TARGETED DRUG THERAPY

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

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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

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.

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%).[4] 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.[5] 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
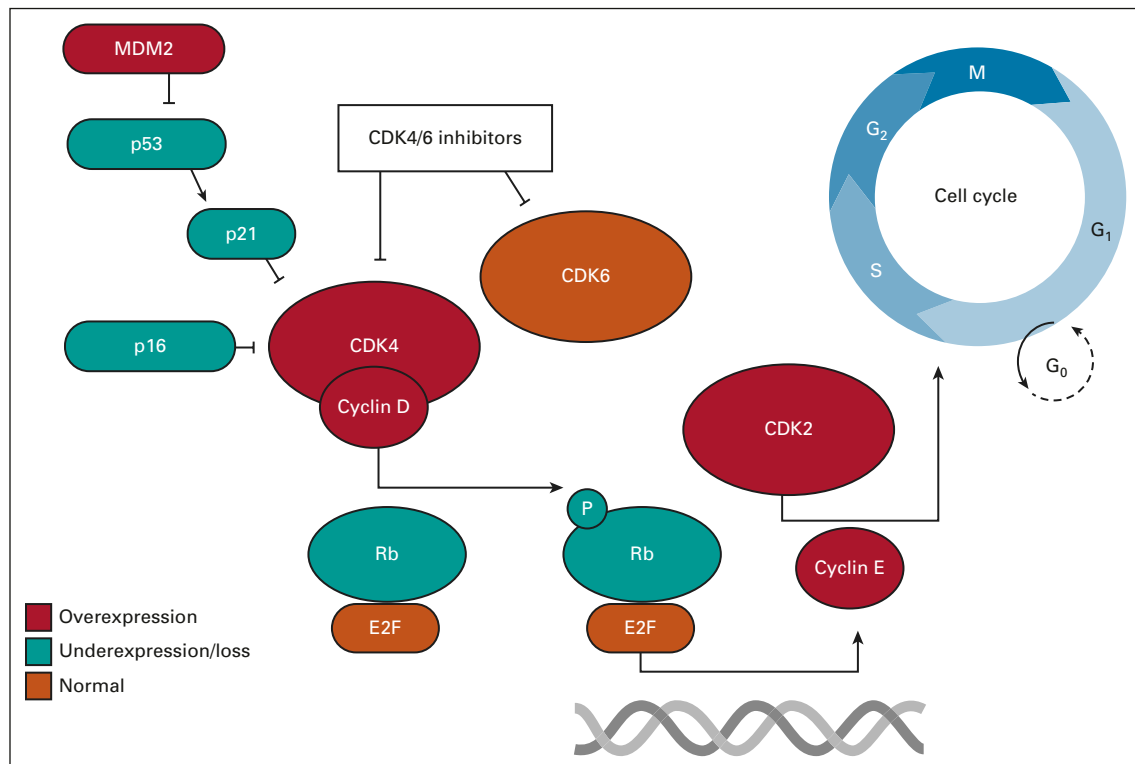

FIG 1. Common derangements in CCND-CDK4/6-RB pathway in sarcoma. The CDK4-cyclin D complex can phosphorylate the Rb-E2F complex to promote cyclin E translation for G1/S progression. CDK4/6 inhibitors can prevent this phosphorylation process. Common genes that are overexpressed (red), underexpressed/loss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

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.[6] 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.[7,8] 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, *CDK4*, *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.[3,54-64]

LPS

LPS, primarily arising from fat tissue in the thigh or retroperitoneum, comprise approximately 13% of all sarcomas.[65,66] 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.[33,34] In addition, amplification and/or activation events of *CDK4* are essential for the growth and survival of WDLPS/DDLPS.[67] MRCLS harbors a *FUS-DDIT3* chimeric gene, which causes abnormal expression of G1 checkpoint regulators including *CCND*, *CCNE*, *CDK4*, and *CDK2*.[47] 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 *GL11*.[49]

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.[68,69] 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.[68] 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.[69] 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.[70]

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.[13]

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.[10] 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.[14] 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).[15]

