Metachronous Medulloblastoma in a Child With Successfully Treated Neuroblastoma: Case Report and Novel Findings of DNA Sequencing

Agda Karina Eterovic, PhD^a; Ossama M. Maher, MD^b; Joya Chandra, PhD^c; Ken Chen, PhD^d; Jason Huse, MD, PhD^e; and Wafik Zaky, MD^c

Abstract

Metachronous neoplasms have rarely been reported in patients with neuroblastoma. This report presents the clinical case of a 23-month-old child who was diagnosed with an anaplastic medulloblastoma 5 months after completing treatment for stage IV neuroblastoma. The patient was treated with complete surgical resection and adjuvant chemoradiation followed by maintenance chemotherapy at an outside institution and came to our institution for further management. A pathologic diagnosis and review of both the suprarenal and posterior fossa masses were performed, as well as a genetic analysis of both cerebellar tumor tissue and blood using next-generation gene sequencing. At our institution, the patient was submitted to induction chemotherapy followed by high-dose chemotherapy and autologous stem cell transplantation and remains free of disease 2 years after completion of treatment. Genetic analysis revealed multiple somatic copy number variations with most deleted genes located in 2q37, a region which harbors genes involved in epigenetic regulation and tumor suppression. A homozygous deletion was found in the *TSC2* gene, which is a clinically actionable gene, and patients with activating deletions in *TSC2* can potentially be eligible for basket clinical trials with mTOR inhibitors. Germline single nucleotide variants were also identified in multiple genes involved in cancer (*ALK*, *FGFR3*, *FLT3/4*, *HNFI1A*, *NCOR1*, and *NOTCH2/3*), cancer predisposition (*TP53*, *TSC1*, and *BRCA1/2*), and genes involved in DNA repair (*MSH6*, *PMS2*, *POLE*, and *ATM*). Metachronous neoplasms are rare and challenging to treat, hence genetic analysis and referral are needed to exclude hereditary cause. DNA sequencing of the tumor and germline can help identify alterations that increase predisposition or can be used to guide treatment decisions on recurrence and when standard options fail.

J Natl Compr Canc Netw 2018;16(6):683–691
doi: 10.6004/jnccn.2018.7009

Embryonal neoplasms of the central nervous system (CNS) represent nearly one-third of brain tumors in children aged <3 years.^{1,2} Medulloblastoma with its different histologic variants belongs to this group, representing up to 20% of pediatric CNS tumors. Cerebellar neuroblastoma is an extremely uncommon entity of this group with few published reports on primary cerebellar neuroblastoma.^{3,4} A number of second neoplasms have been reported in patients with neuroblastoma, including CNS tumors (gliomas and meningioma), but no prior reports of medulloblastoma. These second primary can-

cers have mostly occurred many years after long-term surveillance.^{5–7} This report presents a case of medulloblastoma in a child 5 months after successful treatment of stage IV neuroblastoma, and discusses the findings in the DNA sequencing results of both the resected posterior fossa tumor and the germline mutations.

Methods

Approval was obtained from The University of Texas MD Anderson Cancer Center Institutional Review

^aInstitute for Personalized Cancer Therapy, The University of Texas MD Anderson Cancer Center, Houston, Texas; ^bNicklaus Children's Hospital, Pediatric Hematology and Oncology, Miami, Florida; and the Departments of ^cPediatrics, ^dBioinformatics and Computational Biology, and ^ePathology, The University of Texas MD Anderson Cancer Center, Houston, Texas. Submitted May 27, 2017; accepted for publication January 24, 2018.

The authors have disclosed that they have no financial interests, arrangements, affiliations, or commercial interests with the manufacturers of any products discussed in this article or their competitors.

Correspondence: Wafik Zaky, MD, Department of Pediatrics, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 87, Houston, TX 77030. Email: wzaky@mdanderson.org

Board for this study (Clearing House protocol by Dr. Funda Meric-Bernstam), and informed consent was obtained. Separate consent for the molecular studies and analysis was also obtained. Pathologic diagnosis and review of the suprarenal and posterior fossa masses was performed by members of the Division of Pathology and Laboratory Medicine. Immunohistochemical studies that are pertinent to neuroblastoma and medulloblastoma tumors were used, including CD57 and neurofilaments.

DNA Sequencing and Analysis

Genomic DNA was quantified by PicoGreen (Invitrogen, Ltd.) and quality was assessed using Genomic DNA ScreenTape for the 2200 TapeStation (Agilent Technologies). Library preparation, targeted capture, and data analysis were completed as previously described.⁸ For data analysis, the T200 target-capture deep-sequencing data was aligned to human reference assembly hg19 using the Burrows-Wheeler Alignment tool⁹ and duplicated reads were removed using Picard.¹⁰ Single nucleotide variants and small insertions/deletions were detected using an in-house–developed analysis pipeline,¹¹ which classified variants into 3 categories: somatic, germline, and loss of heterozygosity based on variant allele frequencies in the tumor and the matched normal tissues. Copy number alterations were determined using a previously published algorithm,¹² which reports gain or loss status of each exon. To understand the potential functional consequence of detected variants, we compared them with the SNP Database, Catalogue of Somatic Mutations in Cancer,¹³ and The Cancer Genome Atlas database, and annotated them using VEP,¹⁴ ANNOVAR,¹⁵ CanDrA,¹⁶ and other programs. Translation consequence of the alteration, such as nonsynonymous, missense, stop-gain, frame-shift, and others, follows Sequence Ontology (<http://www.sequenceontology.org>). All aberrations that are nonsynonymous were shown. The software used for predicting consequences and clinical implications has been previously described: PolyPhen (the higher the score, the more damaging the aberration),¹⁷ SIFT (the lower the score, the more deleterious the aberration),¹⁸ and CanDrA¹⁶ to identify whether the mutation is the driver versus the passenger.

