case reports

Sustained Complete Response to Palbociclib in a Refractory Pediatric Sarcoma With *BCOR-CCNB3* Fusion and Germline *CDKN2B* Variant

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Introduction

Genomic alterations in the Ewing sarcoma family of tumors (EFT) were discovered > 30 years ago with the identification of the reciprocal translocation, t(11;22)(q24;q12), otherwise known as EWS-FL1.[1,2] In the time since, multiple other fusion partners with EWS have been identified that fit a similar Ewing sarcoma phenotype.[3,4] When EWS fusions are not identified, tumors with histologic features of Ewing sarcoma have been labeled as primitive neuroectodermal tumors. In 2012, Pierron et al[5] identified a subset of Ewing-like tumors harboring paracentric inversion on the short arm of chromosome X, resulting in the fusion of the *BCOR* and *CCNB3* genes.[5] Since that discovery, several small case series have further elucidated the clinical, morphologic, and genomic differences that make this diagnosis distinct from other round cell sarcomas, most notably Ewing sarcoma.[6-8]

Though distinct from Ewing sarcoma, most *BCOR-CCNB3*–fused sarcomas (BCS) are treated with upfront compressed chemotherapy with vincristine, doxorubicin, cyclophosphamide, ifosfamide, and etoposide plus local control with surgery and/or radiation. BCS shares similar event-free and overall survival rates with the standard EWS-FLI1–fused Ewing sarcoma using this treatment strategy.[6-8] Despite the growing knowledge base related to BCS, little is known about potential drug targets related to this disease entity, especially with regard to treatment of disease recurrence. We highlight the treatment of a young patient who had multiply-relapsed disease with the US Food and Drug Administration–approved cyclin-dependent kinase 4/6 (CDK4/6) inhibitor palbociclib; the tumor harbored a *BCOR-CCNB3* fusion and a germline variant in *CDKN2B*, and treatment resulted in a complete response and no evidence of disease 25 months into therapy.

Case History

Our male patient initially presented in 2010 at 1 year of age with a fixed mass on his back. Magnetic resonance imaging of the pelvis showed a large infiltrating presacral mass measuring 14 × 7.4 × 10.4 cm extending into the lower spinal canal, eroding the posterior right sacrum, and exerting a mass effect on both the rectum and bladder. A core needle biopsy was performed, which revealed a malignant, small, round, blue cell tumor along with small amounts of benign fibrofatty tissue and skeletal muscle. Tumor nuclei were round to oval with a fine-grained chromatin pattern and occasional small nucleoli or chromocenters. Immunohistochemical stains were positive for CD99, Fli1, and vimentin and were negative for NSE, synaptophysin, MYF4, GAF, CD45RB, and TdT—consistent with a primitive neuroectodermal tumor. No polymerase chain reaction–base fusion analysis or breakapart fluorescence in situ hybridization probe for *EWSR1* was performed at the time. Three generations of family history were negative for malignancies on either side of the family, including melanoma or pancreatic cancer. A staging computed tomography scan of the chest and a bone scan showed no evidence of metastatic disease. The patient started chemotherapy per Children's Oncology Group protocol AEWS0031, regimen B2, with ifosfamide, etoposide, vincristine, doxorubicin, and cyclophosphamide. Gross total resection was not feasible at the time per neurosurgery, and the patient received 57.6 Gy of proton beam radiation in October 2010. The patient remained in remission for > 2 years but then developed multiple local recurrences without metastases from 2013 to 2017 and underwent numerous surgeries, along with multiple different early-phase Children's Oncology Group therapeutic studies, as outlined in the timeline in Figure 1A. After the most recent recurrence in October 2016, the patient was referred to our Pediatric Cancer Precision Genomics Program. Because of the findings outlined here in the Results, we chose to start palbociclib in February 2017. This patient has no evidence of disease on imaging 25 months into therapy (Fig 1B) and has had only hematologic toxicity that was grade 2 or less.