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

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

Reference/Clinical Trial No.	Tumor $\left(\frac{\text{patients with subtype}}{\text{total patients}}\right)$	Treatment	Results	Biomarkers
October 2017[9] NCT03096912 NCT02933736 NCT02806648	Sarcoma (59/1,332)	Palbociclib or ribociclib with antiestrogen therapy	Palbociclib was approved as monotherapy or in combination with antiestrogen therapy	
April 2019[10] NCT02187783	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
Octoer 2019[11]	HR+/HER2– breast cancer and WD/DDLPS (1/1)	Letrozole plus palbociclib	PR	
November 2017[11,12] NCT01209598	LPS (3/41)	Palbociclib	PFS at 12 weeks: 57% Median PFS: 17.9 weeks	CDK4 MDM2 Rb is reliable but not enough to make final conclusions by itself
	LPS (4/29)	Palbociclib	PFS at 12 weeks: 66% Median PFS: 18 weeks	
August 2018[13] NCT01237236	LPS (39/132)	Ribociclib	SD (8/41)	CDKN2A CCND1
June 2020[14] NCT02343172	LPS (74/74)	Ribociclib plus HDM201	PR (3) SD (37)	
August 2020[15] NCT02846987	Sarcoma, DDLPS (30/30)	Abemaciclib	PR (1)—PFS at 12 weeks: 76% Median PFS: 30.4 weeks	
October 2018[16]	LMS (1/114)	Palbociclib plus fulvestrant	PD	CDK4
April 2017[17]	Uterine LMS with CDKN2A mutation (1/279)	Palbociclib	SD and Clinical benefit	CDKN2A Rb
August 2019[18] NCT01907607	GIST with CDKN2A loss (29/29)	Palbociclib	No clinical significance as a single agent	CCNE1 CDKN1A Not CDKN2A
February 2019[19] NCT01747876	CDK4-amplified ARMS (1/22)	Ribociclib	PD	SMARCB1 CCND1 CDK4
May 2020[20]	OS (1/1)	Gemcitabine plus ribociclib	SD and well tolerated	
December 2019[12] NCT02644460	OS (2/34) RMS (1/34) Clear cell sarcoma (1/34)	Abemaciclib	Well tolerated at 130 mg/m^2/dose MTD: 130 mg/m^2/dose twice daily on a 28-day cycle	
July 2019[10]	Round blue cell soft tissue sarcoma not otherwise specified with CDK4 amplification (1/106)	Ribociclib	PR	CDK4
April 2020[21]	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.

function, is a biomarker that has been investigated but shows varied biomarker potential.[67,71] The utility of CDKN2A levels as a biomarker for CDK4/6 inhibitor sensitivity in WDLPS/DDLP has also yet to be fully defined.[72]

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.[73] LMS commonly have genomic alterations of TP53, RB1, chromatin remodeling, and homologous recombination DNA repair pathways.[74,75] 21.4% of LMS exhibit RB1-deactivating events, and subpopulations of LMS demonstrate genomic alterations in CDK4, CDK6, CDKN2A, CDKN2B, CCND1, and CCND3.[16,17,28,76] 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

Tumor	Genes in the Cyclin D/Cyclin-Dependent-Kinase 4/Rb Pathway					
	TP53	RB1	CDKN2A/CDKN2B	CCND1/2/3	CDK4/CDK6	CCNE1
ARMS[22,85,86]					Amplification	
AS[24]	Mutation / Amplification					
AFX[104]	Amplification		Mutation	Amplification	Amplification	
CS[26,27]	Mutation		Deletion			
Conventional OS[27-31]	Mutation / Deletion	Mutation / Deletion	Deletion	Amplification	Amplification	Amplification
DDS[127]	Amplification					
DDLPS[23,33-36,128]			Deletion	Amplification	Amplification	Amplification
EHE[37]		Mutation	Deletion			
ES[38]	Mutation	Mutation	Deletion			
GIST[39,40]	Deletion		Deletion	Deletion		
IS[3,129]			Deletion	Mutation	Amplification	
LMS[41,42]	Mutation	Deletion		Amplification		
MPNST[28,35,43-45]	Mutation		Deletion	Deletion		Amplification
MFS[32,46]	Mutation	Mutation	Deletion	Amplification		
MLS/RCLS[47]			Amplification			Amplification
Parosteal OS[103]					Amplification	
PLS[49-51]	Mutation	Mutation	Deletion	Amplification		
SS[21]			Deletion			
UPS[28,35,52,53]	Mutation	Mutation	Deletion	Deletion	Amplification	

Color Key	Mutation	Deletion	Amplification

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.

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

inhibitor, in DDLPS and Rb-positive LMS is currently underway 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.[39] 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* 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 with tumorigenesis of transcription factor, FoxF1- or FoxF2-, elevated RMS.[90] 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%).[92] 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.[97] 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.[21] 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.[98] SS commonly harbor *CDKN2A* deletion (74%) and an array of additional mutations in Rb pathway genes: *CCND1*, *CDK4*, *CDK6*, and *RB1*.[99] It has also been reported that heart sarcomas also have frequent *CDK4* (38%) and *CCND3* alterations (14%)[3]; 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 *CDK4*, mutated *CCND*, and deleted *CDKN2A*.[129] Epithelioid hemangioendothelioma is a rare sarcoma subtype commonly characterized by a *WWTR1-CAMTA1* fusion and altered *CDKN2A/CDKN2B*, *RB1*, *APC*, and *FANCA* genes.[37] 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.[26,100] 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.[10]