Case Description

A 10-month-old girl presented with 2-month history of proptosis and progressive periorbital ecchymosis. She was born full-term via vaginal delivery and has an insignificant past medical history, and her family history was negative for cancer. A CT scan revealed a right retro-orbital mass measuring 2.7 x 1.2 x 1.8 cm. A CT of the abdomen showed a calcified right adrenal mass measuring 6.5 x 5.5 x 5.5 cm with liver metastasis. Evaluation studies for staging including metaiodobenzylguanidine (MIBG) revealed disease at the anterior mediastinum, liver, bone marrow, skull, and third lumbar vertebra. Brain MRI revealed disease in the right orbital fossa extending to the middle cranial fossa, with no intracranial or cerebellar disease. The patient underwent fine-needle aspiration biopsy of the orbital mass, and neuroblastoma was diagnosed. A urine catecholamine test was performed initially and reported to be unremarkable; however, upon patient transfer to a tertiary facility with a pediatric oncology floor, this test was repeated and found to be elevated (91 ng/mL vs a normal institutional laboratory reference range of 15.7–17.0 ng/mL). Further staging evaluation was performed, including spine MRI and spinal tap, and found to be negative for disease.

Chemotherapy with vincristine, cyclophosphamide, cisplatin, and doxorubicin was initiated. After the first 2 cycles of chemotherapy, the patient underwent disease evaluation. Laparotomy and resection of the right adrenal mass and adjacent lymph nodes were performed, and pathology confirmed the diagnosis of neuroblastoma with poor differentiation and extensive treatment-related changes (necrosis and calcification) (Figure 1A). No NMYC amplification or 1p or 17q deletion were seen, and her karyotype was unremarkable. The patient was classified as having intermediate-risk disease and resumed chemotherapy consisting of vincristine, cyclophosphamide, cisplatin, dacarbazine, ifosfamide, and doxorubicin, followed by 5 maintenance cycles of 13-cis retinoic acid. Surveillance MIBG during maintenance chemotherapy revealed no residual disease.

Five months after completing treatment, the patient presented with repeated vomiting and new onset of difficulty walking after experiencing head trauma. MRI of the brain showed a mass in the right cerebellar hemisphere with hydrocephalus (Figure 1E). Com-

Medulloblastoma in Child With History of Neuroblastoma

Figure 1. (A) Posttherapy calcification of adrenalectomy and rare viable neuroblastoma cells showing good therapeutic response to adjuvant chemotherapy (hematoxylin-eosin, original magnification x100). (B) Small round blue cell tumor with anaplastic medulloblastoma features in a section of the brain (hematoxylin-eosin, original magnification x400). Immunohistochemical study (C) positive for anti-CD57 and (D) negative for neurofilament (original magnification x100, for both). (E) Axial T1 MRI of the brain with contrast showing a 5 × 4-cm enhancing mass in the right cerebellum that is compressing the adjacent structures. MRI of the (F) upper and (G) lower spine showing no evidence of metastatic disease, and (H) metaiodobenzylguanidine scan at restaging workup revealing no evidence of neuroblastoma recurrence.

plete surgical resection of the mass was performed and pathology revealed anaplastic medulloblastoma (Figure 1B). Metastatic workup, including spine MRI, spinal tap, urine catecholamine testing, and MIBG scan, was negative for disease (Figure 1F–H).

Chemoradiation with vincristine was initiated, and a brain MRI following completion was negative for disease. The treatment was stopped because disseminated fungal infection was suspected after the patient developed fever and neutropenia, and CT of the liver showed some nodularity but blood culture results were negative. Antifungal therapy was initiated for a total of 6 weeks. The treating team decided to decrease treatment intensity and the patient was switched to metronomic therapy with oral cyclophosphamide and topotecan for 2 cycles. The patient came to The University of Texas MD Anderson Cancer Center (MDACC) for a second opinion and further management. A review of the pathology at MDACC agreed with the findings of a small round blue cell tumor, positive CD57 test results, and the absence of neuroblastic differentiation, which indicate the diagnosis of medulloblastoma, anaplastic variant (Figure 1C, D). Considering the prior treatment of neuroblastoma and the

incomplete treatment of medulloblastoma, the team decided to proceed with second-line chemotherapy with temozolomide and irinotecan for relapsed medulloblastoma and, if the patient responded well, to follow with high-dose chemotherapy and autologous stem cell transplant (SCT) for curative intent. She was started on temozolomide (150 mg/m² × 5 days) and irinotecan (50 mg/m² × 5 days) every 28 days for 5 cycles.