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on March 18, 2020 and published at ascopubs.org/journal/po on April 30, 2020: DOI https://doi.org/10.1200/PO.19.00258

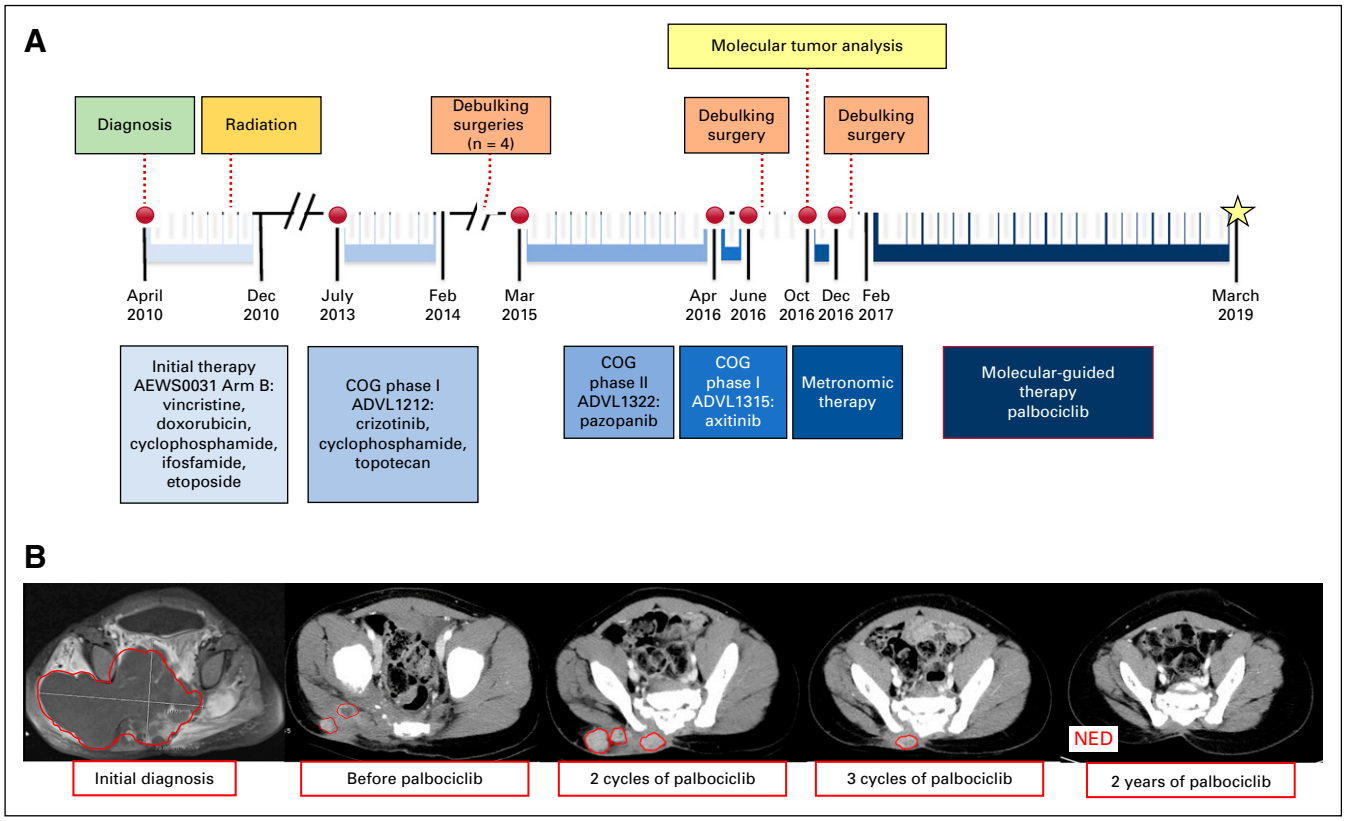

FIG 1. Patient timeline and response imaging. (A) The timeline of treatments and disease recurrences and progression in our patient. Red circles represent time of recurrence or progression. Hash marks represent prolonged period of time progressed through therapy (not to scale of remaining timeline). Patient is still continuing with palbociclib therapy after the most recent scans in March 2019. (B) Patient imaging displayed with initial diagnostic magnetic resonance imaging from 2010; subsequent relapse scans were done via computed tomography imaging. Red lines outline tumor boundaries. There was concern for progression after 2 cycles of palbociclib, but there was a 2-month lag between the "before palbociclib" scan and actually starting drug, so interval progression likely occurred in this timeframe. No new baseline scan was performed on the day palbociclib started. Each cycle of palbociclib was 28 days. NED, no evidence of disease.