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.[101] As *CDKN2A/CDKN2B* loss is a common secondary alteration in sarcomas, potentially combining CDK4/6 inhibition with other agents to target primary and secondary drivers of oncogenesis is an attractive therapy. CDK4/6 inhibition in combination with hormone-directed therapies, DNA-damaging chemotherapy, antibodies against programmed cell death, Wee1, MEK, or mTOR inhibitors have been shown to have promising preliminary outcomes.[102]

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 sarcoma.[21,25,28,39,48,76,103-105] 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.[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.[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).[110] A TAPUR study of 29 patients with advanced non–small-cell lung cancer with *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 *CDKN2A* loss or mutation, there was a lack of clinical activity from CDK4/6 inhibition, indicating that *CDKN2A* may not be a universal biomarker and should be tested in individual sarcoma subtypes.[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.[113] A subgroup of this trial that focused on *CCND1*, *2*, or *3* amplification had prolonged SD in 13% of patients but *CCND1* or *3* amplification was not a predictor of palbociclib sensitivity.[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 *PTEN*. In these sarcoma subtypes, mTOR or PI3K inhibitors may be logical synergistic partners alongside CDK4/6 inhibitors.[102,115] In CDK4/6 inhibitor–resistant ES, which overexpresses *IGF1R*, a combination of CDK4/6 and *IGF1R* inhibitors was synergistic in vitro and in mouse models.[116]

Although PI3K or MEK inhibitors may be predicted to have synergy with combined CDK4/6 inhibition, other cotargets

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.[128] Similarly, in MDM2-amplified sarcomas, the MDM2-p53 binding inhibitor, nutlin, was antagonistic with palbociclib in preclinical sarcoma models.[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.[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.[101] Although chemotherapies 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 non-sarcoma 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.[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.[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

genomic 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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REFERENCES

1. Bleloch JS, Ballim RD, Kimani S, et al: Managing sarcoma: Where have we come from and where are we going? Ther Adv Med Oncol 9:637-659, 2017

2. Katz D, Palmerini E, Pollack SM: More than 50 subtypes of soft tissue sarcoma: Paving the path for histology-driven treatments. Am Soc Clin Oncol Ed Book 38:925-938, 2018

3. Jardim DL, Millis SZ, Ross JS, et al: Cyclin pathway genomic alterations across 190,247 solid tumors: Leveraging large-scale data to inform therapeutic directions. Oncologist 26:e78-e89, 2021

4. Abeshouse A, Adebamowo C, Adebamowo SN, et al: Comprehensive and integrated genomic characterization of adult soft tissue sarcomas. Cell 171:950-965.e28, 2017

5. Hensley ML, Chavan SS, Solit DB, et al: Genomic landscape of uterine sarcomas defined through prospective clinical sequencing. Clin Cancer Res 26:3881-3888, 2020

6. Niu Y, Xu J, Sun T: Cyclin-dependent kinases 4/6 inhibitors in breast cancer: Current status, resistance, and combination strategies. J Cancer 10:5504-5517, 2019

7. Groisberg R, Hong DS, Holla V, et al: Clinical genomic profiling to identify actionable alterations for investigational therapies in patients with diverse sarcomas. Oncotarget 8:39254-39267, 2017

8. Hay MA, Severson EA, Miller VA, et al: Identifying opportunities and challenges for patients with sarcoma as a result of comprehensive genomic profiling of sarcoma specimens. JCO Precis Oncol 4:176-182, 2020

9. Flaherty KT, Lorusso PM, Demichele A, et al: Phase I, dose-escalation trial of the oral cyclin-dependent kinase 4/6 inhibitor PD 0332991, administered using a 21-day schedule in patients with advanced cancer. Clin Cancer Res 18:568-576, 2012

10. Peguero J, Sohal DPS, O'Neil BH, et al: Tissue/site-agnostic study of ribociclib for tumors with cyclin D–CDK4/6 pathway genomic alterations: A phase II, open-label, single-arm basket study. JCO Precis Oncol 3:1-10, 2019

11. Loretan L, Moskovszky LE, Kurrer M, et al: Efficacy of a CDK4/6 inhibitor in a patient with breast cancer and liposarcoma: A case report and review of the literature. Breast Care (Basel) 14:325-328, 2019

12. Cash T, Aguilera D, Macy ME, et al: Phase 1 study of abemaciclib in children with recurrent and refractory solid tumors including malignant brain tumors [abstract. Mol Cancer Ther 18, 2019 (suppl 12; abstr C002). Proceedings of the AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics, October 26-30, 2019, Boston, MA, Philadelphia, PA: AACR

13. Infante JR, Cassier PA, Gerecitano JF, et al: A phase I study of the cyclin-dependent kinase 4/6 inhibitor ribociclib (LEE011) in patients with advanced solid tumors and lymphomas. Clin Cancer Res 22:5696-5705, 2016

14. Razak AA, Bauer S, Blay J-Y, et al: Results of a dose- and regimen-finding Phase Ib study of HDM201 in combination with ribociclib in patients with locally advanced or metastatic liposarcoma. Cancer Res 78, 2018 (suppl; abstr CT009)