No disease recurrence was seen on end-of-induction imaging and spinal tap, and therefore the patient proceeded with a planned 3 tandem autologous SCT with a conditioning regimen of carboplatin and thiopeta. The patient tolerated the treatment well, and end-of-treatment brain and spine MRI and lumbar puncture revealed no evidence of disease. At time of writing, the patient continues to be in remission from both diseases for >2 years posttreatment.

DNA Sequencing and Copy Number Analysis

A genetic analysis of the medulloblastoma sample was performed using a targeted panel of 263 cancer-related genes.⁸ [Supplemental eTable 1](#) (available with this article at JNCCN.org) illustrates the genes

in the panel (all exons). Molecular sequencing of the cerebellar tumor tissue did not reveal any relevant, nonsynonymous somatic mutations. The absence of mutations in the *CTNNB*, *SMO*, *SUFU*, and *PATCH1* genes suggests a non-WNT, non-SHH type of medulloblastoma.

However, copy number analysis of the cerebellar tumor tissue identified homozygous deletions (H. DEL ≤ 1 copy) of various genes, including *ATG4B*, *AXIN1*, *CREBBP*, *D2HGDH*, *FBR5*, *GPC1*, *GPR35*, *HDLBP*, *ING5*, *KIF1A*, *PASK*, *PPP1R7*, *SNED1*, and *TSC2*, as well as high amplification (H. AMP ≥ 4 copies) of the *TOP1* and *SOX4* genes (Table 1, Figure 2A). Interestingly, most of the deleted genes were located in chromosome 2 (q37.3) and, less commonly, chromosome 16 (p11–13). Many other focal areas of lower level deletion or amplification were also observed in the cerebellar tumor, suggesting a moderate level of genomic instability (Figure 2B).

Germline DNA from blood was also tested using the same gene panel. Among hundreds of single nucleotide polymorphisms (SNPs) detected, we selected nonsynonymous alterations that are related to cancer (*ALK*, *FGFR3*, *FLT3/4*, *HNFI1A*, *NCOR1*, and *NOTCH2/3*), cancer predisposition (*TP53*, *TSC1*, and *BRCA1/2*), and DNA repair

(*MSH6*, *PMS2*, *POLE*, and *ATM*) (Table 2). On further analysis of the germline data using state of the art literature-based methodology to identify possible damaging, deleterious, and potentially driver mutations,^{15–18} we refined the list of germline SNPs of interest to include *FGFR3*, *FLT3/4*, *HNFI1A*, *NCOR1*, and *NOTCH3*. Unfortunately, not enough viable tissue was available to perform sequencing of the adrenal tumor.

Discussion

The occurrence of metachronous neoplasms is rare in children, with only a few reported cases in patients with neuroblastoma, including renal cell carcinoma, epithelioid tumor, and astrocytoma, but not medulloblastoma.^{19–21} Furthermore, cancer survivors are at a higher risk of second cancers compared with the general population. Analyses of 9,432 long-term neuroblastoma survivors in 3 major studies indicated that 96 patients developed second cancers, including carcinomas, soft tissue sarcomas, glioblastomas, meningioma, melanomas, and hematologic malignancies, but none developed medulloblastoma (Table 3).^{5–7} Neuroblastoma and, less commonly, medulloblastoma, have been reported in patients with cancer predisposition syn-

Table 1. Somatic Genes With Copy Number Changes					
Gene	Copy Change	Gene Copy	Gene Location	Function	Role in Tumorigenesis
<i>SOX4</i>	Amplification	9.3	6p22.3	Transcription factors	Multiple solid tumors, including osteosarcoma, prostate, and lung
<i>TOP1</i>	Amplification	6	20q12	DNA topoisomerase enzyme	Multiple cancers, including breast, colorectal, and melanoma
<i>ATG4B</i>	Deletion	1	2q37.3	Autophagy	Multiple cancers, including breast
<i>AXIN1</i>	Deletion	0.9	16p13.3	Tumor suppressor	Medulloblastoma and colorectal and gynecologic cancers
<i>CREBBP</i>	Deletion	0.9	16p13.3	Gene transcription and epigenetic regulation	Multiple cancers, including leukemia, brain, breast, and lung
<i>D2HGDH</i>	Deletion	1	2q37.3	Mitochondrial enzyme and epigenetic regulation	<i>IDH</i> -mutant cancers (gliomas) and prostate cancer
<i>FBR5</i>	Deletion	1	16p11.2	Fibrogenic lymphokine	Not known
<i>GPC1</i>	Deletion	1	2q37.3	Membrane proteoglycan	Not known
<i>GPR35</i>	Deletion	1	2q37.3	G-protein receptor	Gastric and breast cancers
<i>HDLBP</i>	Deletion	1	2q37.3	Protein regulate lipid metabolism	Involved in breast and prostate cancers, and hepatocellular carcinoma
<i>ING5</i>	Deletion	1	2q37.3	Tumor suppressor and epigenetic regulator	Multiple solid tumors, including glioblastoma, lung, and bladder
<i>KIF1A</i>	Deletion	1	2q37.3	Kinesin family protein and epigenetic regulation	Multiple solid tumors, including neuroblastoma, breast, and lung
<i>PASK</i>	Deletion	1	2q37.3	Serine/Threonine kinase and epigenetic regulator	Neuroblastoma and others
<i>PPP1R7</i>	Deletion	1	2q37.3	Protein phosphatase and epigenetic regulator	Multiple solid tumors
<i>SNED1</i>	Deletion	1	2q37.3	Structural protein	Possible role in cancer metastasis
<i>TSC2</i>	Deletion	0.9	16p13.3	Tumor suppressor	Medulloblastoma and lung and liver cancers