Results

Whole-genome sequencing, RNA sequencing (RNA-Seq) analysis, germline exome sequencing, and protein evaluation were performed at the Clinical Laboratory Improvement Amendment (CLIA)–approved laboratory, NantOmics (Culver City, CA). Somatic DNA changes were determined by comparing the whole-genome DNA sequence from the tumor with the patient's germline sequence at 33X coverage. The mutational burden of the tumor was relatively low at 75,046 somatic mutations, with only 88 somatic mutations mapping to protein coding regions (Circos plot in Fig 2A). The tumor harbored an in-frame fusion of the second base in the last codon of BCOR exon 15 (chrX: 39,911,366) and the first base of CCNB3 exon 5 (chrX: 50,051,505) (Fig 2B). Additionally, an undescribed somatic mutation in the *SMO* gene (*SMO* N476S) was identified in the tumor, and germline sequencing revealed a *CDKN2B* N41D missense variant, which was heterozygous in both the germline and tumor genomes of this patient. RNA-Seq was also performed by NantOmics, and mRNA transcripts were ranked by abundance, which could be associated with increased pathway activity and sensitivity to a targeted drug. Overexpression of relevant tumor-promoting pathways is displayed in Table 1 and Figure 2C.

Discussion

Germline and somatic whole-genome DNA sequencing combined with RNA sequencing was used with the goal of developing a treatment plan, and it surprisingly provided our team with a more defined diagnosis of a recurrent BCS. The specific intrachromosomal fusion between *BCOR* and *CCNB3* in our patient's tumor is identical to previously described cases.[5,6] The *BCOR* gene itself can fuse to a number of 3′ partner genes in round cell sarcomas or additionally have internal tandem duplications, which have been reported to drive similar transcriptional patterns in a variety of sarcomas.[5,8,9] Similar to previous studies of mRNA transcripts in BCS,[5,8,9] both *BCOR* and *CCNB3* transcripts were highly overexpressed in our patient's tumor, as were the *HOX-A*, *-B*, and *-C* gene clusters (Table 2). Furthermore, analysis of our patient's tumor (Table 1) matched the BCS-specific fingerprint of genes used in