15. Dickson MA, Koff A, D'Angelo SP, et al: Phase 2 study of the CDK4 inhibitor abemaciclib in dedifferentiated liposarcoma. J Clin Oncol 37, 2019 (suppl; abstr 11004)

16. Boddu S, Walko CM, Bienasz S, et al: Clinical utility of genomic profiling in the treatment of advanced sarcomas: A single-center experience. JCO Precis Oncol 2:1-8, 2018

17. Elvin JA, Gay LM, Ort R, et al: Clinical benefit in response to palbociclib treatment in refractory uterine leiomyosarcomas with a common CDKN2A alteration. Oncologist 22:416-421, 2017

18. Toulmonde M, Blay JY, Bouche O, et al: Activity and safety of palbociclib in patients with advanced gastrointestinal stromal tumors refractory to imatinib and sunitinib: A biomarker-driven phase II study. Clin Cancer Res 25:4611-4615, 2019

19. Geoerger B, Bourdeaut F, Dubois SG, et al: Phase I study of Lee011 (Cdk4/6 inhibitor) in patients with malignant rhabdoid tumors, neuroblastoma, and cyclin D–Cdk4/6 pathway-activated tumors. Ann Oncol 25:iv151, 2014

20. Lazow MA, Johnson SL, Johnson ND, et al: Genome-driven therapy for chemotherapy-resistant metastatic CDK6-amplified osteosarcoma. JCO Precis Oncol 4:498-504, 2020

21. Tramontana TF, Marshall MS, Helvie AE, et al: Sustained complete response to palbociclib in a refractory pediatric sarcoma with BCOR-CCNB3 fusion and germline CDKN2B variant. JCO Precis Oncol 4:466-471, 2020

22. Khan J, Simon R, Bittner M, et al: Gene expression profiling of alveolar rhabdomyosarcoma with cDNA microarrays. Cancer Res 58:5009-5013, 1998

23. Italiano A, Bianchini L, Gjernes E, et al: Clinical and biological significance of CDK4 amplification in well-differentiated and dedifferentiated liposarcomas. Clin Cancer Res 15:5696-5703, 2009

24. Naka N, Tomita Y, Nakanishi H, et al: Mutations of p53 tumor-suppressor gene in angiosarcoma. Int J Cancer 71:952-955, 1997

25. Pappo AS, Dirksen U: Rhabdomyosarcoma, Ewing sarcoma, and other round cell sarcomas. J Clin Oncol 36:168-179, 2017

26. Schrage YM, Lam S, Jochemsen AG, et al: Central chondrosarcoma progression is associated with pRb pathway alterations: CDK4 down-regulation and p16 overexpression inhibit cell growth in vitro. J Cell Mol Med 13:2843-2852, 2009

27. Speetjens FM, de Jong Y, Gelderblom H, et al: Molecular oncogenesis of chondrosarcoma: Impact for targeted treatment. Curr Opin Oncol 28:314-322, 2016

28. Kohlmeyer JL, Gordon DJ, Tanas MR, et al: CDKs in sarcoma: Mediators of disease and emerging therapeutic targets. Int J Mol Sci 21:3018, 2020

29. Martin JW, Squire JA, Zielenska M: The genetics of osteosarcoma. Sarcoma 2012:627254, 2012

30. Kansara M, Teng MW, Smyth MJ, et al: Translational biology of osteosarcoma. Nat Rev Cancer 14:722-735, 2014

31. Zhou Y, Shen JK, Yu Z, et al: Expression and therapeutic implications of cyclin-dependent kinase 4 (CDK4) in osteosarcoma. Biochim Biophys Acta Mol Basis Dis 1864:1573-1582, 2018

32. Ogura K, Hosoda F, Arai Y, et al: Integrated genetic and epigenetic analysis of myxofibrosarcoma. Nat Commun 9:2765, 2018

33. Bill KLJ, Seligson ND, Hays JL, et al: Degree of MDM2 amplification affects clinical outcomes in dedifferentiated liposarcoma. Oncologist 24:989-996, 2019

34. De Vita A, Mercatali L, Recine F, et al: Current classification, treatment options, and new perspectives in the management of adipocytic sarcomas. Onco Targets Ther 9:6233-6246, 2016

35. Bui NQ, Przybyl J, Trabucco SE, et al: A clinico-genomic analysis of soft tissue sarcoma patients reveals CDKN2A deletion as a biomarker for poor prognosis. Clin Sarcoma Res 9:12, 2019

36. Cuppens T, Moisse M, Depreeuw J, et al: Integrated genome analysis of uterine leiomyosarcoma to identify novel driver genes and targetable pathways. Int J Cancer 142:1230-1243, 2018

37. Seligson ND, Awasthi A, Millis SZ, et al: Common secondary genomic variants associated with advanced epithelioid hemangioendothelioma. JAMA Netw Open 2:e1912416, 2019