Medulloblastoma in Child With History of Neuroblastoma

Figure 2. Somatic copy number changes in the medulloblastoma sample. (A) Chromosome location of genes identified with high amplification (H. AMP) and homozygous deletion (H. DEL). (B) Overview of global copy number changes by chromosome. Red lines above and below the gray (normal) line indicate areas of gene amplification and deletion, respectively. Numbers on the y axis represent log-R ratios, and numbers on the x axis represent the gene index.

dromes such as neurofibromatosis type 1 and overgrowth syndromes such as Simpson-Golabi-Behmel syndrome.^{22–24} Our patient did not have clinical stigmata of these conditions and her genetic testing was negative for these syndromes.

Remarkably, the patient had several somatic copy number alterations in the cerebellar tumor, including deletions of *ATG4B*, *AXIN1*, *CREBBP*, *D2HGDH*, *FBRS*, *GPC1*, *GPR35*, *HDLBP*, *ING5*, *KIF1A*, *PASK*, *PPP1R7*, *SNED1*, and *TSC2* genes, as well as amplifications of *TOP1* and *SOX4* genes (Table 1, Figure 2A). Some of the deleted areas are in genes involved in the epigenetic regulation (*D2HGDH*, *ING5*, *PASK*, *PPP1R7*, *KIF1A*, and *CREBBP*), whereas others act as putative tumor suppressor genes (*TSC2*, *AXIN*, *KIF1A*, and *ATG4B*). Importantly, deletions of *TSC2* are potentially actionable, and on clinical validation, patients harboring these aberrations can be eligible for targeted therapy using mTOR inhibitors.²⁵ Therefore, this particular somatic deletion could guide treatment decisions for this patient after recurrence.

Recent studies have shown chromosome 2 microdeletions in several cancers, such as neuroblastoma and breast, lung, and cervical cancers.^{26–29} Strikingly, most of the deletions detected in this patient sample are in genes located in Chr2 q37.3 (Table 1, Figure 2A), an area that harbors many genes involved in skeletal and neuronal development. Although the 2q37.3 deletion was a somatic

event for our patient, it is interesting to mention that the 2q37 deletion syndrome consists of a germline loss of Chr2 (q37.1, q37.2, q37.3).³⁰ Children with this syndrome present with developmental delays and learning disabilities, as well as autism-like symptoms, seizures, and low muscle tone, among other symptoms. However, no germline 2q37 deletion was observed in our patient.

Another intriguing finding was the presence of multiple nonsynonymous germline alterations in genes involved in DNA repair (*BRCA 1/2*, *MSH6*, *PMS2*, *POLE*, *ATM*, and *ATR*) and cancer-predisposing syndromes (*TP53* and *TSC1*). On further analysis, few alterations were found to be clinically relevant; for example, we found *POLE* to be deleterious, *BRCA1* to be damaging, and *MSH6* to be both damaging and deleterious. Surprisingly, a group of genes that were found to be damaging, deleterious, and possible drivers includes *NOTCH3*, *FGFR3*, *FLT3*, *FLT4*, *HNF1A*, and *NCOR1*, which have been recognized in literature to play a role in cancer tumorigenesis. Several past studies suggest that genetic alterations in DNA damage response genes can determine the individual risk of developing cancer.^{29,31} *MSH6* and *PMS2* genes belong to the mismatch repair system and play a basic role in genome integrity maintenance by correcting single base pair mismatches after DNA replication. Mutations of the aforementioned genes have been reported in cancer predisposition syndromes, including Turcot and

Eterovic et al

Table 2. Germline Variations Detected by a Targeted Sequencing Panel of 263 Cancer-Related Genes

