Clinical Utility of CDK4/6 Inhibitors in Sarcoma: Successes and Future Challenges

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Abstract

Purpose Soft tissue and bone sarcomas are rare malignancies that exhibit significant pathologic and molecular heterogeneity. Deregulation of the CDKN2A-CCND-CDK4/6-retinoblastoma 1 (Rb) pathway is frequently observed in about 25% of unselected sarcomas and is pathognomonic for specific sarcoma subtypes. This genomic specificity has fueled the clinical evaluation of selective CDK4/6 inhibitors in sarcomas. Here, we highlight successes, opportunities, and future challenges for using CDK4/6 inhibitors to treat sarcoma.

Materials and Methods This review summarizes the current evidence for the use of CDK4/6 inhibitors in sarcoma while identifying molecular rationale and predictive biomarkers that provide the foundation for targeting the CDK4/6 pathway in sarcoma. A systematic review was performed of articles indexed in the PubMed database and the National Institutes of Health Clinical Trials Registry (ClinicalTrials.gov). For each sarcoma subtype, we discuss the preclinical rationale, case reports, and available clinical trials data.

Results Despite promising clinical outcomes in a subset of sarcomas, resistance to CDK4/6 inhibitors results in highly heterogeneous clinical outcomes. Current clinical data support the use of CDK4/6 inhibitors in subsets of sarcoma primarily driven by CDK4/6 deregulation. When dysregulation of the Rb pathway is a secondary driver of sarcoma, combination therapy with CDK4/6 inhibition may be an option. Developing strategies to identify responders and the mechanisms that drive resistance is important to maximize the clinical utility of these drugs in patients with sarcoma. Potential biomarkers that indicate CDK4/6 inhibitor sensitivity in sarcoma include CDK4, CCND, CCNE, RB1, E2F1, and CDKN2A.

Conclusion CDK4/6 inhibitors represent a major breakthrough for targeted cancer treatment. CDK4/6 inhibitor use in sarcoma has led to limited, but significant, early clinical success. Targeted future clinical research will be key to unlocking the potential of CDK4/6 inhibition in sarcoma.

INTRODUCTION

Sarcomas are rare malignant tumors that originate from the connective tissues of bone or soft tissues including fat, muscle, blood vessels, or nerves. Sarcomas make up < 1% of all adult malignancies and approximately 20% of pediatric cancers.1 The rarity and heterogeneity of these tumors have led to challenges in both diagnosis and treatment development.2 Even with multimodal therapy including surgery, radiation, and chemotherapy in the advanced setting, sarcomas are incurable malignancies associated with dismal prognosis.1

Despite the molecular and cellular heterogeneity of sarcoma, with more than 100 subtypes exhibiting unique and defining genomic alterations, dysregulation of the cyclin D (CCND)-cyclin-dependent kinase 4/6 (CDK4/6)-retinoblastoma 1 (Rb) pathway is common across several sarcoma subtypes (Fig 1). These recurrent genetic aberrations result in the deregulation of activator E2 promoter binding factors 1-3 (E2F1-3). These potent transcription factors directly bind to and induce the transcription of genes required for the G1/S, G2/M cell cycle transition, apoptosis, and metabolism. The activity of E2F1-3 is tightly controlled by the Rb protein, which acts as a key cell cycle checkpoint regulator and tumor suppressor. In sarcomas, RB1 deletion and/or mutation, changes to kinase regulators of Rb stability (CDK4/6 and CCND1-3), or deletion/silencing of the CDK inhibitor family (p16 [CDKN2A]/p15[CDKN2B]) all result in diminished Rb-mediated repression of E2F1-3 activity and deregulated cellular growth.

DNA sequencing of nearly 10,000 sarcomas identified widespread alterations in RB1 (14.6%) and CDK4 (12%).3 A similar analysis of soft tissue sarcomas in...
The Cancer Genomic Atlas (TCGA) also found that alterations in the CDKN2A-CCND-CDK4-Rb pathway are highly prevalent. Common alterations in the TCGA data included RB1 deletion (16.1%), CDKN2A/CDKN2B deletion (12.9%), CCND3 amplification (4.4%), and CDK4 amplification (18.5%). An additional study explored the genetic alterations in leiomyosarcoma and found frequent genomic alterations including TP53 mutation (34%), TP53 deletion (8%), CDKN2A deletion (21%), RB1 deletion (11%), MDM2 amplification (8%), and PIK3CA mutation (6%). In this study, CDKN2A deletion was correlated with poor overall survival. Collectively, these genomic data highlight that the CDKN2A-CCND-CDK4/6-Rb pathway is commonly altered in more than 25% of sarcoma and represents a key oncogenic driver in these tumors. On the basis of these results, targeting this pathway may be effective.

**Context**

**Key Objective**

Sarcomas exhibit significant molecular heterogeneity including dysregulation of the CDKN2A-CCND-CDK4/6-retinoblastoma 1 (Rb) pathway. The use of CDK4/6 inhibitors in sarcomas has led to mixed results, indicating the need to identify biomarkers of sensitivity and resistance across sarcomas.