38. Toomey EC, Schiffman JD, Lessnick SL: Recent advances in the molecular pathogenesis of Ewing's sarcoma. Oncogene 29:4504-4516, 2010

39. Heinrich MC, Patterson J, Beadling C, et al: Genomic aberrations in cell cycle genes predict progression of KIT-mutant gastrointestinal stromal tumors (GISTs). Clin Sarcoma Res 9:3, 2019

40. Yang J, Du X, Lazar AJ, et al: Genetic aberrations of gastrointestinal stromal tumors. Cancer 113:1532-1543, 2008

41. Agaram NP, Zhang L, LeLoarer F, et al: Targeted exome sequencing profiles genetic alterations in leiomyosarcoma. Genes Chromosomes Cancer 55:124-130, 2016

42. Dei Tos AP, Maestro R, Doglioni C, et al: Tumor suppressor genes and related molecules in leiomyosarcoma. Am J Pathol 148:1037-1045, 1996

43. Brohl AS, Kahen E, Yoder SJ, et al: The genomic landscape of malignant peripheral nerve sheath tumors: Diverse drivers of Ras pathway activation. Sci Rep 7:14992, 2017

44. Kourea HP, Cordon-Cardo C, Dudas M, et al: Expression of p27(kip) and other cell cycle regulators in malignant peripheral nerve sheath tumors and neurofibromas: The emerging role of p27(kip) in malignant transformation of neurofibromas. Am J Pathol 155:1885-1891, 1999

45. Krawczyk MA, Karpinsky G, Izycka-Swieszewska E, et al: Immunohistochemical assessment of cyclin D1 and p53 is associated with survival in childhood malignant peripheral nerve sheath tumor. Cancer Biomark 24:351-361, 2019

46. Wang T, Goodman MA, McGough RL, et al: Immunohistochemical analysis of expressions of RB1, CDK4, HSP90, cPLA2G4A, and CHMP2B is helpful in distinction between myxofibrosarcoma and myxoid liposarcoma. Int J Surg Pathol 22:589-599, 2014

47. Olofsson A, Willén H, Göransson M, et al: Abnormal expression of cell cycle regulators in FUS-CHOP carrying liposarcomas. Int J Oncol 25:1349-1355, 2004

48. Weidema ME, Versleijen-Jonkers YMH, Flucke UE, et al: Targeting angiosarcomas of the soft tissues: A challenging effort in a heterogeneous and rare disease. Crit Rev Oncol Hematol 138:120-131, 2019

49. Fritz B, Schubert F, Wrobel G, et al: Microarray-based copy number and expression profiling in dedifferentiated and pleomorphic liposarcoma. Cancer Res 62:2993-2998, 2002

50. Gardner JM, Dandekar M, Thomas D, et al: Cutaneous and subcutaneous pleomorphic liposarcoma: A clinicopathologic study of 29 cases with evaluation of MDM2 gene amplification in 26. Am J Surg Pathol 36:1047-1051, 2012

51. Hofvander J, Jo VY, Ghanei I, et al: Comprehensive genetic analysis of a paediatric pleomorphic myxoid liposarcoma reveals near-haploidization and loss of the RB1 gene. Histopathology 69:141-147, 2016

52. Pérot G, Chibon F, Montero A, et al: Constant p53 pathway inactivation in a large series of soft tissue sarcomas with complex genetics. Am J Pathol 177:2080-2090, 2010

53. Reid AH, Tsai MM, Venzon DJ, et al: MDM2 amplification, P53 mutation, and accumulation of the P53 gene product in malignant fibrous histiocytoma. Diagn Mol Pathol 5:65-73, 1996

54. Marra A, Curigliano G: Are all cyclin-dependent kinases 4/6 inhibitors created equal? NPJ Breast Cancer 5:1-9, 2019

55. Ibrance (palbociclib) package insert. New York, NY: Pfizer, 2019

56. Kisqali (ribociclib) package insert. East Hanover, NJ: Novartis Pharmaceuticals, 2019

57. Verzenio (abemaciclib) package insert. Indianapolis, IN: Eli Lilly; , 2019

58. Asghar U, Witkiewicz AK, Turner NC, et al: The history and future of targeting cyclin-dependent kinases in cancer therapy. Nat Rev Drug Discov 14:130-146, 2015

59. Spring L, Bardia A, Modi S: Targeting the cyclin D-cyclin-dependent kinase (CDK) 4/6-retinoblastoma pathway with selective CDK 4/6 inhibitors in hormone receptor-positive breast cancer: Rationale, current status, and future directions. Discov Med 21:65-74, 2016

60. Yuan K, Wang X, Dong H, et al: Selective inhibition of CDK4/6: A safe and effective strategy for developing anticancer drugs. Acta Pharmaceutica Sinica B 11:30-54, 2020