Gene Symbol	Chromosome Location	Nucleotide	AA Position	AA Change	dbSNP ID	Polyphen1	SIFT2	Candra3
ALK	2_29444076_C/A	237	79	F/L	rs1534545	benign(0)	unknown	unknown
ALK	2_29416572_A/G	4381	1461	I/V	rs1670283	benign(0)	tolerated(1)	Passenger(0.0020182)
ATM	11_108183167_T/C	5948	1983	N/S	rs659243	benign(0.001)	tolerated(0.89)	Passenger(0.097881)
ATR	3_142281612_T/C	632	211	M/T	rs2227928	benign(0)	tolerated(1)	Passenger(0.7336)
ATR	3_142178144_G/A	7274	2425	R/Q	rs2229032	benign(0.001)	tolerated(0.37)	Passenger(0.59435)
BRCA1	17_41244936_C/T	2612	871	P/L	rs799917	benign(0)	tolerated(1)	Passenger(0.51463)
BRCA1	17_41244000_A/G	3548	1183	K/R	rs16942	benign(0.001)	tolerated(1)	Passenger(0.069627)
BRCA1	17_41244435_A/G	3113	1038	E/G	rs16941	possibly_damaging(0.458)	tolerated(0.06)	Passenger(0.74874)
BRCA1	17_41223094_A/G	4900	1634	S/G	rs1799966	probably_damaging(0.925)	deleterious(0.04)	Passenger(0.58022)
BRCA2	13_32929387_T/C	7397	2466	V/A	rs169547	benign(0)	tolerated(1)	Passenger(0.53481)
CHEK1	11_125525195_A/G	1411	471	I/V	rs506504	benign(0.001)	tolerated(1)	Passenger(0.067608)
FGFR3	4_1806629_C/T	1351	451	P/S	rs61735104	possibly_damaging(0.479)	deleterious(0.04)	Driver(0.81385)
FLT3	13_28624294_C/T	680	227	T/M	rs1933437	possibly_damaging(0.817)	deleterious(0.03)	Driver(0.77501)
FLT4	5_180046344_C/G	2670	890	H/Q	rs448012	benign(0.32)	deleterious(0.03)	Driver(0.66398)
HNF1A	12_121416650_T/G	79	27	I/L	rs1169288	benign(0.004)	deleterious(0.05)	Driver(0.71491)
HNF1A	12_121437221_A/G	1652	551	L/S	rs1169304	unknown	deleterious(0)	unknown
MSH6	2_48025764_G/A	472	158	R/C	rs1800937	possibly_damaging(0.88)	unknown	unknown
NCOR1	17_16068463_G/A	448	150	G/R	rs74453660	probably_damaging(0.957)	deleterious(0.02)	Driver(0.58923)
NCOR1	17_15973774_A/C	4218	1406	L/F	rs61753150	probably_damaging(0.96)	deleterious(0.01)	Driver(0.23452)
NOTCH2	1_120611554_A/G	515	172	N/S	rs2794165	unknown	unknown	unknown
NOTCH2	1_120611555_A/C	514	172	N/H	rs2603925	unknown	unknown	unknown
NOTCH2	1_120611758_C/T	313	105	R/W	rs61790276	unknown	unknown	unknown
NOTCH3	19_15273335_G/A	5854	1952	V/M	rs115582213	probably_damaging(0.936)	deleterious(0)	Driver(0.57713)
PMS2	7_6026775_A/G	1621	541	K/E	rs2228006	benign(0)	tolerated(1)	Passenger(0.25227)
PMS2	7_6026865_A/G	1531	511	T/A	rs2228007	benign(0)	tolerated(1)	Passenger(0.12916)
PMS2	7_6026988_C/T	1408	470	P/S	rs1805321	benign(0.008)	tolerated(0.83)	Passenger(0.036327)
POLE	12_133201947_A/G	458	153	H/R	rs4883544	benign(0)	tolerated(0.35)	unknown
POLE	12_133220526_A/G	4187	1396	N/S	rs5744934	benign(0.076)	deleterious(0.05)	Passenger(0.77497)
PRG4	1_186273994_G/A	538	180	R/W	rs2273779	unknown	unknown	Passenger(0.4551)
PRG4	1_186275564_G/A	713	238	T/M	rs12128607	unknown	unknown	Passenger(0.024218)
PRG4	1_186276661_T/C	1810	604	T/A	rs61831150	unknown	unknown	unknown
PRG4	1_186277088_C/G	2237	746	C/S	rs68069719	unknown	unknown	Passenger(0.25328)
SMARCA4	19_11106600_G/A	1783	595	R/W	rs11672232	unknown	deleterious(0)	unknown
TP53	17_7579472_C/G	215	72	P/R	rs1042522	benign(0.145)	tolerated(0.16)	Passenger(0.79213)
TSC1	9_135786904_T/C	965	322	M/T	rs1073123	benign(0)	tolerated(1)	Passenger(0.29465)

All aberrations shown are non-synonymous. The software used for predicting consequences and clinical implications have been previously described: (1) PolyPhen (the higher the score, the more damaging is the aberration), (2) SIFT (the lower the score the more deleterious the aberration), and (3) and CanDrA. Abbreviations: AA, amino acid; dbSNP, database single nucleotide polymorphism.

Lynch syndromes, and are associated with colorectal, brain, and other cancers.^{32,33} Furthermore, other genes belonging to the DNA repair machinery, including *ATM* and *POLE*, constitute a network that is crucial for genomic stability, acting as a barrier against tumorigenesis.

Other germline alterations were detected in genes that also seem to play a role in tumorigenesis and tumor progression, but mostly in a somatic context.^{34–38} For example, somatic aberrations in fibroblast growth factor receptor 3 (*FGFR3*), which is a tyrosine kinase receptor for the FGF ligands, confer a selectable survival advantage in multiple solid tumors.³⁹ The *FGFR3*IIIS is a splice variant that may act as a dominant negative, inhibiting *FGFR3*-

induced growth arrest and differentiation, and has been detected in both medulloblastoma and neuroblastoma.⁴⁰ Another example, the *FLT3* gene, which encodes a receptor tyrosine kinase, is a cytokine receptor that regulates hematopoiesis and mutations in this receptor, and can result in acute myeloid leukemia and acute lymphoblastic leukemia.⁴¹ Treatment with *FLT3* ligand induces proliferation and growth in neuroblastoma cell lines, which was significantly inhibited with *FLT3* antisense, indicating a role in tumorigenesis. *FLT4* is a receptor tyrosine kinase for VEGF-C and VEGF-D ligands, and plays vital role in angiogenesis through activation of AKT and MAPK signaling pathways, which promote proliferation, survival, and migration of endothelial cells, and reg-

