in a complete radiographic response, now 12 months after starting therapy (Fig. 3D). He continues to receive dabrafenib and trametinib with minimal treatment-emergent toxicities. Two episodes of pyrexia have been attributed to dabrafenib/trametinib treatment, each of several days' duration and resolving in less than 48 h upon holding medication, at which point the patient resumed treatment at the previous dose.

DISCUSSION

WT is a malignant embryonal neoplasm originating from metanephric blastema (Argani and Beckwith 2015). Until recently, knowledge of the genetic basis for WT was restricted to

aberrations of *WT1*, abnormalities of 11p15 methylation, and mutations that activate the WNT signaling pathway (*CTNNB1*, *AMER1/WTX*). Recent comprehensive genomic analyses of WT have identified additional mutations involved in Wilms tumorigenesis, including *DROSHA*, *DGCR8*, *DICER1*, *SIX1*, *SIX2*, *MYCN*, and *MLLT1* (Gadd et al. 2017). No clinically targetable oncogene mutations, however, have been identified in population-based sets of WT cases (Dalpa et al. 2017; Gadd et al. 2017). Recently, BRAF V600E mutations have been described in a subset of renal malignancies with overlapping histologies of WT and MA (Wobker et al. 2019). MA was previously thought to be a differentiated form of epithelial WT (Argani et al. 2016), with BRAF mutations occurring in upward of 85% of cases (Chami et al. 2015; Udager et al. 2015). A case series (including this case) from our institution described the novel finding of epithelial-predominant WT with areas of MA with BRAF V600E mutations (Wobker et al. 2019). This finding raised the possibility that it could represent a targetable alteration that could yield a sustained clinical response.

BRAF (sarcoma viral oncogene homolog B) is a member of the RAF serine/threonine protein kinase family and functions to transmit signals from upstream RAS to regulate ERK activity through direct phosphorylation of its substrates MEK1 and MEK2 (Ikawa et al. 1988; Marais and Marshall 1996). BRAF is commonly mutated in cancer, with the most common mutation (80%–90% of cases) a T to A transversion at nucleotide 1799 (T1799A), which results in a substitution of valine (V) for glutamic acid (E) at codon 600 (V600E) (Davies et al. 2002). The resulting mutation results in elevated BRAF kinase activity and thereby constitutive activation of ERK signaling (Wan et al. 2004). BRAF mutations are most often seen in melanoma, thyroid and colon carcinomas, and histiocytic disorders; they occur less frequently in breast and lung carcinoma, sarcomas, gliomas, leukemias, and lymphomas (Brose et al. 2002; Davies et al. 2002; Kimura et al. 2003). BRAF mutations have been shown to induce cellular transformation and increased cell survival in many model systems (Pritchard et al. 2007).

Vemurafenib was the first selective ATP-competitive inhibitor of mutant BRAF to gain FDA approval, based on demonstration of improved progression-free survival (PFS) seen in patients with metastatic BRAF-mutated melanoma compared to dacarbazine (McArthur et al. 2014). Similarly, dabrafenib was studied in patients with BRAF V600E-mutated metastatic melanoma and resulted in significantly improved PFS compared to dacarbazine (Hauschild et al. 2012). Dabrafenib gained FDA approval for treatment of unresectable or metastatic melanoma with BRAF V600E mutation in 2013.

Since then, BRAF inhibitors have demonstrated efficacy in a number of other tumor types, including colon (limited benefit as single agent) (Falchook et al. 2012; Kopetz et al. 2015); lung cancer (Planchard et al. 2016b) (FDA-approved); and histiocytic disorders (Langerhans cell histiocytosis [LCH] and Erdheim–Chester disease [ECD]) (Haroche et al. 2015). Among a histology-agnostic population of patients enrolled in a BRAF V600E selected basket trial, an overall objective response rate of 42% was seen in patients with non-small-cell lung cancer (NSCLC) and 43% in patients with ECD or LCH, confirming the driver role of the oncogene in a diverse selection of tumor types (Hyman et al. 2015). Likewise, a pediatric histology-agnostic phase 1 trial of dabrafenib in BRAF V600E tumors has been conducted in patients with low- or high-grade gliomas, LCH, and thyroid cancer (Kieran et al. 2019).

Despite the initial exciting responses seen in patients with BRAF-mutant cancers treated with vemurafenib and dabrafenib, most patients developed acquired resistance over time. In addition, in cells with wild-type RAF, including normal tissues, RAF inhibitors induce ERK signaling through transactivation of CRAF leading to MEK activation (Hatzivassiliou et al. 2010; Heidorn et al. 2010; Joseph et al. 2010; Poulidakos et al. 2010); this phenomenon underlies the spectrum of treatment-emergent hyperproliferative toxicities that may be seen (Su et al. 2012). MEK inhibitors in combination with BRAF inhibitors were trialed as a strategy

to overcome both acquired resistance as well as paradoxical MEK activation (Planchard et al. 2016a). Dual pathway inhibition (dabrafenib plus trametinib) showed superior efficacy to dabrafenib monotherapy in V600E metastatic melanoma (Long et al. 2015) and NSCLC (Planchard et al. 2016a). Recent publications report responses to dabrafenib plus trametinib in patients with high-grade gliomas (Schreck et al. 2018; Toll et al. 2019). A phase 2 study investigating the activity of dabrafenib in combination with trametinib in pediatric patients with low-grade glioma or refractory high-grade glioma with BRAF V600 mutation (NCT02684058) is ongoing (Hargrave et al. 2018).