61. Rugo HS: What Differentiates Abemaciclib, Ribociclib, and Palbociclib? https://oncpracticemanagement.com/videos/187-what-differentiates-abemaciclib-ribociclib-and-palbociclib

62. Kim S, Tiedt R, Loo A, et al: The potent and selective cyclin-dependent kinases 4 and 6 inhibitor ribociclib (LEE011) is a versatile combination partner in preclinical cancer models. Oncotarget 9:35226-35240, 2018

63. Xie N, Qin T, Ren W, et al: Efficacy and safety of cyclin-dependent kinases 4 and 6 inhibitors in HR+/HER2- advanced breast cancer. Cancer Manag Res 12:4241-4250, 2020

64. Dickler MN, Tolaney SM, Rugo HS, et al: MONARCH 1, A phase II study of abemaciclib, a CDK4 and CDK6 inhibitor, as a single agent, in patients with refractory HR+/HER2– metastatic breast cancer. Clin Cancer Res 23:5218-5224, 2017

65. Crago AM, Dickson MA: Liposarcoma: Multimodality management and future targeted therapies. Surg Oncol Clin N Am 25:761-773, 2016

66. Yang L, Chen S, Luo P, et al: Liposarcoma: Advances in cellular and molecular genetics alterations and corresponding clinical treatment. J Cancer 11:100-107, 2020

67. Zhang YX, Sicinska E, Czaplinski JT, et al: Antiproliferative effects of CDK4/6 inhibition in CDK4-amplified human liposarcoma in vitro and in vivo. Mol Cancer Ther 13:2184-2193, 2014

68. 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 clinical trial. JAMA Oncol 2:937-940, 2016

69. 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

70. von Mehren M et al: Soft tissue sarcoma, version 2.2018, NCCN Clinical Practice Guidelines in Oncology. JNCCN 16:536-563, 2018

71. Knudsen ES, Witkiewicz AK: The strange case of CDK4/6 inhibitors: Mechanisms, resistance, and combination strategies. Trends Cancer 3:39-55, 2017

72. Perez M, Muñoz-Galván S, Jiménez-García MP, et al: Efficacy of CDK4 inhibition against sarcomas depends on their levels of CDK4 and p16ink4 mRNA. Oncotarget 6:40557-40574, 2015

73. Gladdy RA, Qin LX, Moraco N, et al: Predictors of survival and recurrence in primary leiomyosarcoma. Ann Surg Oncol 20:1851-1857, 2013

74. Guo X, Jo VY, Mills AM, et al: Clinically relevant molecular subtypes in leiomyosarcoma. Clin Cancer Res 21:3501-3511, 2015

75. Seligson ND, Kautto EA, Passen EN, et al: BRCA1/2 functional loss defines a targetable subset in leiomyosarcoma. Oncologist 24:973-979, 2019

76. 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 2019:3914232, 2019

77. Schaefer IM, Hornick JL, Sholl LM, et al: Abnormal p53 and p16 staining patterns distinguish uterine leiomyosarcoma from inflammatory myofibroblastic tumour. Histopathology 70:1138-1146, 2017

78. Movva S, von Mehren M, Handorf EA, et al: SAR-096: A phase II trial of ribociclib in combination with everolimus in advanced dedifferentiated liposarcoma (DDL), and leiomyosarcoma (LMS). J Clin Oncol 38, 2020 (suppl; abstr 11544)

79. Dickson MA: Molecular pathways: CDK4 inhibitors for cancer therapy. Clin Cancer Res 20:3379-3383, 2014

80. Koumarianou A, Economopoulou P, Katsaounis P, et al: Gastrointestinal stromal tumors (GIST): A prospective analysis and an update on biomarkers and current treatment concepts. Biomark Cancer 7:1-7, 2015

81. Schmieder M, Wolf S, Danner B, et al: p16 expression differentiates high-risk gastrointestinal stromal tumor and predicts poor outcome. Neoplasia 10:1154-1162, 2008

82. Li J, Yang Z, Li Y, et al: Cell apoptosis, autophagy and necroptosis in osteosarcoma treatment. Oncotarget 7:44763-44778, 2016

83. Nouri H, Ben Maitigue M, Abid L, et al: Surface osteosarcoma: Clinical features and therapeutic implications. J Bone Oncol 4:115-123, 2015

84. Chen PC, Yen CC, Hung GY, et al: Gene amplification and tumor grading in parosteal osteosarcoma. J Chin Med Assoc 82:889-894, 2019

85. Parham DM, Barr FG: Classification of rhabdomyosarcoma and its molecular basis. Adv Anat Pathol 20:387-397, 2013

86. Barr FG, Duan F, Smith LM, et al: Genomic and clinical analyses of 2p24 and 12q13-q14 amplification in alveolar rhabdomyosarcoma: A report from the Children's Oncology Group. Genes Chromosomes Cancer 48:661-672, 2009

87. Arnold MA, Barr FG: Molecular diagnostics in the management of rhabdomyosarcoma. Expert Rev Mol Diagn 17:189-194, 2017