**Knowledge Generated**

While highlighting the promising clinical outcomes in subsets of sarcomas, we present the current knowledge of intrinsic and/or acquired resistance to CDK4/6 inhibitors identified across sarcomas. Current data support the use of CDK4/6 inhibitors in sarcomas primarily driven by CDK4/6 signaling. In sarcomas where Rb pathway dysregulation is a secondary driver, combinations of CDK4/6 inhibition therapy with additional therapies may be effective.

**Relevance**

The use of CDK4/6 inhibitors across sarcomas remains limited; however, subtypes of the disease are sensitive to this therapeutic strategy as either monotherapy or combination therapy.

![FIG 1. Common derangements in CCND-CDK4/6-RB pathway in sarcoma. The CDK4-cyclin D complex can phosphorylate the Rb-E2F complex to promote cyclin E translation for G1/S progression. CDK4/6 inhibitors can prevent this phosphorylation process. Common genes that are overexpressed (red), underexpressed/loss (teal), or normal (orange) in the majority of sarcoma subtypes are depicted.](image-url)
pathway represents a promising therapeutic strategy for treating sarcoma.

Currently, the US Food and Drug Administration (FDA) has approved the clinical use of several CDK4/6 inhibitors that prevent G1/S cell cycle progression by blocking CDK4 and/or CDK6 from binding to their regulatory partner, CCND. The dependence of sarcomas on activation of the CDKN2A-CCND-CDK4/6-Rb pathway has opened opportunities for the use of CDK4/6 inhibitors in the clinic. This review outlines the current state of CDK4/6 inhibition as a treatment option for soft tissue and bone sarcomas. In addition, we focus on new clinical and preclinical research on the use of predictive biomarkers for CDK4/6-directed therapy in sarcoma.

**CLINICAL USE OF CDK4/6 INHIBITORS IN SARCOMA**

Given the diversity of sarcoma types, this review focuses primarily on the six common sarcoma subtypes: liposarcoma (LPS), leiomyosarcoma (LMS), gastrointestinal stromal tumors (GIST), osteosarcoma (OS), rhabdomyosarcoma (RMS), and Ewing’s sarcoma (ES). The methods for reviewing the literature are included in Appendix 1. In each section, we discuss the preclinical rationale, available clinical trials and case reports specific to the sarcoma subtype, and potential biomarkers for therapeutic efficacy (Table 1). Using TCGA data and PubMed searches, subtypes with a characteristic genetic alteration in the CDKN2A-CDK4/6-CCND-Rb pathway including, but not limited to, CD4K, CDK6, CCND1, RB1, E2F, CDKN2A, CDKN1A, CCNE1, and MDM2 were identified and related studies were analyzed for significant biomarkers (Fig 2). A brief overview regarding CDK4/6 inhibitor development is included in Appendix 2.

**LPS**

LPS, primarily arising from fat tissue in the thigh or retroperitoneum, comprise approximately 13% of all sarcomas. Subclasses of LPS, including well-differentiated/dedifferentiated liposarcomas (WDLPS/DDLPS), myxoid/round cell liposarcoma (MRCLS), and pleomorphic liposarcoma (PLS), have demonstrated consistent dysregulation of the Rb pathway. DDLPS frequently present with amplifications of the 12q region of chromosome 12, resulting in MDM2 and CDK4 overexpression. In addition, amplification and/or activation events of CDK4 are essential for the growth and survival of WDLPS/DDLPS. MRCLS harbors a FUS-DDIT3 chimeric gene, which causes abnormal expression of G1 checkpoint regulators including CCND, CCNE, CDK4, and CDK2. Widespread genetic alterations in PLS frequently present with the loss of TP53 and RB1 tumor suppressor genes as well as overexpression of CCND1, CDK4, MDM2, MYB, and GLI1.

To date, LPS represents the largest subgroup of sarcomas to be treated with CDK4/6 inhibitors and these trials have resulted in clinical success. In a pair of phase II clinical trials, two different doses of palbociclib were compared in WDLPS and DDLPS. Patients treated with a 200-mg daily dose for days 1-14 of a 21-day cycle showed a median progression-free survival (PFS) of 18 weeks and 66% of patients reached a PFS of at least 12 weeks or longer on this regimen. The second trial examined the effects of a 125-mg dose for days 1-21 of a 28-day cycle where the median PFS was also 17.9 weeks and 57% of patients reached a PFS of 12 weeks or longer. As a single agent in CDK4 amplified, Rb-intact LPSs, palbociclib demonstrated significant clinical activity and a favorable adverse event profile. On the basis of these results, palbociclib is currently listed as category 2A evidence in the soft tissue sarcoma National Comprehensive Cancer Network (NCCN) guidelines but is not currently approved by the FDA or EMA for this indication.