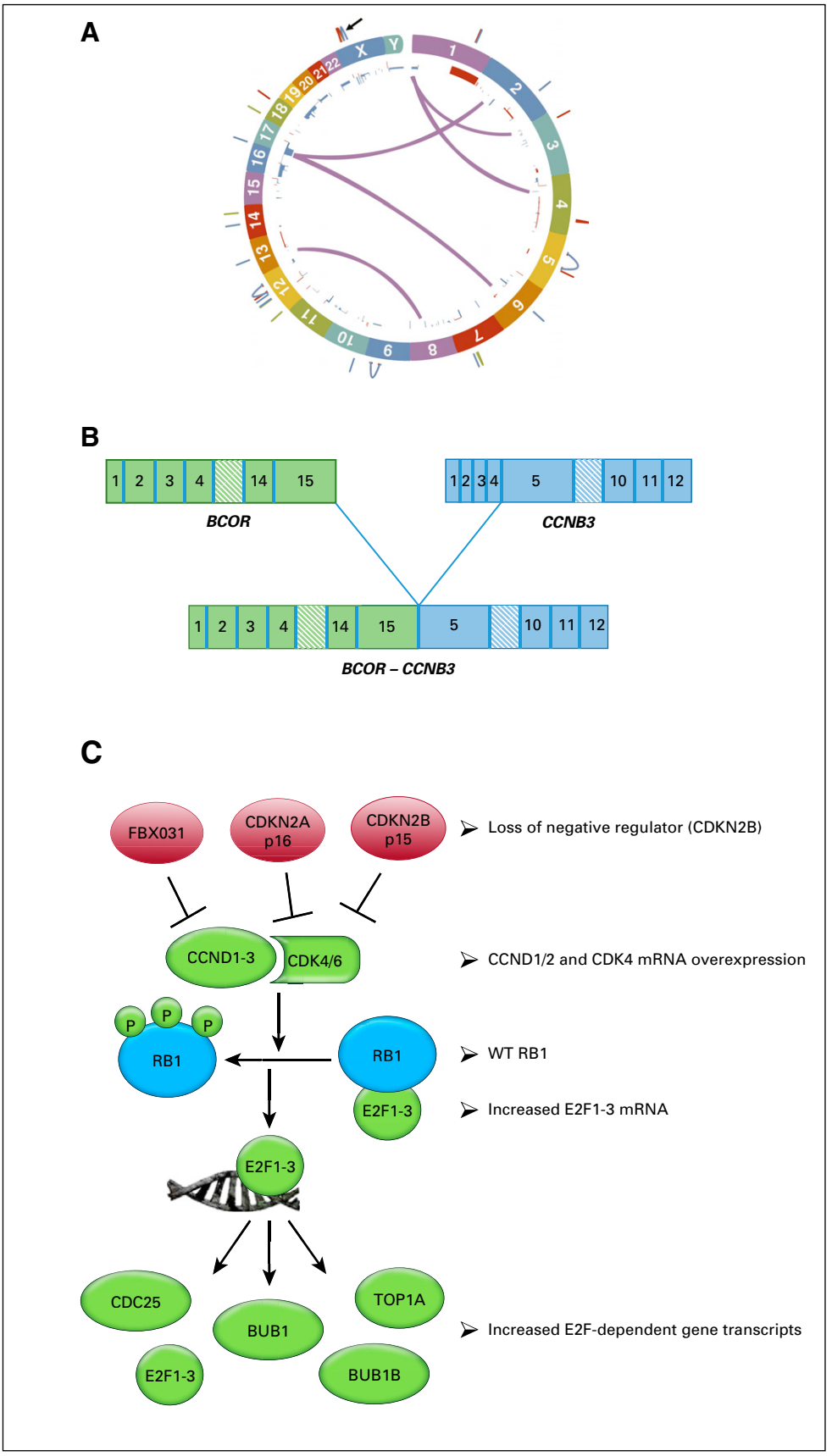

FIG 2. Transcriptome analysis reveals activation of the cyclin-dependent kinase 4/6 (CDK4/6)-RB pathway. (A) Circos plot of patient's tumor genome. (B) Identification of BCOR-CCNB3 fusion. This graphic illustrates the fusion of BCOR exon 15 to CCNB3 exon 5. With the exception of the destruction box in CCNB3, all functional domains from each encoded protein remain intact in the BCOR-CCNB3 fusion protein. (C) The CDK4/6 pathway is a gene regulatory program controlled by multiple tiers of protein kinases and transcriptional regulators. Increases in cyclin-D or CDK4 or 6 protein can lead to phosphorylation of the RB1-E2F tumor suppressor complex. Upon phosphorylation of RB1, the E2F1-3 transcription factors are released from the complex and are able to bind to the promoters of target genes, driving activation of transcription. These target genes include E2F1-3 themselves, the *CDC25* genes, *TOP2A*, *BUB1*, and *BUB1B*. WT, wild type.

the Riggi_Ewing_sarcoma_progenitor signature, which can be used to distinguish between BCS and EWS.[5,10] Most notable for this patient was the observation that multiple genes in the CDK4/6–RB pathway (Table 1; Fig 2C) were overexpressed, which made palbociclib, which has known pediatric dosing information, an attractive drug to use in this case.