88. Olanich ME, Sun W, Hewitt SM, et al: CDK4 amplification reduces sensitivity to CDK4/6 inhibition in fusion-positive rhabdomyosarcoma. Clin Cancer Res 21:4947-4959, 2015

89. Nguyen TH, Barr FG: Therapeutic approaches targeting PAX3-FOXO1 and its regulatory and transcriptional pathways in rhabdomyosarcoma. Molecules 23:2798, 2018

90. Milewski D, Pradhan A, Wang X, et al: FoxF1 and FoxF2 transcription factors synergistically promote Rhabdomyosarcoma carcinogenesis by repressing transcription of p21Cip1 CDK inhibitor. Oncogene 36:850-862, 2017

91. Dauphinot L, De Oliveira C, Melot T, et al: Analysis of the expression of cell cycle regulators in Ewing cell lines: EWS-FLI-1 modulates p57KIP2and c-Myc expression. Oncogene 20:3258-3265, 2001

92. Bridge JA, Sumegi J, Druta M, et al: Clinical, pathological, and genomic features of EWSR1-PATZ1 fusion sarcoma. Mod Pathol 32:1593-1604, 2019

93. Cidre-Aranaz F, Alonso J: EWS/FLI1 target genes and therapeutic opportunities in Ewing sarcoma. Front Oncol 5:162, 2015

94. Lerman DM, Monument MJ, McIlvaine E, et al: Tumoral TP53 and/or CDKN2A alterations are not reliable prognostic biomarkers in patients with localized Ewing sarcoma: A report from the Children's Oncology Group. Pediatr Blood Cancer 62:759-765, 2015

95. Grünewald TGP, Cidre-Aranaz F, Surdez D, et al: Ewing sarcoma. Nat Rev Dis Primers 4:5, 2018

96. 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

97. Lin DI, Hemmerich A, Edgerly C, et al: Genomic profiling of BCOR-rearranged uterine sarcomas reveals novel gene fusion partners, frequent CDK4 amplification and CDKN2A loss. Gynecol Oncol 157:357-366, 2020

98. Schettini F, De Santo I, Rea CG, et al: CDK 4/6 inhibitors as single agent in advanced solid tumors. Front Oncol 8:608, 2018

99. Subramaniam MM, Noguera R, Piqueras M, et al: p16INK4A (CDKN2A) gene deletion is a frequent genetic event in synovial sarcomas. Am J Clin Pathol 126:866-874, 2006

100. Ouyang Z, Wang S, Zeng M, et al: Therapeutic effect of palbociclib in chondrosarcoma: Implication of cyclin-dependent kinase 4 as a potential target. Cell Commun Signal 17:17, 2019

101. Francis AM, Alexander A, Liu Y, et al: CDK4/6 inhibitors sensitize Rb-positive sarcoma cells to Wee1 kinase inhibition through reversible cell-cycle arrest. Mol Cancer Ther 16:1751-1764, 2017

102. Cortés J, Im SA, Holgado E, et al: The next era of treatment for hormone receptor-positive, HER2-negative advanced breast cancer: Triplet combination-based endocrine therapies. Cancer Treat Rev 61:53-60, 2017

103. Gamberi G, Ragazzini P, Benassi MS, et al: Analysis of 12q13-15 genes in parosteal osteosarcoma. Clin Orthop Relat Res:195-204, 2000

104. Helbig D, Ihle MA, Pütz K, et al: Oncogene and therapeutic target analyses in atypical fibroxanthomas and pleomorphic dermal sarcomas. Oncotarget 7:21763-21774, 2016

105. Ragazzini P, Gamberi G, Pazzaglia L, et al: Amplification of CDK4, MDM2, SAS and GLI genes in leiomyosarcoma, alveolar and embryonal rhabdomyosarcoma. Histol Histopathol 19:401-411, 2004

106. Grupo Espanol en Investigacion en Sarcomas: Phase II Multicenter Trial of Palbociclib in Second Line of Advanced Sarcomas With CDK4 Overexpression, 2020. https://clinicaltrials.gov/ct2/show/NCT03242382

107. Charlson J: Abemaciclib for Treatment of Advanced Bone and Soft Tissue Sarcoma Identified as Having CDK Pathway Alteration, 2020. https://clinicaltrials.gov/ct2/show/NCT04040205

108. Institut National de la Santé Et de la Recherche Médicale, France: Molecular Profiling of Advanced Soft-Tissue Sarcomas. A Phase III Study, 2020. https://clinicaltrials.gov/ct2/show/NCT03784014

109. Malorni L, Piazza S, Ciani Y, et al: A gene expression signature of retinoblastoma loss-of-function is a predictive biomarker of resistance to palbociclib in breast cancer cell lines and is prognostic in patients with ER positive early breast cancer. Oncotarget 7:68012-68022, 2016