In a phase I clinical trial to determine the maximum tolerated dose of ribociclib in advanced solid tumors, patients with LPS made up 30% (39 of 132) of the study population with six patients with LPS demonstrating stable disease (SD) at 6 months on treatment. Three of the four patients who remained on therapy for the longest durations harbored CCND1 amplification without CDKN2A/CDKN2B codeletion. Comparatively, seven (29%) of the 24 patients who received treatment for < 8 weeks had CDKN2A/CDKN2B codeletion. In a different tissue agnostic phase II trial of ribociclib, three of 13 (23.1%) enrolled patients with sarcoma exhibited clinical benefit, as defined by a response of stabilized disease or better at 16 weeks of therapy. Of these 13 enrolled patients with sarcoma, only four were considered DDLPS with none of these patients achieving clinical benefit by the study’s definition. Across tumor types, cancers with a single hit in the cyclin D-CDK4/6 complex tended to see the greatest benefit from ribociclib. Interim analysis was conducted of a phase Ib study of the MDM2 inhibitor, HDM201, in combination with ribociclib in 74 patients with WDLPS/DDLPS with multiple dosing regimens. Partial response was achieved in three patients (4%), whereas 36 patients achieved stabilized disease (49%). Median PFS ranged from 2.1 to 4.8 months across three dosing regimens. In a key interim analysis of a phase II study of abemaciclib in patients with DDLPS, the observed PFS at 12 weeks was 76% (95% CI, 57 to 90), whereas the median PFS was 30.4 weeks (95% CI, 28.9 to not evaluable). The use of CDK4/6 inhibitors shows promise as an active therapy regimen in patients with WDLPS/DDLPS. Although phase I and II trials with CDK4/6 inhibitors include both DDLPS and WDLPS, varied representation of patients with DDLPS/WDLPS makes interpretation of each subtype difficult to determine. Overall, the genomic amplification of CDK4 and possibly CCND1 are likely biomarkers for CDK4/6 inhibitor sensitivity in this disease. MDM2, which is commonly upregulated in LPS and results in altered Rb...
function, is a biomarker that has been investigated but shows varied biomarker potential. The utility of CDKN2A levels as a biomarker for CDK4/6 inhibitor sensitivity in WDLPS/DDLP has also yet to be fully defined.

**LMS**

LMS are smooth muscle sarcomas primarily arising from cells of the abdomen, uterus, and gastrointestinal tract, and less commonly from the vasculature or cutaneous structures. LMS commonly have genomic alterations of TP53, RB1, chromatin remodeling, and homologous recombination DNA repair pathways. 21.4% of LMS exhibit RB1-deactivating events, and subpopulations of LMS demonstrate genomic alterations in CDK4, CDK6, CDKN2A, CDKN2B, CCND1, and CCND3. Uterine myxoid LMS also frequently harbor TP53 and/or CDKN2A genomic alterations. In cultured CDKN2A-deleted LMS cell lines, palbociclib exerts a strong growth-inhibitory effect.

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**TABLE 1. Completed Case Studies and Clinical Trials With CDK4/6 Inhibitors**

| Reference/Clinical Trial No. | Tumor (subtype) | Treatment | Results | Biomarkers |
|-----------------------------|----------------|-----------|---------|------------|
| October 2017<sup>9</sup> | Sarcoma (59/1,332) | Palbociclib or ribociclib with antiestrogen therapy | Palbociclib was approved as monotherapy or in combination with antiestrogen therapy | PI3KA, CDK4, CDK6, MDM2, p53 |
| April 2019<sup>10</sup> | Sarcoma (13/106) | Ribociclib | No clinical benefit (10/13) Clinical benefit (3/13) SD > 16 weeks (2/13) Median PFS: 6 weeks | PI3KA, CDK4, CDK6, MDM2, p53 |
| October 2019<sup>11</sup> | HR+/HER2− breast cancer and WD/DDLP (1/1) | Letrozole plus palbociclib | PR | CDK4, MDM2 |
| November 2017<sup>11,12</sup> | LPS (3/41) | Palbociclib | PFS at 12 weeks: 57% Median PFS: 17.9 weeks | CDK4, MDM2 |
| | LPS (4/29) | Palbociclib | PFS at 12 weeks: 66% Median PFS: 18 weeks | CDK4, MDM2 |
| August 2018<sup>13</sup> | LPS (39/132) | Ribociclib | SD (8/41) | CDKN2A, CCND1 |
| June 2020<sup>14</sup> | LPS (74/74) | Ribociclib plus HDM201 | PR (3) SD (37) | |
| August 2020<sup>15</sup> | Sarcoma, DDLPS (30/30) | Abemaciclib | PR (1)—PFS at 12 weeks: 76% Median PFS: 30.4 weeks | |
| October 2018<sup>16</sup> | LMS (1/114) | Palbociclib plus fulvestrant | PD | CDK4 |
| April 2017<sup>17</sup> | Uterine LMS with CDKN2A mutation (1/279) | Palbociclib | SD and Clinical benefit | CDKN2A, Rb |
| August 2019<sup>18</sup> | GIST with CDKN2A loss (29/29) | Palbociclib | No clinical significance as a single agent | CCNE1, CDKN1A, Not CDKN2A |
| February 2019<sup>19</sup> | CDK4-amplified ARMS (1/22) | Ribociclib | PD | SMARCB1, CCND1, CDK4 |
| May 2020<sup>20</sup> | OS (1/1) | Gemcitabine plus ribociclib | SD and well tolerated | |
| December 2019<sup>12</sup> | Clear cell sarcoma (1/34) | Abemaciclib | Well tolerated at 130 mg/m²/dose MTD: 130 mg/m²/dose twice daily on a 28-day cycle | |
| July 2019<sup>10</sup> | Round blue cell soft tissue sarcoma not otherwise specified with CDK4 amplification (1/106) | Ribociclib | PR | CDK4 |
| April 2020<sup>21</sup> | BCOR-CCNB3-fused sarcoma (1/1) | Palbociclib | CR | Cyclin D |

