Sustained Complete Response to Palbociclib in a Refractory Pediatric Sarcoma With \textit{BCOR-CCNB3} Fusion and Germline \textit{CDKN2B} Variant

Timothy F. Tramontana, MD\textsuperscript{1}; Mark S. Marshall, PhD\textsuperscript{2}; Amy E. Helvie, PharmD\textsuperscript{2}; Morgan R. Schmitt, BSN\textsuperscript{2}; Jennifer Ivanovich, MS\textsuperscript{1}; Jacquelyn L. Carter, MD, MS\textsuperscript{2}; Jamie L. Renbarger, MD, MS\textsuperscript{2}; and Michael J. Ferguson, MD, MS\textsuperscript{2}

\section*{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-FLI1.\textsuperscript{1,2} In the time since, multiple other fusion partners with EWS have been identified that fit a similar Ewing sarcoma phenotype.\textsuperscript{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\textsuperscript{5} identified a subset of Ewing-like tumors harboring paracentric inversion on the short arm of chromosome X, resulting in the fusion of the \textit{BCOR} and \textit{CCNB3} genes.\textsuperscript{6} 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.\textsuperscript{6,8}

Though distinct from Ewing sarcoma, most \textit{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.\textsuperscript{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 \textit{BCOR-CCNB3} fusion and a germline variant in \textit{CDKN2B}, and treatment resulted in a complete response and no evidence of disease 25 months into therapy.

\section*{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 \textit{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 AEW5001, 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.
**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.\textsuperscript{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.\textsuperscript{5,8,9} Similar to previous studies of mRNA transcripts in BCS,\textsuperscript{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
Loss of negative regulator (CDKN2B)

CCND1/2 and CDK4 mRNA overexpression

WT RB1

Increased E2F1-3 mRNA

Increased E2F-dependent gene transcripts
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.}

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

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 al12 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 al19 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 al20 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, CDKN2B 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.

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 liposarcoma26,27 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
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 benefitted 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.

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

| Gene | BCS Signature Reference | Patient With BCS | Watson et al Reference | Pierron et al Reference |
|------|--------------------------|-----------------|------------------------|-------------------------|
| HOX-A family | Watson,9 Pierron5 | Overexpressed | Overexpressed | Overexpressed |
| HOX-B family | Watson,9 Pierron5 | Overexpressed | Overexpressed | Overexpressed |
| HOX-C family | Watson,9 Pierron5 | Overexpressed | Overexpressed | Overexpressed |
| HOX-D family | Watson,9 Pierron5 | Overexpressed | Overexpressed | Overexpressed |
| HMX1 | Watson9 | Overexpressed | Overexpressed | Underexpressed |
| PITX1 | Watson9 | Normal | Overexpressed | Overexpressed |
| ALX4 | Watson9 | Overexpressed | Overexpressed | ND |
| DLX1 | Watson9 | Overexpressed | Overexpressed | Overexpressed |
| RET | Watson9 | Normal | Overexpressed | Normal |
| FGFR2 | Watson9 | Overexpressed | Overexpressed | Overexpressed |
| FGFR3 | Watson9 | Overexpressed | Overexpressed | Normal |
| EGFR | Watson9 | Overexpressed | Overexpressed | Normal |
| PDGFRα | Watson9 | Overexpressed | Overexpressed | Overexpressed |
| NTRK3 | Watson9 | Overexpressed | Overexpressed | Normal |
| KIT | Watson9 | Normal | Overexpressed | Overexpressed |
| NGFR | Watson9 | 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.

Manuscript writing: All authors
Final approval of manuscript: All authors

AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO’s conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