110. Andrews A: ASCO and NCI launch largest precision medicine trials using real-world evidence. Am Health Drug Benefits 8:37, 2015

111. Al Baghdadi T, Halabi S, Garrett-Mayer E, et al: Palbociclib in patients with pancreatic and biliary cancer with CDKN2A alterations: Results from the targeted agent and profiling utilization registry study. JCO Precis Oncol 3:1-8, 2019

112. Ahn ER, Mangat PK, Garrett-Mayer E, et al: Palbociclib in patients with non–small-cell lung cancer with CDKN2A alterations: Results from the targeted agent and profiling utilization registry study. JCO Precis Oncol 4:757-766, 2020

113. National Cancer Institute (NCI): Molecular Analysis for Therapy Choice (MATCH), 2021. https://clinicaltrials.gov/ct2/show/NCT02465060

114. Clark AS, Hong F, Finn RS, et al. Molecular analysis for therapy choice (NCI-MATCH, EAY131) arm Z1B: Phase II trial of palbociclib for CCND1, 2 or 3 amplified tumors. American Association for Cancer Research annual meeting, Atlanta, GA, March 29–April 3, 2019 (abstr LB-010)

115. Dillon LM, Miller TW: Therapeutic targeting of cancers with loss of PTEN function. Curr Drug Targets 15:65-79, 2014

116. Guenther LM, Dharia NV, Ross L, et al: A combination CDK4/6 and IGF1R inhibitor strategy for Ewing sarcoma. Clin Cancer Res 25:1343-1357, 2019

117. Laroche-Clary A, Chaire V, Algeo MP, et al: Combined targeting of MDM2 and CDK4 is synergistic in dedifferentiated liposarcomas. J Hematol Oncol 10:123, 2017

118. Sriraman A, Dickmanns A, Najafova Z, et al: CDK4 inhibition diminishes p53 activation by MDM2 antagonists. Cell Death Dis 9:918, 2018

119. Fassl A, Sicinski P: Chemotherapy and CDK4/6 inhibition in cancer treatment: Timing is everything. Cancer Cell 37:265-267, 2020

120. Dean JL, McClendon AK, Knudsen ES: Modification of the DNA damage response by therapeutic CDK4/6 inhibition. J Biol Chem 287:29075-29087, 2012

121. Hamilton G, Klameth L, Rath B, et al: Synergism of cyclin-dependent kinase inhibitors with camptothecin derivatives in small cell lung cancer cell lines. Molecules 19:2077-2088, 2014

122. Gao Y, Shen J, Choy E, et al: Inhibition of CDK4 sensitizes multidrug resistant ovarian cancer cells to paclitaxel by increasing apoptosiss. Cell Oncol (Dordr) 40:209-218, 2017

123. Roberts PJ, Kumarasamy V, Witkiewicz AK, et al: Chemotherapy and CDK4/6 inhibitors: Unexpected bedfellows. Mol Cancer Ther 19:1575-1588, 2020

124. Huang X, Di Liberto M, Jayabalan D, et al: Prolonged early G(1) arrest by selective CDK4/CDK6 inhibition sensitizes myeloma cells to cytotoxic killing through cell cycle-coupled loss of IRF4. Blood 120:1095-1106, 2012

125. Zhang XH, Cheng Y, Shin JY, et al: A CDK4/6 inhibitor enhances cytotoxicity of paclitaxel in lung adenocarcinoma cells harboring mutant KRAS as well as wild-type KRAS. Cancer Biol Ther 14:597-605, 2013

126. Min A, Kim JE, Kim YJ, et al: Cyclin E overexpression confers resistance to the CDK4/6 specific inhibitor palbociclib in gastric cancer cells. Cancer Lett 430:123-132, 2018

127. Bovée JV, Cleton-Jansen AM, Rosenberg C, et al: Molecular genetic characterization of both components of a dedifferentiated chondrosarcoma, with implications for its histogenesis. J Pathol 189:454-462, 1999

128. Singer S, Socci ND, Ambrosini G, et al: Gene expression profiling of liposarcoma identifies distinct biological types/subtypes and potential therapeutic targets in well-differentiated and dedifferentiated liposarcoma. Cancer Res 67:6626-6636, 2007

129. Roszik J et al: Unique aberrations in intimal sarcoma identified by Next-Generation Sequencing as potential therapy targets. Cancers (Basel) 11:1283, 2019

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.[3] Progression through this checkpoint requires adequate cell size, nutrients, and growth factors, in addition to a low threshold of DNA damage in the cell.[54]

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.[54-57] CDK4/6 inhibitors directly compete with CCND for binding to the ATP cleft of CDK4 and CDK6.[58] 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.[59]

Current US Food and Drug Administration (FDA)-approved CDK4/6 inhibitors include palbociclib, ribociclib, and abemaciclib with an additional 15 new agents under development.[60] 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.[55] 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.[56-61] 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.[54-64]