VOLUME 31 · NUMBER 16 · JUNE 1 2013

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Phase II Trial of the CDK4 Inhibitor PD0332991 in Patients With Advanced *CDK4*-Amplified Well-Differentiated or Dedifferentiated Liposarcoma

Mark A. Dickson, William D. Tap, Mary Louise Keohan, Sandra P. D'Angelo, Mrinal M. Gounder, Cristina R. Antonescu, Jonathan Landa, Li-Xuan Qin, Dustin D. Rathbone, Mercedes M. Condy, Yelena Ustoyev, Aimee M. Crago, Samuel Singer, and Gary K. Schwartz

All authors: Memorial Sloan-Kettering Cancer Center, New York, NY.

Published online ahead of print at www.jco.org on April 8, 2013.

Supported by Pfizer and in part by National Cancer Institute, National Institutes of Health, Soft Tissue Sarcoma Program Project Grant No. P01 CA047179 (C.R.A., S.S, G.K.S.).

Presented in part at the 48th Annual Meeting of the American Society for Clinical Oncology, Chicago, IL, June 1-5, 2012.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical trial information: NCT01209598.

Corresponding author: Mark A. Dickson, MD, 300 E 66th St, New York, NY 10065; e-mail: dicksonm@mskcc.org.

0732-183X/13/3116w-2024w/$20.00

DOI: 10.1200/JCO.2012.46.5476

ABSTRACT

Purpose

CDK4 is amplified in > 90% of well-differentiated (WDLS) and dedifferentiated liposarcomas (DDLS). The selective cyclin-dependent kinase 4 (CDK4)/CDK6 inhibitor PD0332991 inhibits growth and induces senescence in cell lines and xenografts. In a phase I trial of PD0332991, several patients with WDLS or DDLS experienced prolonged stable disease. We performed an open-label phase II study to determine the safety and efficacy of PD0332991 in patients with advanced WDLS/DDLS.

Patients and Methods

Patients age ≥ 18 years experiencing disease progression while receiving systemic therapy before enrollment received PD0332991 200 mg orally once per day for 14 consecutive days in 21-day cycles. All were required to have *CDK4* amplification by fluorescence in situ hybridization and retinoblastoma protein (RB) expression by immunohistochemistry (≥ 1+). The primary end point was progression-free survival (PFS) at 12 weeks, with 12-week PFS of ≥ 40% considered promising and ≤ 20% not promising. If ≥ nine of 28 patients were progression free at 12 weeks, PD0332991 would be considered active.

Results

We screened 48 patients (44 of 48 had *CDK4* amplification; 41 of 44 were RB positive). Of those, 30 were enrolled, and 29 were evaluable for the primary end point. Grade 3 to 4 events included anemia (17%), thrombocytopenia (30%), neutropenia (50%), and febrile neutropenia (3%). At 12 weeks, PFS was 66% (90% CI, 51% to 100%), significantly exceeding the primary end point. The median PFS was 18 weeks. There was one partial response.

Conclusion

Treatment with the CDK4 inhibitor PD0332991 was associated with a favorable progression-free rate in patients with *CDK4*-amplified and RB-expressing WDLS/DDLS who had progressive disease despite systemic therapy.

J Clin Oncol 31:2024-2028. © 2013 by American Society of Clinical Oncology

INTRODUCTION

Liposarcomas are malignant mesenchymal tumors that are classified into three main biologic groups: well-differentiated (WDLS) and dedifferentiated liposarcomas (DDLS), myxoid/round-cell liposarcoma, and pleiomorphic liposarcoma.[1] WDLS/DDLS are considered a biphasic disease. The dedifferentiated component, which can be rapidly growing, aggressive, and metastatic, is thought to arise from the well-differentiated component, which can grow slowly.[2] Both WDLS and DDLS are relatively resistant to chemotherapy, and few viable treatments exist for patients with locally advanced or metastatic disease.[3]