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.
REFERENCES

1. Whang-Peng J, Triche TJ, Knutsen T, et al: Cytogenetic characterization of selected small round cell tumors of childhood. Cancer Genet Cytogenet 21:185-208, 1986
2. Turc-Carel C, Aurias A, Mugneret F, et al: Chromosomes in Ewing’s sarcoma: I. An evaluation of 85 cases of remarkable consistency of t(11;22)(q24;q12). Cancer Genet Cytogenet 32:229-238, 1988
3. Ginsberg JP, de Alava E, Ladanyi M, et al: EWS-FLI1 and EWS-ERG gene fusions are associated with similar clinical phenotypes in Ewing’s sarcoma. J Clin Oncol 17:1809-1814, 1999
4. Shing DC, McMullan DJ, Roberts P, et al: FUS/ERG gene fusions in Ewing’s tumors. Cancer Res 63:4568-4576, 2003
5. Pierro G, Tierde F, Lucchesi C, et al: A new subtype of bone sarcoma defined by BCOR-CCNB3 gene fusion. Nat Genet 44:461-466, 2012
6. Peters TL, Kumar V, Polkepahad S, et al: BCOR-CCNB3 fusions are frequent in undifferentiated sarcomas of male children. Mod Pathol 28:575-586, 2015
7. Puls F, Niblett A, Marland G, et al: BCOR-CCNB3 (Ewing-like) sarcoma: A clinicopathologic analysis of 10 cases, in comparison with conventional Ewing sarcoma. Am J Surg Pathol 38:1307-1318, 2014
8. Kao, YC, Owosho AA, Sung YS, et al: BCOR-CCNB3 fusion-positive sarcomas: A clinicopathologic and molecular analysis of 36 cases with comparison to morphologic spectrum and clinical behavior of other round cell sarcomas. Am J Surg Pathol, 42:604-615, 2018
9. Watson S, Perrin V, Guillermot D, et al: Transcriptomic definition of molecular subgroups of small round cell sarcomas. J Pathol 245:29-40, 2018
10. Riggi N, Suva ML, Suva D, et al: EWS-FLI-1 expression triggers a Ewing’s sarcoma initiation program in primary human mesenchymal stem cells. Cancer Res 68:2176-2185, 2008
11. Matsuyama A, Shiba E, Umekita Y, et al: Clinicopathologic diversity of undifferentiated sarcoma with BCOR-CCNB3 fusion: Analysis of 11 cases with a reappraisal of the utility of immunohistochemistry for BOCF and CCNB3. Am J Pathol 211:171-1721, 2017
12. Alfaro-Cervello C, Andrade-Gama Roa V, Nieto G, et al: Congenital undifferentiated sarcoma associated to BOCF-CCNB3 gene fusion. Pathol Res Pract 213:1435-1439, 2017
13. Judkins AR, Mauger J, Ht A, et al: Immunohistochecmical analysis of hSNF5/NI1 in pediatric CNS neoplasms. Am J Surg Pathol 28:644-650, 2004
14. Modena P, Lualdi E, Facchini F, et al: SMARCB1/IN11 tumor suppressor gene is frequently inactivated in epithelioid sarcomas. Cancer Res 65:4012-4019, 2005
15. Hornick JL, Dal Cin P, Fletcher CD: Loss of INI1 expression is characteristic of both conventional and proximal-type epithelioid sarcoma. Am J Surg Pathol 33:542-550, 2009
16. Sredni ST, Tomita T: Rhabdoid tumor predisposition syndrome. Pediatr Dev Pathol 18:49-58, 2015
17. Chan AK, Han SJ, Choy W, et al: Familial melanoma-astrocytoma syndrome: Synchronous diffuse astrocytoma and pleomorphic xanthoastrocytoma in a patient with germline CDKN2A/B deletion and a significant family history. Clin Neuropathol 36:213-221, 2017
18. Campa D, Pastore M, Gentiluomo M, et al: Functional single nucleotide polymorphisms within the cyclin-dependent kinase inhibitor 2A/B region affect pancreatic cancer risk. Oncotarget 7:57011-57020, 2016
19. Jouenne F, Chauvet de Beauchene I, Bollaert E, et al: Functional single nucleotide polymorphisms within the cyclin-dependent kinase inhibitor 2A/B region affect pancreatic cancer risk. Oncotarget 7:57011-57020, 2016
20. Agarwal SK, Mateo CM, Marx SJ: Rare germline mutations in cyclin-dependent kinase inhibitor genes in multiple endocrine neoplasia type 1 and related states. J Clin Endocrinol Metab 94:1826-1834, 2009
21. Knudsen ES, Witkiewicz AK: The strange case of CDK4 inhibitors: Mechanisms, resistance, and combination strategies. Trends Cancer 3:39-55, 2017
22. Murakami T, Singh AS, Kiyuna T, et al: Effective molecular targeting of CDK4/6 and IGF-1R in a rare FUS-ERG fusion CDKN2A-deletion doxorubicin-resistant Ewing sarcoma patient-derived orthotopic xenograft (PDX) nude-mouse model. Oncotarget 7:47556-47564, 2016
23. Perez M, Muñoz-Galván S, Jiménez-Garcia MP, et al: Efficacy of CDK4 inhibition against sarcomas depends on their levels of CDK4 and p16INK4 mRNA. Oncotarget 6:40557-40574, 2015
24. Vlenterie M, Hillebrandt-Roeffen MH, Schaars EW, et al: Targeting cyclin-dependent kinases in synovial sarcoma: Palbociclib as a potential treatment for synovial sarcoma patients. Ann Surg Oncol 23:2745-2752, 2016
25. Böhm MJ, Marienfeld R, Jäger D, et al: Analysis of the CDK4/6 cell cycle pathway in leiomyosarcomas as a potential target for inhibition by palbociclib. Sarcoma 2019:9914223, 2019
26. Dickson MA, Tap WD, Keohan ML, et al: Phase II trial of the CDK4 inhibitor PD0332991 in patients with advanced CDK4-amplified well-differentiated or dedifferentiated liposarcoma. J Clin Oncol 31:2024-2028, 2013
27. Dickson MA, Schwartz GK, Keohan ML, et al: Progression-free survival among patients with well-differentiated or dedifferentiated liposarcoma treated with CDK4 inhibitor palbociclib: A phase 2 trial. JAMA Oncol 2:937-940, 2016
28. Elvin JA, Gay LM, Orr R, et al: Clinical benefit in response to palbociclib treatment in refractory uterine leiomyosarcomas with a common CDKN2A alteration. Oncologist 22:416-421, 2017
29. Schulz KA, Petronio J, Bendel A, et al: PD0332991 (palbociclib) for treatment of pediatric intracranial growing teratoma syndrome. Pediatr Blood Cancer 62:1072-1074, 2015
30. Li B, Dewey CN: RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12:323, 2011