Curiously, our patient was diagnosed at a very young age compared with the literature on BCS. In the several case series describing BCS, the median age of diagnosis is in the teenage years, with the youngest patient recorded at age 2.[5-8,11] Again, because of the age of presentation, one could be concerned about an inherited cancer syndrome. Alfaro-Cervello et al[12] published a case report of a congenital undifferentiated sarcoma with *BCOR-CCNB3* fusion possibly similar to our patient case. This congenital tumor also harbored a *SMARCB1*/*INI1* gene deletion common to malignant rhabdoid tumor, epithelioid sarcomas, and epithelioid malignant peripheral nerve sheath tumor that also, when found germline, is known to cause rhabdoid tumor predisposition syndrome.[12-16] In the case reported by Alfaro-Cervello et al,[12] *INI1* germline analysis was not performed. Our patient's tumor had functionally intact *INI1*, which precludes an effective comparison. Our patient also harbored a germline heterozygous missense variant, *CDKN2B* N41D. It is unclear what role this germline *CDKN2B* N41D variant could play in sarcomagenesis, as cancer risks associated with *CDKN2A*/*B* gene variants include melanoma, pancreatic cancer, and astrocytomas.[17,18] There is a recent short report from Jouenne et al[19] that found an increased risk of soft tissue sarcoma development with germline loss of *CDKN2A*, though no data exist confirming this risk with *CDKN2B* variants. Additionally, little is known about this actual variant in *CDKN2B*. Sunita et al[20] showed that the specific *CDKN2B* N41D variant, which encodes p15(INK4B), is unable to bind to the CDK6 protein, leading to loss of function of *CDKN2B*, which could lead to dysregulated control of S-phase entry. Though this variant's contribution to tumorigenesis is intriguing, *CKDN2B* was normally expressed in our patient's tumor, and there are no data suggesting that this impaired binding to CDK6 leads to mRNA overexpression along multiple levels of the CDK4/6 pathway.

TABLE 1. Overexpression of Relevant Tumor-Promoting Pathways

Gene	TPM	Status	Gene Function
CCND1	935	Overexpressed	Activating cyclin for CDK4 and CDK6
CCND2	69	Overexpressed	Activating cyclin for CDK4 and CDK6
CDK4	168	Overexpressed	RB1 protein kinase
E2F1	47	Overexpressed	RB1-regulated transcription factor
E2F2	24	Overexpressed	RB1-regulated transcription factor
E2F3	21	Overexpressed	RB1-regulated transcription factor
CDC25A	11	Overexpressed	E2F-regulated gene
CDC25C	7	Overexpressed	E2F-regulated gene
CDC25B	96	Overexpressed	E2F-regulated gene
TOP2A	162	Overexpressed	E2F-regulated gene
BUB1	35	Overexpressed	E2F-regulated gene
BUB1B	35	Overexpressed	E2F-regulated gene

NOTE. RNA sequencing (RNA-Seq) data highlights the transcripts of the CDK4/6 pathway that were overexpressed in our patient's tumor. processed by RNA-seq by Expectation-Maximization (RSEM) to estimate TPMs. Gene-level TPMs were used to determine if the gene was overexpressed using the upper 5th percentiles of per-gene RSEM TPM values for a collection of RNA-Seq datasets from The Cancer Genome Atlas normal samples. The expression status for a gene was classified as overexpressed if its TPM exceeded the gene's upper 5th percentile.[30]

Abbreviations: CDK, cyclin-dependent kinase; E2F, E2 transcription factor; RB1, retinoblastoma gene; TPMs, transcripts per million.

Despite discovering alterations of several key regulators of the CDK4/6 pathway in this tumor, none have been proven to serve as clinical biomarker for sensitivity to CDK4/6 inhibitors.[21] In a preclinical Ewing sarcoma orthotopic xenograft model with *CDKN2A* deletion, palbociclib was able to greatly suppress growth despite doxorubicin resistance of this model.[22] In other sarcoma subtypes, palbociclib reduced tumor burden in murine preclinical models.[23-25] Clinically, there is phase II evidence of palbociclib's efficacy in adults with liposarcoma[26,27] and leiomyosarcoma.[28] Despite growing evidence in these sarcomas, there are no published data testing CDK4/6 inhibitors in BCS. Additionally, though phase I/II trials are underway, the only published response data for palbociclib in pediatrics is a case report of growing teratoma syndrome,[29] thus making our use of this drug in a child novel.