The oncogene cyclin-dependent kinase 4 (*CDK4*) is amplified in > 90% of WDLS/DDLS,[4,5] and it is also highly amplified.[6] Gene expression array studies have shown that that CDK4 expression is > 10× as high in WDLS/DDLS as in normal fat tissue.[7] Inhibition of CDK4 expression with short hairpin RNA inhibits growth of WDLS/DDLS cells in vitro.[8]

PD0332991 is a potent oral inhibitor of CDK4 and CDK6 that prevents downstream phosphorylation of the retinoblastoma (RB) protein.[9,10] The drug inhibits the growth of WDLS/DDLS cells in vitro and in xenograft models.[8] In a phase I study of PD0332991, two patients with RB-positive WDLS/DDLS had prolonged stable disease lasting several

years.[11] We performed a single-arm phase II clinical trial of PD0332991 in patients with progressive advanced WDLS/DDLS.

PATIENTS AND METHODS

To be eligible, patients had to be adults (age ≥ 18 years) with locally advanced or metastatic WDLS/DDLS. In addition, patients had to have *CDK4* amplification, as detected by fluorescence in situ hybridization (FISH), and RB expression by immunohistochemistry, both determined on an archival tumor specimen.

Main inclusion criteria were histologically confirmed WDLS/DDLS, adequate organ and marrow function, Eastern Oncology Cooperative Group (ECOG) performance status of 0 or 1, and measurable disease by RECIST (version 1.1).[12] Patients must have received at least one other systemic treatment for advanced disease. All patients had evidence of clinical disease progression before enrolling onto this trial. The protocol was approved by the institutional review board of Memorial Sloan-Kettering Cancer Center, and all patients provided written informed consent.

Study Design and Statistical Analysis

This was a single-institution nonrandomized open-label phase II study. The primary end point was progression-free survival (PFS) at 12 weeks. On the basis of historical controls, PFS > 40% at 3 months was considered promising for second-line therapy, and PFS < 20% was considered not promising.[13] A one-stage design was used.[14] The initial study design called for a sample size of 28. The study would meet its primary end point if at least nine patients were progression free at 12 weeks. This design has a type I error rate of 0.09 and a type II error rate of 0.15.

CDK4 and RB Assessment

CDK4 amplification testing by FISH was performed using a probe comprising BAC clones RP11-571M6 (Wellcome Trust Sanger Institute, Hinxton, United Kingdom) and RP11-970A5 (BACPAC Resources, Oakland, CA) spanning *CDK4*, labeled with red deoxyuridine triphosphate, together with chromosome 12 centromeric clone pα12H8, labeled with green deoxyuridine triphosphate (Enzo Life Sciences, Farmingdale, NY; supplied by Abbott Molecular, Chicago, IL).

FISH was performed on formalin-fixed, paraffin-embedded sections according to standard procedures. Briefly, paraffin sections were dewaxed in xylenes, microwaved in 10 mmol/L sodium citrate (pH, 6.0) solution for approximately 10 minutes, cooled to room temperature, rinsed, and treated with approximately 150 units/mL pepsin–hydrochloric acid for approximately 5 minutes at 37°C before being rinsed and dehydrated. Prewarmed probe mixture was applied to the slides, and a coverslip was sealed in place with rubber cement. The slides were then denatured at 80°C for 8 minutes on a HYBrite automated hybridizer (Vysis, Des Plaines, IL) and incubated overnight at 37°C. After standard nonformamide posthybridization washes, the slides were stained with 4′,6-diamidino-2-phenylindole and mounted in antifade (Vectashield; Vector Laboratories, Burlingame, CA).

Analysis was performed using a Zeiss Axioplan epifluorescence microscope (Carl Zeiss Microscopy, Thornwood, NY) with motorized stage and Isis 5 imaging software (MetaSystems Group, Waltham, MA). Image records consisted of collapsed stacks captured at 0.5-micron intervals through the depth of the tissue. Amplification was defined as *CDK4*-to-centromere ratio > 2.5, with a ratio > 10 representing high-level amplification.