**Abbreviations:** ARMS, alveolar rhabdomyosarcoma; CR, complete response; DDLPS, dedifferentiated liposarcoma; GIST, gastrointestinal stromal tumors; LMS, leiomyosarcoma; LPS, liposarcoma; MTD, maximum tolerated dose; OS, osteosarcoma; PD, progressive disease; PFS, progression-free survival; PR, partial response; RMS, rhabdomyosarcoma; SD, stable disease.
and, conversely, high levels of p16 in combination with Rb loss correlate with palbociclib resistance.76,77

To date, there are no formal clinical studies with published results for CDK4/6 inhibitors in LMS. Retrospective analyses and case reports have documented mixed success. In particular, one patient with CDKN2A/CDKN2B loss, estrogen receptor–positive, LMS was treated with a combination of fulvestrant and palbociclib without evidence of clinical benefit.16 A second report described a patient with uterine LMS harboring loss of CDKN2A and heterozygous mutation in NF2 treated with palbociclib resulting in 8 months of disease stabilization.17 Although the Rb pathway is frequently dysregulated by genomic variants at RB1, in Rb-intact disease with dysregulating upstream variants, CDK4/6 inhibition may be a therapeutic option. A phase II trial of ribociclib and everolimus, an mTOR

| Tumor          | Genes in the Cyclin D/Cyclin-Dependent-Kinase 4/Rb Pathway |
|---------------|---------------------------------------------------------|
|               | TP53 | RB1 | CDKN2A/CDKN2B | CCND1/2/3 | CDK4/CDK6 | CCNE1 |
| ARMS22,45,86  |      |     |                |          |          |      |
| AS34          |      |     |                |          |          |      |
| AFX104        |      |     |                |          |          |      |
| CS26,27       |      |     |                |          |          |      |
| Conventional OS27-31 |     |     |                |          |          |      |
| DDLPS25,33-36,128 |   |     |                |          |          |      |
| EHE37         |      |     |                |          |          |      |
| ES38          |      |     |                |          |          |      |
| GIST39,40     |      |     |                |          |          |      |
| IS3,129       |      |     |                |          |          |      |
| LMS31,42      |      |     |                |          |          |      |
| MPNST39,53,43-45 |    |     |                |          |          |      |
| MFS32,46      |      |     |                |          |          |      |
| MLS/RCLS47    |      |     |                |          |          |      |
| Parosteal OS103 |    |     |                |          |          |      |
| PLS49-51      |      |     |                |          |          |      |
| SS21          |      |     |                |          |          |      |
| UPS39,36,52,53 |     |     |                |          |          |      |

**FIG 2.** Common genetic alterations in the CDKN2A-CCND-CDK4/6-Rb pathway in various sarcoma subtypes. Dysregulation of the CDKN2A-CCND-CDK4/6-Rb pathway is common across sarcomas. Despite the common pathway dysregulation in sarcomas, molecular variations exist between subtypes. Subtypes with common mutations (red), deletions (blue), and amplifications (teal) in TP53, RB1, CDKN2A/CDKN2B, CCND1/2/3, CDK4/CDK6, and CCNE1 are indicated by the colored table cells. References are indicated by the numbers in each cell. ARMS, alveolar rhabdomyosarcoma; AS, angiosarcoma; AFX, atypical fibroxanthoma; CS, chondrosarcoma; DDS, dedifferentiated chondrosarcoma; DDLPS, dedifferentiated liposarcoma; EHE, epithelioid hemangioendothelioma; ES, Ewing's sarcoma; GIST, gastrointestinal sarcoma tumors; IS, intimal sarcoma; LMS, leiomyosarcoma; MPNST, malignant peripheral nerve sheath tumors; MFS, myxofibrosarcoma; MLS/RCLS, myxoid/round cell liposarcoma; OS, osteosarcoma; PLS, pleomorphic liposarcoma; SS, synovial sarcoma; UPS, undifferentiated pleomorphic sarcoma.
inhibitor, in DDLPS and Rb-positive LMS is currently under way and will provide insight into the benefits of CDK4/6 inhibitors in Rb-intact diseases. 78 Given the predilection for alterations in RB1 in LMS, de novo or secondary resistance to CDK4/6 inhibitors may complicate, or limit the efficacy, of single-agent treatment. 71, 79