Medulloblastoma in Child With History of Neuroblastoma

Table 3. Major Recent Studies Evaluating SMNs in Neuroblastoma Survivors

Study	Median Age of SMN Diagnosis (Range)	N	Result	RT	Chemotherapy	Outcome
Applebaum et al, ⁵ 2015	15 y (1–46 y)	2,801	SEER database analysis from 1973–2006 34 patients developed a SMN Diagnosis included renal cell carcinoma (n=7), thyroid carcinoma (n=5), hematologic malignancies or lymphoma (n=10), sarcoma (n=7), melanoma (n=1), meningioma (n=1), and other (n=3)	RT in 25% of patients	Multiple regimens	Cumulative incidence of SMN at 30 years for high-risk patients was 10.44% (95% CI, 3.98%–20.52%) vs 3.57% (95% CI, 1.87%–6.12%) for non-high-risk patients (P<.001)
Applebaum et al, ⁶ 2017	6.7 y (9–23.6 y)	5,987	INRG database from 1990–2010 43 patients developed a SMN Diagnosis included ALL or lymphoma (n=9), AML (n=10), sarcoma (n=12), carcinoma (n=6), and brain tumor (n=6)	Not defined	Multiple regimens	10-year cumulative incidence of SMN for high-risk patients was 1.8% (95% CI, 1.0%–2.6%) vs 0.38% (95% CI, 0.22%–0.94%) for low-risk patients (P=.01) High-risk patients had an almost 18-fold higher incidence of SMN vs age- and sex-matched controls (SIR = 17.5; 95% CI, 11.4–25.3)
Huibregtse et al, ⁷ 2016	4.4 y (0.5–37.8 y)	644	Multi-institutional retrospective study between 1984 to 2014 19 patients developed a SMN after treatment with ¹³¹ I-MIBG Diagnosis included ALL (n=2), AML (n=10), thyroid carcinoma (n=2), sarcoma (n=2), inflammatory myofibroblastic tumor (n=2), and other (n=1)	RT in 17/19 patients	Multiple regimens	Cumulative incidence of SMN was 7.6% (95% CI, 4.4–13.0%) and 14.3% (95% CI, 8.3–23.9%) at 5 and 10 years, respectively

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; INRG, International Neuroblastoma Risk Group; MIBG, metaiodobenzylguanidine; RT, radiation therapy; SIR, standardized incidence ratio; SMNs, second malignant neoplasms.

ulates angiogenic sprouting. FLT4 has been found to be overexpressed in tumors, including medulloblastoma, glioblastoma, and neuroblastoma, and confers poor outcome in neuroblastoma.^{42,43} Finally, Notch receptors play an essential role in the regulation of central cellular processes during embryonic and postnatal development, and has been implicated in several cancers, including leukemia, glioma and medulloblastoma.⁴⁴ The Notch pathway prevents neuronal differentiation in the CNS by driving neural stem cell maintenance, and is therefore often implicated in the growth, survival, invasion, and recurrence of brain tumors. Moreover, neuroblastoma has high expression of Notch3, and its downstream-regulated genes have mesenchymal characteristics and are associated with an increased incidence of metastases and poor prognosis.⁴⁵

When this patient presented to MDACC, we were faced with 2 challenges: (1) determining whether the cancer was medulloblastoma versus neuroblastoma, and (2) dealing with the nonstandard treatment approach that was used at the abroad institution. Neuroaxial metastasis of neuroblastoma is not uncommon, and usually occurs at a median of 18 months in high-risk groups with elevated catecholamine. Furthermore, metastatic CNS neuroblastoma

frequently appears as a leptomeningeal or intraventricular mass, and less commonly as a parenchymal mass (with the frontal lobe the most common location), and not in the cerebellum. In our patient's case, results of the catecholamine test were negative and the tumor was in the cerebellum, and the lack of neurofilament staining and absence of neuroblastic differentiation favored the diagnosis of medulloblastoma. An entity called *cerebellar neuroblastoma* has been reported rarely in the literature, but there has been no report of its coexistence with extracranial neuroblastoma.

In infant medulloblastoma, high-dose chemotherapy and autologous SCT is a preferred treatment strategy to delay radiation and avoid its deleterious late effect.⁴⁶ Despite complete tumor resection and postoperative radiation therapy in our patient, and because the medulloblastoma was high-risk (due to its anaplastic histologic type and the patient's young age), we proceeded with induction chemotherapy followed by high-dose chemotherapy and autologous SCT to augment the suboptimal dose of radiation and incomplete treatment that was administered at the previous institution.