RB expression by immunohistochemistry was determined using standard methods (RB [4H1] mouse monoclonal antibody; Cell Signaling Technology, Danvers, MA). Tumor samples were required to express RB at a level ≥ 1+ (above background).

Treatment

Patients were treated with 200 mg of PD0332991 once per day for 14 days, followed by 7 days of rest. This was the maximum-tolerated dose determined in the phase I study.[11] Cycles were repeated every 3 weeks, provided the following criteria were met on the first day of the next cycle: platelet count ≥ 50,000/μL, absolute neutrophil count ≥ 1000/μL, and hemoglobin ≥ 8.0 g/dL. If these criteria were not met, the start of the cycle was delayed up to 7 days to allow for hematologic recovery. If the start of the next cycle had to be delayed > 7 days, the dose was reduced to 150 mg. In addition, the dose was reduced to 150 mg for grade 4 hematologic toxicity occurring at any time. If a second dose reduction was required for the same reasons, the dose was reduced to 100 mg.

Response Assessment

Clinical examinations and laboratory testing were performed at a screening visit, at the start of treatment, and at the start of each cycle of therapy for the first 12 cycles and thereafter at every other cycle. In addition, a complete blood count was performed once per week during the first cycle. Tumor response was assessed by a reference radiologist by computed tomography (CT) scan once every 6 weeks (regardless of dose delays) for 36 weeks and once every 12 weeks thereafter. Toxicities were assessed and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0).

RESULTS

Between October 2010 and November 2011, 51 patients gave consent to the protocol. The flow of patients and tumor testing is described

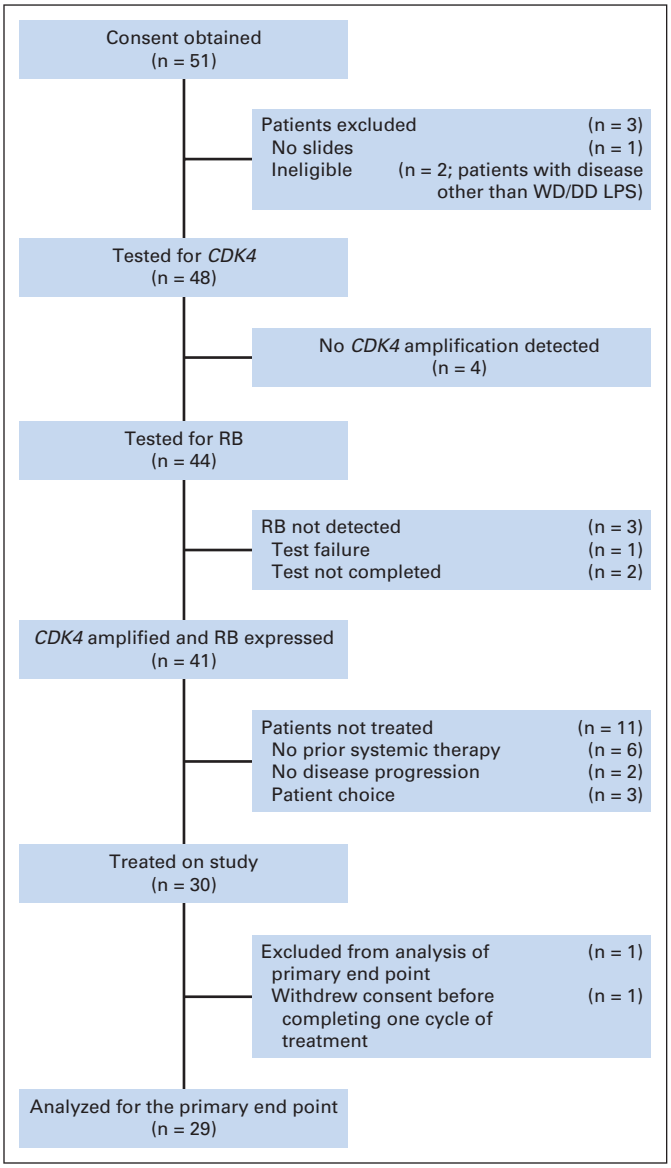

Fig 1. Diagram showing flow of patients and testing for *CDK4* and retinoblastoma protein (RB). DD, dedifferentiated; LPS, liposarcoma; WD, well differentiated.