GIST

GIST are often misdiagnosed as LMS as they develop in the intestinal tract. However, GIST present with genomic features distinct from LMS, as 85% of patients with GIST have oncogenic changes within either KIT or PDGFRA. 39, 80 Less frequent GIST driver alterations include NF1, BRAF, or SDH. Interestingly, concomitant secondary alterations in CDKN2A, RB1, MDM2, and CCND1 amplifications are common. Recent work has shown that loss or deregulation of the Rb-CDK4 pathway is linked to increased risk of metastasis in patients with GIST. 40, 81

Genomic analysis of these tumors supports this hypothesis. Recent whole-exome sequencing on 29 high-grade metastatic KIT-mutant GIST found that CDKN2A, RB1, or TP53 mutation or loss was associated with poor patient prognosis. 82 However, to date, targeting GIST with CDK4/6 inhibitors has not provided clinical utility. A phase II study examining palbociclib in CDKN2A-deleted advanced GIST demonstrated no significant clinical activity as a single agent with 19 of 22 (86.4%) patients experiencing progressive disease at 4 months on therapy. Therefore, the clinical and biological relevance of CDK4/6 inhibitors as a single-agent in GIST is unclear. Combination therapy and biomarkers to better understand the pathways fueling tumor growth are required, and the changes within the CDK4/CCND pathway remain under investigation. 18

OS

OS is the most common bone cancer in children and is usually found around the knee or long bones of the extremities where immature bone is produced by mesenchymal cells. 82 OS is prone to aberrations of cell cycle control regulators including RB1, TP53, CDKN2A, PTEN, CDK4, MDM2, MYC, TWIST1, CCND3, and CCNE1. 28 Parosteal OS, not to be confused with periosteal OS that arises from the inner layer of the periosteum, originates from the outer fibrous layer of the periosteum, and has frequent alterations in 12q13-15 of chromosome 12 that include SAS, CDK4, and MDM2 genes. In particular, SAS and CDK4 genes were found to be amplified commonly in grade II and dedifferentiated tumors. 83 One important consideration for the use of CDK4/6 inhibitor use in OS is that CDK4 amplification may be related to parosteal tumor grade, which should be considered with designing these trials. 84

Because of its rarity, prospective trials with CDK4-directed therapy in OS are not available. The limited data we have are anecdotal. A patient with chemotherapy-resistant, metastatic, CDK6-amplified OS experienced SD for 10 cycles of treatment with ribociclib and gemcitabine. Treatment was ultimately stopped because of toxicity and not progression. Similar to CDK4, CDK6 may serve as a biomarker for CDK4/6 inhibitor success and these findings set the foundation for further testing. 20

RMS

RMS develops from rhabdomyoblasts in soft tissues, especially skeletal muscle tissue, bladder, or uterus. 85 RMS, specifically alveolar rhabdomyosarcoma (ARMS), is prone to gene amplifications of 12q13-q14 and 2p24, leading to an increase in the expression of MYCN, CDK4, CDK6, CDC25A, and SKP2. RMS comprises two major subtypes: fusion-positive and fusion-negative. Although most fusion-positive RMS tumors are characterized by the PAX3-FOXO1 gene fusion, a smaller subset of cases have a PAX7-FOXO1 gene fusion. Fusion-positive RMS tumors have equal rates of amplification of 2p24, but amplifications of 12q13-q14 are specific to PAX3-FOXO1 fusion-positive RMS. 86 In ARMS, 80% of cases have PAX3-FOXO1 and PAX7-FOXO1 gene fusions. Fusion-negative RMS tumors have lower Rb protein levels than fusion-positive RMS. 87-89

Downregulation of p21 has also been linked to tumorigenesis of transcription factor, FoxF1- or FoxF2-, elevated protein levels than fusion-positive RMS. 87-89 In contrast to other sarcoma subtypes, in vitro experimental data in RMS cultured samples show little correlation between CDK4 overexpression and CDK4/6 inhibitor sensitivity. Additional data also suggest that the RMS cells lines with CDK4 amplification have reduced sensitivity to ribociclib. These findings suggest that CDK4 may represent a biomarker for resistance to CDK4/6 inhibitors in RMS. 87 In a phase I trial of ribociclib in pediatric solid tumors, a patient with CDK4-amplified RMS progressed after 5 months of therapy. 19 It appears that CDK4 amplification may be related to CDK4/6 inhibitor sensitivity for patients with RMS; however, further research is required to confirm these findings.

ES and Ewing Family of Tumors

ES represents a large family of mesenchymal tumors with varied molecular fusions. Classical ES is a small round cell tumor that develops in the bones and/or soft tissue around the bones. ES’ characteristic fusion gene, EWS-FLI1, is an oncogenic transcription factor that upregulates MYC and CDK4 and downregulates CDK inhibitors, CDKN1A and CDKN1C. 91 Secondary genomic alterations in ES include STAG2 (15%-17%), TP53 (6%-9%), CDKN2A (11%-22%), RB1, and CCND1. 91, 95 In EWSR1-PATZ1-positive sarcomas, secondary alterations in CDKN2A are highly prevalent (71%). 90 The clinical evaluation of CDK4/6 inhibitors in the treatment of ES has yet to be described.