Regarding management and the therapeutic impact of molecular testing, the patient and parents

were referred to genetic services and the parents were offered genetic testing but decided not to proceed. Our recommendation for follow-up was to perform annual whole-body surveillance imaging with dedicated brain MRI and blood counts. Regarding treatment, future target agents to be considered in the event of recurrence include PARP inhibitors, with literature supporting their use in tumors with DNA damage repair genes alterations (*POLE*, *MSH6*, *BRCA1/2*).⁴⁷ Recently, PD-L1 inhibitors were postulated to be efficacious in treating patients with cancer with mismatch gene repair mutations (*MSH6*) and/or hypermutation.⁴⁸ This is in addition to other potential targets, such as mTOR inhibition (for tumors with *TSC2* deletion).²⁵

Conclusions

Metachronous neoplasms are rare and challenging to treat; hence, genetic testing and referral are needed to exclude familial cancer syndromes and to aid in treatment planning. In addition, DNA sequencing of the tumor can help to identify actionable somatic alterations that could guide treatment decisions when standard treatment fail upon recurrence.

Acknowledgments

The genetic analysis presented in this case reported was supported by the Khalifa Foundation and The Institute for Personalized Cancer Therapy (IPCT-MDACC). The patient was enrolled in the IPCT Clearing House protocol led by Dr. Funda Meric-Bernstam.

References

- Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol* 2016;131:803–820.
- Ostrom QT, Gittleman H, Farah P, et al. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2006–2010. *Neuro Oncol* 2013;15(Suppl 2):ii1–56.
- Pearl GS, Takei Y. Cerebellar “neuroblastoma”: nosology as it relates to medulloblastoma. *Cancer* 1981;47:772–779.
- Yagishita S, Itoh Y, Chiba Y, et al. Cerebellar neuroblastoma. A light and ultrastructural study. *Acta Neuropathol* 1980;50:139–142.
- Applebaum MA, Henderson TO, Lee SM, et al. Second malignancies in patients with neuroblastoma: the effects of risk-based therapy. *Pediatr Blood Cancer* 2015;62:128–133.
- Applebaum MA, Vaksman Z, Lee SM, et al. Neuroblastoma survivors are at increased risk for second malignancies: a report from the International Neuroblastoma Risk Group Project. *Eur J Cancer* 2017;72:177–185.
- Huibregtse KE, Vo KT, DuBois SG, et al. Incidence and risk factors for secondary malignancy in patients with neuroblastoma after treatment with (131)I-metaiodobenzylguanidine. *Eur J Cancer* 2016;66:144–152.
- Chen K, Meric-Bernstam F, Zhao H, et al. Clinical actionability enhanced through deep targeted sequencing of solid tumors. *Clin Chem* 2015;61:544–553.
- Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009;25:1754–1760.
- DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet* 2011;43:491–498.
- Zhou W, Chen T, Zhao H, et al. Bias from removing read duplication in ultra-deep sequencing experiments. *Bioinformatics* 2014;30:1073–1080.
- Lonigro RJ, Grasso CS, Robinson DR, et al. Detection of somatic copy number alterations in cancer using targeted exome capture sequencing. *Neoplasia* 2011;13:1019–1025.
- Bamford S, Dawson E, Forbes S, et al. The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website. *Br J Cancer* 2004;91:355–358.
- McLaren W, Pritchard B, Rios D, et al. Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. *Bioinformatics* 2010;26:2069–2070.
- Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* 2010;38:e164.
- Mao Y, Chen H, Liang H, et al. CanDrA: cancer-specific driver missense mutation annotation with optimized features. *PLoS One* 2013;8:e77945.
- Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. *Nucleic Acids Res* 2002;30:3894–3900.
- Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 2009;4:1073–1081.
- Kato K, Ijiri R, Tanaka Y, et al. Metachronous renal cell carcinoma in a child cured of neuroblastoma. *Med Pediatr Oncol* 1999;33:432–433.
- Tanaka M, Kato K, Gomi K, et al. Perivascular epithelioid cell tumor with SFPQ/PSF-TFE3 gene fusion in a patient with advanced neuroblastoma. *Am J Surg Pathol* 2009;33:1416–1420.
- Krieger JN, Chasko SB, Keuhnelian JG. Paratesticular neuroblastoma associated with subependymal giant cell astrocytoma. *J Urol* 1980;124:736–738.
- Jongmans MC, Loeffen JL, Waanders E, et al. Recognition of genetic predisposition in pediatric cancer patients: an easy-to-use selection tool. *Eur J Med Genet* 2016;59:116–125.
- Thomas M, Enciso V, Stratton R, et al. Metastatic medulloblastoma in an adolescent with Simpson-Golabi-Beckwith syndrome. *Am J Med Genet A* 2012;158A:2534–2536.
- Varan A, Sen H, Aydin B, et al. Neurofibromatosis type 1 and malignancy in childhood. *Clin Genet* 2016;89:341–345.
- Huynh H, Hao HX, Chan SL, et al. Loss of tuberous sclerosis complex 2 (*TSC2*) is frequent in hepatocellular carcinoma and predicts response to mTORC1 inhibitor everolimus. *Mol Cancer Ther* 2015;14:1224–1235.
- Takita J, Yang HW, Chen YY, et al. Allelic imbalance on chromosome 2q and alterations of the caspase 8 gene in neuroblastoma. *Oncogene* 2001;20:4424–4432.
- Piao Z, Lee KS, Kim H, et al. Identification of novel deletion regions on chromosome arms 2q and 6p in breast carcinomas by aneuploidy analysis. *Genes Chromosomes Cancer* 2001;30:113–122.
- Narayan G, Pulido HA, Koul S, et al. Genetic analysis identifies putative tumor suppressor sites at 2q35-q36.1 and 2q36.3-q37.1 involved in cervical cancer progression. *Oncogene* 2003;22:3489–3499.
- Rube CE, Fricke A, Schneider R, et al. DNA repair alterations in children with pediatric malignancies: novel opportunities to identify patients at risk for high-grade toxicities. *Int J Radiat Oncol Biol Phys* 2010;78:359–369.
- Falk RE, Casas KA. Chromosome 2q37 deletion: clinical and molecular aspects. *Am J Med Genet C Semin Med Genet* 2007;145C:357–371.
- Sijmons RH, Hofstra RM. Review: clinical aspects of hereditary DNA mismatch repair gene mutations. *DNA Repair (Amst)* 2016;38:155–162.
- Lindsay H, Jubran RF, Wang L, et al. Simultaneous colonic adenocarcinoma and medulloblastoma in a 12-year-old with biallelic deletions in *PMS2*. *J Pediatr* 2013;163:601–603.
- Scott RH, Mansour S, Pritchard-Jones K, et al. Medulloblastoma, acute myelocytic leukemia and colonic carcinomas in a child with biallelic *MSH6* mutations. *Nat Clin Pract Oncol* 2007;4:130–134.
- Privalsky ML. The role of corepressors in transcriptional regulation by nuclear hormone receptors. *Annu Rev Physiol* 2004;66:315–360.