(Fig 1). *CDK4* amplification was detected in 44 (92%) of 48 tumors tested. Of these 44 patients, two did not complete RB testing because of clinical deterioration rendering them ineligible for the study. For one patient, there was a technical failure with the test. Of the remaining 41 patients, all had RB expression, and thus, all were eligible for treatment in the study. Eleven patients did not start study treatment either because they had not yet shown evidence of disease progression during prior systemic therapy or because of patient choice.

Thirty patients were treated with PD0332991. The characteristics of these patients are listed in Table 1. The primary site of disease was the retroperitoneum in 97% of patients. Only five patients (17%) had purely well-differentiated tumors. The remaining 83% had either dedifferentiated or well-differentiated plus dedifferentiated disease on pathology review. All had received at least one prior regimen of systemic therapy, and some had received up to five prior regimens. Nineteen (63%) had received prior doxorubicin-based treatment.

Toxicity

The incidence of grade 2 to 4 adverse events possibly, probably, or definitely related to treatment is summarized in Table 2. Most toxicities were hematologic. Grade 3 to 4 toxicities included anemia (17%), neutropenia (50%), and thrombocytopenia (30%). Despite the frequency of neutropenia, there was only one episode of neutropenic fever, which resolved without complications. The drug was otherwise well tolerated, with no other common serious adverse events. Dose reduction for hematologic toxicity was required for 24% of patients.

Table 1. Demographic and Clinical Characteristics of Patients Treated With PD0332991 (n = 30)

Characteristic	No.	%
Sex		
Male	16	52
Female	14	48
Age, years		
Median	65	
Range	37-83	
ECOG PS		
0	20	67
1	10	33
Primary site		
Retroperitoneum	29	97
Extremity	1	3
Histology		
Well differentiated	5	17
Dedifferentiated	25	83
No. of prior systemic treatments		
Median	1	
Range	1-5	
Prior systemic treatments		
Doxorubicin or liposomal doxorubicin	19	
Gemcitabine	4	
Gemcitabine and docetaxel	4	
Ifosfamide	5	
Trabectedin	3	
Other cytotoxics (dacarbazine, cyclophosphamide, irinotecan)	3	
Other targeted agents (imatinib, sunitinib, brivanib, flavopiridol, and inhibitors of notch, hedgehog, MDM2)	18	

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; MDM2, mouse double minute 2 homolog.

Table 2. Incidence of Grade 2 to 4 Adverse Events Possibly, Probably, or Definitely Related to Treatment

Toxicity	Grade 2	Grade 3	Grade 4	Total
Hematologic				
Anemia	6	5		11
WBC decreased	13	13	1	27
Platelet count decreased	2	5	4	11
Lymphocyte count decreased	4	6	2	12
Neutrophil count decreased	13	13	2	28
Febrile neutropenia		1		1
Nonhematologic				
Anorexia	1			1
Constipation		1		1
Diarrhea	1			1
Dry skin	1			1
Epistaxis	1			1
Fatigue	1	2		3
Hematuria		1		1
Upper respiratory infection		1		1

Efficacy

One patient withdrew consent before completing the first cycle of treatment after experiencing nausea, diarrhea, and fatigue (all grade 1). This patient did not have a repeat CT scan during the study and was not evaluable for the primary end point. Of 29 patients evaluable for the primary end point, one died 3 weeks after starting treatment. This was probably related to advanced disease and unlikely related to treatment. Three patients had clinical deterioration and were considered to have progressed even though scans did not show objective progression per RECIST. An additional six patients had progression by RECIST on