In addition, the BCOR-CCNB3 fusion-positive sarcoma, another Ewing’s family variant, upregulates CCND1, SATB2, TLE1, and BCL2. 96 Comprehensive genomic profiling in BCOR-fusion uterine sarcoma revealed CDK4 and MDM2 coamplifications or homozygous deletion of
CDKN2A. These genetic changes closely mirror the genomic profile of DDLPS and, on the basis of the successes of CDK4/6 inhibitors in DDLPS, a therapeutic opportunity to use CDK4/6 inhibitors as a single agent or in combination with MDM2 inhibitors to treat BCOR-CCNB3 fusion-positive sarcomas exists. A report of a patient with a BCOR-CCNB3 fusion sarcoma and germline CDKN2B missense variant pediatric sarcoma demonstrated sustained complete response following treatment with palbociclib. Despite harboring a CDKN2B variant, CDKN2B was normally expressed, although RNA analysis suggested overactivation of the Rb pathway. It is unclear how CDK4/6 inhibition might provide benefit in the Ewing family of tumors.

Other Sarcomas

Synovial sarcomas (SS) frequently upregulate EGFR, MDM2, CDK2, and CDK4, and downregulate CCND1 to fuel tumor development. SS commonly harbor CDKN2A deletion (74%) and an array of additional mutations in Rb pathway genes: CCND1, CDK4, CDK6, and RB1. It has also been reported that heart sarcomas also have frequent CDK4 (38%) and CCND3 alterations (14%); this heart analysis did not delineate a more specific diagnosis, indicating more research is necessary to identify enriched disease subtypes harboring these CDK4 and CCND3 alterations. Similarly, intimal sarcoma also have amplified CD4, mutated CCND, and deleted CDKN2A. Epithelioid hemangioendothelioma is a rare sarcoma subtype commonly characterized by a WWTR1-CAMTA1 fusion and altered CDKN2A/CDKN2B, RB1, APC, and FANCA genes. An estimated 96% of high-grade chondrosarcoma have affected RB1 pathways via TP53, RB1, or CDKN2A loss, CDK4 or CCND1 overexpression, or inactivated CDK inhibitors such as p16INK4a. On the basis of the continued theme of CDKN2A-CCND-CDK4-Rb pathway mutations and altered G1/S checkpoint regulations, these tumors may represent excellent candidates for CDK4/6 inhibitor treatment. However, similar to LMS, sarcoma subtypes with loss of Rb or further downstream proteins may limit the efficacy of CDK4/6 inhibitors as a single-agent treatment.

Sarcoma histology-agnostic studies of CDK4/6 inhibition have yielded a mixed response. A phase II study in CDK4/6 pathway activated soft tissue sarcomas had a median PFS of 6 weeks when treated with 600 mg doses of ribociclib for days 1-21 of a 28-day cycle. Out of 13 patients with sarcoma on this trial, 10 showed no clinical benefit. Of the three who did have clinical benefits, two had SD over 16 weeks on CDK4/6 inhibitor treatment. These patients presented with CDKN2A/CDKN2B loss and CCND1 variants plus CDK4 and MDM2 amplifications, respectively. The patient with partial response had a CDK4 amplification. Notably, the 10 patients with progressive disease also frequently harbored CDKN2A/CDKN2B loss and one had CCND3 amplification. Additionally, a patient with poorly differentiated, round blue cell sarcoma not otherwise specified with CDK4 amplification had a 100% reduction after ribociclib treatment. More work is required in this space; however, these findings do further support CDK4 as a potential biomarker for CDK4 inhibitor utility in treating sarcoma.

FUTURE DIRECTIONS

Dysregulation of the CDKN2A-CDK4-CCND-Rb pathway in patients with sarcoma represents a promising opportunity for therapeutic treatments with CDK4/6 inhibitors. Although sensitivity to CDK4/6 inhibitors varies across sarcoma subtypes, biomarkers for CDK4/6 inhibitor sensitivity may serve as a driving force in diagnosing and treating sarcoma subtypes. Since CDK4/6 inhibitor sensitivity in many sarcoma subtypes remain untested or have yet to be reported, further in vitro, in vivo, and clinical testing of these agents is necessary for advancing our understanding of these compounds in sarcoma. To expand biomarker development, clinical trials with non-LPS subtypes treated with CDK4/6 inhibitors would help to draw connections between their shared genomic aberrations. Overall, CDK4/6 inhibitors have demonstrated modest clinical success when used as monotherapy; however, data from other cancer types suggest that combination strategies for CDK4/6 inhibitors may offer improved PFS and patient outcome.

In many sarcomas defined by characteristic genomic events, often an oncogenic fusion or activating kinase mutation, variants in the Rb pathway represent a second hit. These alterations are highly prevalent, but not exclusively seen, across the disease. This suggests that Rb pathway alterations are not necessary for the development of the initial disease but are enriched through the evolution of the tumor. These variants are potentially reasonable targets for CDK4/6 inhibitors either as single agents or in combination.