Medulloblastoma in Child With History of Neuroblastoma

35. Heldring N, Nyman U, Lonnerberg P, et al. NCoR controls glioblastoma tumor cell characteristics. *Neuro Oncol* 2014;16:241–249.
36. Pugh TJ, Weeraratne SD, Archer TC, et al. Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. *Nature* 2012;488:106–110.
37. Bluteau O, Jeannot E, Bioulac-Sage P, et al. Bi-allelic inactivation of TCF1 in hepatic adenomas. *Nat Genet* 2002;32:312–315.
38. Rebouissou S, Vasiliu V, Thomas C, et al. Germline hepatocyte nuclear factor 1alpha and 1beta mutations in renal cell carcinomas. *Hum Mol Genet* 2005;14:603–614.
39. Kelleher FC, O'Sullivan H, Smyth E, et al. Fibroblast growth factor receptors, developmental corruption and malignant disease. *Carcinogenesis* 2013;34:2198–2205.
40. Sturla LM, Merrick AE, Burchill SA. FGFR3IIIS: a novel soluble FGFR3 spliced variant that modulates growth is frequently expressed in tumour cells. *Br J Cancer* 2003;89:1276–1284.
41. Frohling S, Scholl C, Gilliland DG, Levine RL. Genetics of myeloid malignancies: pathogenetic and clinical implications. *J Clin Oncol* 2005;23:6285–6295.
42. Eggert A, Ikegaki N, Kwiatkowski J, et al. High-level expression of angiogenic factors is associated with advanced tumor stage in human neuroblastomas. *Clin Cancer Res* 2000;6:1900–1908.
43. Huber H, Eggert A, Janss AJ, et al. Angiogenic profile of childhood primitive neuroectodermal brain tumours/medulloblastomas. *Eur J Cancer* 2001;37:2064–2072.
44. Teodorczyk M, Schmidt MH. Notching on cancer's door: notch signaling in brain tumors. *Front Oncol* 2014;4:341.
45. van Nes J, Chan A, van Groningen T, et al. A NOTCH3 transcriptional module induces cell motility in neuroblastoma. *Clin Cancer Res* 2013;19:3485–3494.
46. Cohen BH, Geyer JR, Miller DC, et al. Pilot study of intensive chemotherapy with peripheral hematopoietic cell support for children less than 3 years of age with malignant brain tumors, the CCG-99703 phase I/II study. A report from the Children's Oncology Group. *Pediatr Neurol* 2015;53:31–46.
47. Pihlak R, Valle JW, McNamara MG. Germline mutations in pancreatic cancer and potential new therapeutic options. *Oncotarget* 2017;8:73240–73257.
48. Viale G, Trapani D, Curigliano G. Mismatch repair deficiency as a predictive biomarker for immunotherapy efficacy. *Biomed Res Int* 2017;2017:4719194.

See JNCCN.org for supplemental online content.

Includes RT recommendations for 43 disease sites

NCCN Radiation Therapy Compendium™

The NCCN Radiation Therapy Compendium™ is designed to support decision-making around the use of radiation therapy in patients with cancer. The radiation therapy recommendations contained in the Compendium are derived directly from the library of NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®), which document evidence-based, consensus-driven management to ensure that all patients receive care most likely to lead to optimal outcomes.

Subscribe and learn more at:
NCCN.org/RTcompendium

The NCCN Radiation Therapy Compendium™ is copyrighted by NCCN. All rights reserved. NCCN Content may not be reproduced in any form for any purpose without the express written permission of NCCN. For licensing and permissions, please visit the Permissions Requests Section of NCCN.org.

JNCCN-N-0256-0518