Dabrafenib plus trametinib in combination has been well-tolerated, with rash, fatigue, joint pain, photosensitivity, and pyrexia being the most frequently reported toxicities (Grimaldi et al. 2015). High-dose chemotherapy followed by ASCR is established as effective therapy for first relapse in patients with high-risk or very-high-risk relapse, and is not required in standard-risk patients—that is, those treated initially with VCR/actinomycin (Ha et al. 2013). Its role, however, is not established in patients in second relapse. Further, high-dose chemotherapy with ASCR would have been accompanied by prolonged cytopenias, hospitalizations, and potentially life-threatening complications, with unproven benefit either in second relapse or in the unique subset of WT with BRAF mutations. Based on these factors, combination therapy with dabrafenib and trametinib was an attractive therapeutic option in the case of our patient.

This report provides clinical details of the first case, to our knowledge, of a complete response to RAF/MEK inhibition in a pediatric patient with metastatic epithelial-predominant BRAF V600E-mutated WT. This case adds to the ongoing discovery of driver mutations in WT and highlights the importance of adding molecular diagnostic studies to the histologic diagnosis. The finding of a BRAF V600E mutation allowed for the application of a targeted therapy that had not been previously reported in a child with WT. Of note, a recent single-patient case report described the rare case of an adult with metastatic epithelial-predominant WT with BRAF V600E mutation who achieved a partial response with BRAF inhibitor therapy (de Vries-Brilland et al. 2019). Together, these findings highlight the utility of histology-independent genomically selected basket studies for rare tumor types with targetable mutations. Objective responses seen across tissue types support the use of targeted therapies in tumor types which otherwise may not be studied in a tissue-specific trial.

Several questions remain unanswered, however. The optimal duration of combination therapy following a sustained CR is not established. The feasibility of maintaining the response remains to be seen, as acquired resistance has been shown to develop with dual RAF/MEK inhibition, as with single-agent RAF inhibitors. The majority of melanoma patients receiving RAF/MEK inhibitors will relapse within 1 year following an initial response (Arozarena and Wellbrock 2017). Various resistance mechanisms have been described with the majority of them converging on ERK reactivation (Nazarian et al. 2010; Villanueva et al. 2010; Poulidakos et al. 2011; Samatar and Poulidakos 2014; Wang et al. 2018). Preclinical studies investigating an optimal dosing schedule to prevent the development of resistance have suggested that intermittent dosing may forestall tumor adaptive changes (Abdel-Wahab et al. 2014). A new class of RAF dimer inhibitors is under clinical investigation and their use following the emergence of resistance to first-generation RAF inhibitors warrants continued exploration (Peng et al. 2015; Yao et al. 2015, 2019; Zhang et al. 2015). One could also consider the possibility of ASCR therapy to consolidate a response to molecularly targeted therapy, but this approach is one that has not yet been tested or validated in clinical trials.

Pediatric WT with BRAF V600E mutation has only recently been described. This is the first case of a patient with metastatic recurrence responsive to therapeutic RAF/MEK inhibition. This case supports the use of RAF/MEK inhibition as a therapeutic option in an increasing range of tumor types with BRAF V600E mutation.

METHODS

DNA was isolated from formalin-fixed paraffin-embedded tumor tissue using the automated Siemens Tissue Preparation System (Siemens Healthcare Diagnostics). NGS was also conducted using the HiSeq 2500 platform (Illumina). Briefly, libraries were prepared using the SureSelect-XT Target Enrichment System (Agilent Technologies) and an Agilent custom panel covering full coding regions of 432 cancer-related genes for clinical reporting of solid tumors. Sequencing was performed to an average 500×–1000× read depth on the HiSeq 2500 platform (Illumina). All reads were aligned to the human genome (GRCh37/hg19), using the Burrows–Wheeler alignment (BWA) algorithm. BWA files were generated using Picard Tools v1.119, and variant calling was performed using an in-house variant caller algorithm (MDLVC v5.0) and the HaplotypeCaller (Genome Analysis Tool Kit 3.3). Variants were reviewed using the Integrated Genomics Viewer (Broad Institute) and annotated using the Single Nucleotide Polymorphism Database (dbSNP) and Catalogue of Somatic Mutations in Cancer (COSMIC) database (Gondek et al. 2016; Palsgrove et al. 2018).

ADDITIONAL INFORMATION

Data Deposition and Access

Coding variants identified in the Johns Hopkins Solid Tumor Panel analysis are reported in the body of the manuscript. The patient did not provide consent for public deposition of all raw sequencing data for the 400 genes included in the Solid Tumor Panel version 3.0. The variants were submitted to ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) and can be found under accession numbers SCV001147030–SCV001147043.

Ethics Statement

The minor patient's parent has provided written consent to publication of relevant clinical information presented in the manuscript and was given the opportunity to read the manuscript prior to publication. The patient's parent understands that material will be freely available on the web and can be freely redistributed and that complete anonymity cannot be guaranteed.

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Author Contributions

S.S., D.S.R., J.S.D., A.D.F., and C.A.P. were responsible for patient care and clinical decision-making. P.O. and C.A.P. were responsible for the concept and design. E.D. provided radiographic interpretation and figures. P.A. provided pathologic diagnosis and figures. L.J. was responsible for the next-generation sequencing assay. P.O. and C.A.P. wrote the manuscript. All authors performed a final review of the manuscript.

Competing Interest Statement

C.A.P. is a paid consultant for Genentech/Roche (cobimetinib).

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