Notably, subtypes with Rb loss or mutation may have limited sensitivity to CDK4/6 inhibitors. In Rb-positive cell lines, combination with palbociclib and doxorubicin or Wee1 inhibitor, AZD1775, was synergistic; however, Rb knockdown cell lines displayed resistance to palbociclib treatment. As CDKN2A/CDKN2B loss is a common secondary alteration in sarcomas, potentially combining CDK4/6 inhibition with other agents to target primary and secondary alteration in sarcoma subtypes remains untested or has yet to be reported.

A significant number of sarcoma types, at least in part, driven by CDK4 amplifications include DDLPS, undifferentiated pleomorphic sarcoma, LMS, ARMS, ES, SS, and parosteal sarcomas. Ongoing clinical trials including the phase II multicenter trial of palbociclib in advanced sarcomas with CDK4 overexpression, phase II...
study in bone and soft tissue sarcoma with CDK pathway alterations treated with abemaciclib, and the phase III study on molecular profiling of soft tissue sarcomas will provide a wealth of data for biomarker development and CDK4/6 inhibitor clinical utility.\textsuperscript{106–108}

Further research examining the effect that CDK4, CCND, CCNE, Rb, E2F, and p16 proteins have on CDK4/6 inhibitor treatments in sarcoma is necessary to make any concrete decisions. While researchers continue to look for biomarkers that can predict sensitivity to CDK4/6 inhibitors in sarcomas, data from other tumor types may help guide these decisions. Recently published transcriptome profiling studies from CDK4/6 inhibitor–resistant and –sensitive breast cancer cells lines identified an Rb-loss signature RBsig that can discriminate between CDK4/6 inhibitor–resistant and –sensitive lines.\textsuperscript{109} Currently, no single biomarker can accurately predict CDK4/6 inhibitor sensitivity; however, gene signatures may provide insight into potential genetic profiles, specifically in sarcomas.

**Precision Medicine: NCI-MATCH and TAPUR Studies**

Pairing a patient's genomic profile with available treatments has recently been available from the National Cancer Institute’s Molecular Analysis for Therapy Choice (NCI-MATCH) and ASCO’s Targeted Agent and Profiling Utilization Registry (TAPUR).\textsuperscript{110} A TAPUR study of 29 patients with advanced non–small-cell lung cancer with \textit{CDKN2A} loss or mutation and no RB mutations demonstrated antitumor effects from CDK4/6 inhibition. In a previous study including pancreatic adenocarcinoma and cholangiocarcinoma with \textit{CDKN2A} loss or mutation, there was a lack of clinical activity from CDK4/6 inhibition, indicating that \textit{CDKN2A} may not be a universal biomarker and should be tested in individual sarcoma subtypes.\textsuperscript{111,112}

More information on cell cycle biomarkers for sarcoma subtypes will be collected from an ongoing phase II Pediatric MATCH trial of palbociclib in Rb-intact solid tumors with active mutations in cell cycle genes.\textsuperscript{113} A subgroup of this trial that focused on \textit{CCND1, 2}, or 3 amplification had prolonged SD in 13% of patients but \textit{CCND1} or 3 amplification was not a predictor of palbociclib sensitivity.\textsuperscript{114}

**Combination Therapies and the Role of Treatment Sequencing**

Several sarcoma subtypes have dual characteristic molecular alterations that may make them attractive targets for combination therapies. For example, LMS, angiosarcoma, and OS tend to exhibit activation of the mTOR pathway either through mTOR overexpression or direct loss of \textit{PTEN}. In these sarcoma subtypes, mTOR or PI3K inhibitors may be logical synergistic partners alongside CDK4/6 inhibitors.\textsuperscript{102,115} In CDK4/6 inhibitor–resistant ES, which overexpresses \textit{IGF1R}, a combination of CDK4/6 and \textit{IGF1R} inhibitors was synergistic in vitro and in mouse models.\textsuperscript{116}

Although PI3K or MEK inhibitors may be predicted to have synergy with combined CDK4/6 inhibition, other targets including Wee1 require further preclinical testing. Combination therapies with the CDK4 inhibitor, palbociclib, and MDM2 inhibitors have also been found to have both synergistic and antagonistic results in sarcomas. Reduced tumor growth and increased progression-free survival were evident in DDLPS when treated with palbociclib and MDM2 inhibitor, RG7388, but antagonistic effects were evident in myxofibrosarcoma and LMS cell lines.\textsuperscript{128} Similarly, in MDM2-amplified sarcomas, the MDM2-p53 binding inhibitor, nutlin, was antagonistic with palbociclib in preclinical sarcoma models.\textsuperscript{118}