To summarize, 3 independent observations supported consideration of therapeutic inhibition of the CDK4/6-RB1 pathway for this patient: (1) the presence of the *BCOR-CCNB3* gene fusion believed to drive entry into the cell cycle, (2) direct detection of an active CDK4/6-RB1

TABLE 2. Comparison of Patient Transcriptome With Published BCS Reference Publications

Gene	BCS Signature Reference	Patient With BCS	Watson et al[9] Reference	Pierron et al[5] Reference
HOX-A family	Watson,[9] Pierron[5]	Overexpressed	Overexpressed	Overexpressed
HOX-B family	Watson,[9] Pierron[5]	Overexpressed	Overexpressed	Overexpressed
HOX-C family	Watson,[9] Pierron[5]	Overexpressed	Overexpressed	Overexpressed
HOX-D family	Watson,[9] Pierron[5]	Overexpressed	Overexpressed	Overexpressed
HMX1	Watson[9]	Overexpressed	Overexpressed	Underexpressed
PITX1	Watson[9]	Normal	Overexpressed	Overexpressed
ALX4	Watson[9]	Overexpressed	Overexpressed	ND
DLX1	Watson[9]	Overexpressed	Overexpressed	Overexpressed
RET	Watson[9]	Normal	Overexpressed	Normal
FGFR2	Watson[9]	Overexpressed	Overexpressed	Overexpressed
FGFR3	Watson[9]	Overexpressed	Overexpressed	Normal
EGFR	Watson[9]	Overexpressed	Overexpressed	Normal
PDGFRA	Watson[9]	Overexpressed	Overexpressed	Overexpressed
NTRK3	Watson[9]	Overexpressed	Overexpressed	Normal
KIT	Watson[9]	Normal	Overexpressed	Overexpressed
NGFR	Watson[9]	Overexpressed	Overexpressed	Overexpressed

NOTE. For our patient with BCS, the expression status for a gene was classified as overexpressed if its TPM exceeded the gene's upper 5th percentile of per-gene RNA-Seq by Expectation-Maximization (RSEM) transcript-per-million (TPM) values for a collection of RNA sequencing (RNA-Seq) datasets from The Cancer Genome Atlas normal samples. In the study by Watson et al,[9] 7 tumor transcriptomes from patients with BCS were analyzed; overexpression was determined as described in the Watson manuscript methods. In the study by Pierron et al,[5] 10 BCS tumors were analyzed for certain mRNA expression; overexpression was determined as described in Pierron manuscript methods.

Abbreviations: BCS, BCOR-CCNB3–fused sarcomas; ND, not disclosed.

pathway, and (3) the presence of a germline *CDKN2B* variant. Using this information, our Precision Genomics team chose to place our patient with multiply-relapsed disease on palbociclib; the patient has now benefited from > 2 years of disease remission. The sustained complete response with palbociclib in our patient makes this case a novel and interesting application of palbociclib use and argues for additional research using CDK4/6 inhibitors in BCS.

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SUPPORT

Supported by Grant No. U54HD16014 (to J.L.R. on behalf of the Precision Genomics Team at Indiana University School of Medicine) with the Indiana University Center for Pediatric Pharmacology and Precision Medicine.

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Provision of study material or patients: Amy E. Helvie
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Final approval of manuscript: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Michael J. Ferguson
Consulting or Advisory Role: Bayer

No other potential conflicts of interest were reported.

ACKNOWLEDGMENT

We thank Grzegorz J. Nalepa, Department of Pediatrics, Indiana University School of Medicine, the physician of this patient and who laid the clinical and scientific foundation for this work, which came to fruition after his passing.

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