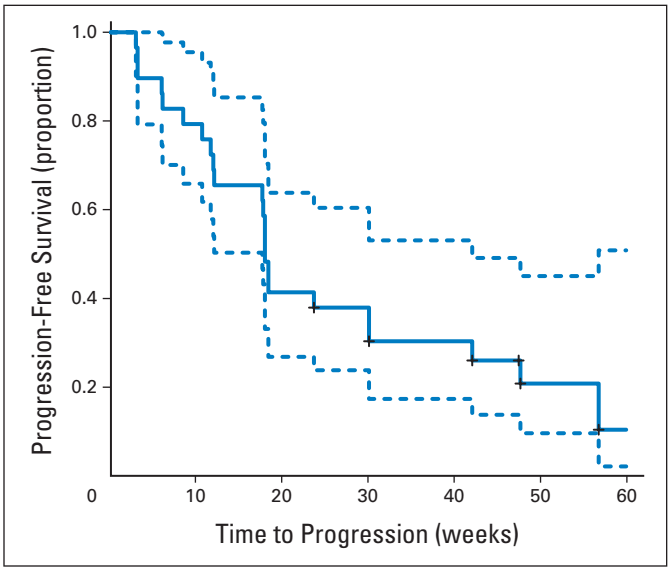

Fig 2. Kaplan-Meier curve of progression-free survival. Dashed lines indicate 95% CI.

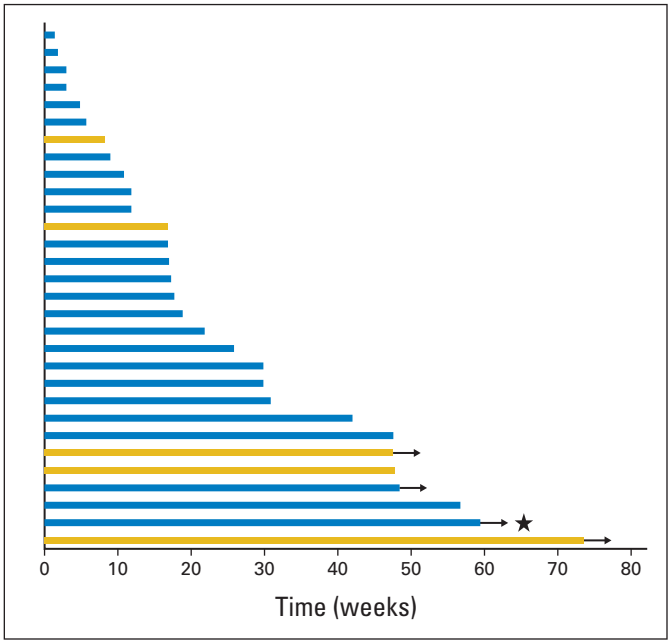

Fig 3. Time in study for all evaluable patients. Gold bars represent patients with purely well-differentiated liposarcoma; blue bars represent dedifferentiated tumors. Arrows indicate patients who remained in study at the data cutoff. Star indicates patient with partial response.

or before week 12. Thus, 19 patients were progression free at 12 weeks. The estimated 12-week PFS rate was 66%, with a one-sided 90% CI of 51% to 100%. This significantly exceeded the 12-week PFS rate of 40% to consider the study positive. A Kaplan-Meier curve of PFS is shown in Figure 2. The median PFS was 17.9 weeks. At data cutoff, four patients remained in the study, at 48 to 74 weeks of follow-up.