It should be noted that CDK4/6 inhibitors are frequently found to be antagonistic of drugs that require cells to enter the mitotic phase of the cell cycle to exert their effect. Nevertheless, the sequencing and timing of drug delivery can combat potential antagonism. Successful synergy was seen in combinations of CDK4/6 inhibitors and taxanes or microtubule stabilizers when taxanes were administered after CDK4/6 inhibitors.\textsuperscript{119} Another preclinical study suggested that Wee1 inhibitor, AZD1775, should only be treated after palbociclib treatment and a recovery period for cells to traverse through S phase.\textsuperscript{101} Although chemotherapy that target faster growing cells may be less likely to work when CDK4 is inhibited and slows cell growth, a study in ES and other nonsarcoma models had success when CDK4/6 inhibitors were used in combination with chemotherapy. Other in vitro and in vivo models in nonsarcoma tumors showed synergy between CDK4/6 inhibitors and chemotherapies using concurrent and sequential dosing schedules. CDK4/6 inhibitors’ ability to deregulate DNA repair, metabolism, and cell plasticity, and reduce thymidylate synthase, topoisomerase 1, and topoisomerase 2 alpha expression limit the dose required for chemotherapy efficacy and may enhance chemotherapy-induced apoptosis.\textsuperscript{115,116,120–126}

Despite the various successful combination therapies and the fact that preclinical reports that described antagonistic relationships only recorded short-term effects, the potential for antagonism should not be discounted.\textsuperscript{119}

Rational drug combination strategies in sarcoma will require a molecular understanding of both the interactions between genomic drivers of disease as well as the interactions between the drug combinations themselves. In conclusion, CDK4/6 inhibitors represent a major breakthrough for targeted cancer treatment. CDK4/6 inhibitor use in sarcoma has led to limited, but significant, early clinical success. These therapies, as single agents, represent relatively well-tolerated therapies with the flexibility of oral administration, both of which are uncommon in these diseases. Current clinical data support the use of CDK4/6 inhibitors in subsets of sarcoma, which are primarily driven by CDK4/6 deregulation such as DDLPS and WDLPS. Alteration in the Rb-CDK4/6 pathway also serves a role as secondary drivers of sarcoma oncogenicity. Thus, combination therapy with CDK4/6 inhibition to target dual
Clinical Utility of CDK4/6 Inhibitors in Sarcoma

 genic derangements is attractive. Combination therapies with mTOR or PI3K inhibitors may provide promising response in subtypes with mTOR overexpression or PTEN loss such as LMS, angiosarcoma, and OS. However, further research should be conducted to determine the synergy of MDM2 inhibitors with CDK4 inhibitors. Overall, targeted future clinical research will be key to unlocking the potential of CDK4/6 inhibition in sarcoma.

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APPENDIX 1. LITERATURE REVIEW

A systematic review was performed of articles indexed in the PubMed database and the National Institutes of Health Clinical Trials Registry (ClinicalTrials.gov) between January 1, 1996, and October 1, 2021. Search terms included sarcoma, CDK4/6 inhibitor, palbociclib, and biomarkers. Only articles published in English and articles with results were included. Gene expression of a sarcoma data set from 255 patients was obtained from The Cancer Genome Atlas.

APPENDIX 2. CDK4/6 INHIBITORS

CCND-dependent kinase activities of CDK4/6 are essential for progression through G1. CDK4/6-CCND phosphorylation of Rb and subsequent activation of E2F family of transcription factors then enhance transcription of CCNE. The CDK2/CCNE complex then starts a phosphorylation cascade that results in hyperphosphorylation and the degradation of Rb, which then allows transitioning through the G1/S checkpoint. The G1/S checkpoint represents the most important checkpoint for ensuring genome and cellular fidelity. Progression through this checkpoint requires adequate cell size, nutrients, and growth factors, in addition to a low threshold of DNA damage in the cell.

CDK4/6 inhibitors are small molecules (approximately 500 Da) that were first approved in 2015. These agents were identified for their ability to selectively target and block CDK4/6-CCND activation compared with older, pan-CDK inhibitors. CDK4/6 inhibitors directly compete with CCND for binding to the ATP cleft of CDK4 and CDK6. By blocking the formation of the CDK4/6-CCND, the Rb tumor suppressor remains unphosphorylated and tightly bound to E2F1-3. This directly inhibits cell cycle progression by preventing E2F-mediated transcription of genes required for G1/S progression. Dysregulation of the Rb pathway and its role in genome integrity, cellular programming, and proliferation in sarcoma are key topics for continuing research.

Current US Food and Drug Administration (FDA)-approved CDK4/6 inhibitors include palbociclib, ribociclib, and abemaciclib with an additional 15 new agents under development. Palbociclib (IBRANCE) was the first CDK4/6 inhibitor FDA-approved in 2015 for the treatment of hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced or metastatic breast cancer. Ribociclib (KISQALI) and abemaciclib (VERZENIO) were both approved in 2017 for the treatment of HR-positive, HER2-negative advanced or metastatic breast cancer. Although palbociclib and ribociclib demonstrate significant selectivity for CDK4/6, abemaciclib has additional affinity for CDK9. Despite an increase in off-target activity associated with abemaciclib, preferential binding to CDK4 over CDK6 results in a lower prevalence of severe neutropenia with abemaciclib compared with either palbociclib or ribociclib.