The duration of study treatment for each patient is depicted in Figure 3. One patient (3%) achieved a partial response according to RECIST at 74 weeks and remains in the study. Three other patients had evidence of favorable response to treatment that did not meet RECIST, specifically, decreases in tumor size of at least 10%. For example, one patient had gradual regression of a component of DDLS within a larger well-differentiated tumor (Fig 4). This occurred over a period of $>$ 1 year of treatment with PD0332991. At the point of maximal response, the patient had a 30% decrease in tumor size per RECIST; however, the patient developed a new enlarging nontarget lesion, which was considered progressive disease.

DISCUSSION

To our knowledge, this is the first phase II clinical trial performed specifically for patients with WDLS/DDLS. In addition to testing a targeted therapy in a set of patients with a single biologic group of sarcomas characterized by *CDK4* amplification, this study specifically enriched for patients with a molecularly defined target. As expected, RB expression was common, and *CDK4* amplification was detected in $>$ 90% of samples, consistent with prior published series.[5]

Treatment with PD0332991 was generally well tolerated. Although myelosuppression was common, this rarely resulted in serious sequelae. Only a minority of patients required dose reductions or delays. Overall, 74% of cycles were administered on schedule. Moreover, myelosuppression was an isolated adverse effect, with no significant systemic symptoms such as nausea, diarrhea, or alopecia, which are commonly associated with conventional myelosuppressive chemotherapy.

The natural history of WDLS/DDLS can be highly variable. To address this heterogeneity, all patients in the study were required to have evidence of disease progression despite systemic therapy. Most patients had experienced prior treatment failure with doxorubicin-containing regimens. This eligibility criterion selected for patients with advanced and progressive disease and increases the importance of the prolonged PFS that was observed. A potential weakness is that progression at the time of study entry was not formally defined but rather was assessed by the treating physician. A future randomized study would obviate this concern. Retrospective review of the scans of patients treated in this study showed that all had enlarging tumors before enrollment. Representative tumors grew by an average of 25% over serial CT scans performed on average 10 weeks apart. In most cases, this growth occurred while patients were receiving other systemic therapy, thus demonstrating that the disease was refractory and progressive.

This study met its primary end point. The 12-week PFS of 66% significantly exceeded the expected PFS rate of 40% for an active second-line agent and even further exceeded the expected PFS rate of 20% for an inactive agent.[13] The expected PFS rates were derived from an assessment of pretreated patients with soft tissue sarcoma across 12 clinical trials. The patients constituting these historical controls, like those in our study, all had progressive sarcoma despite prior chemotherapy and similar ECOG

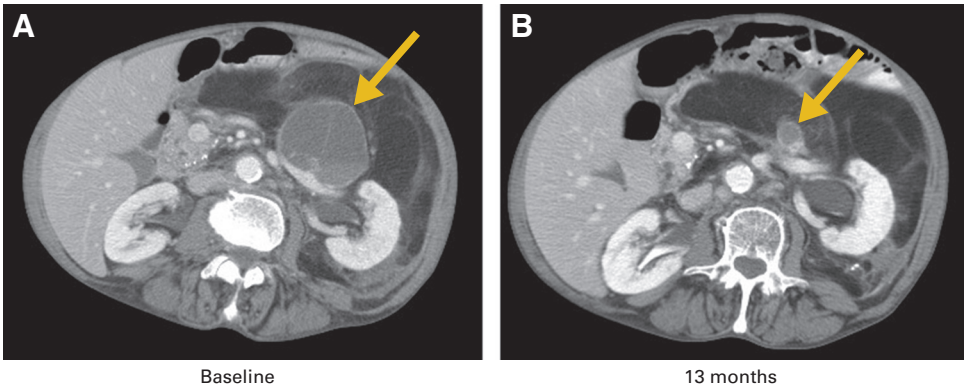

Fig 4. Computed tomography scans at (A) baseline and (B) after 13 months of treatment with PD0332991, demonstrating favorable tumor response (arrows) in dedifferentiated liposarcoma surrounded by well-differentiated liposarcoma.

performance status. However, the historical controls encompassed multiple sarcoma types, not just WDLS/DDLS. Also, as with any historical controls, comparisons were hindered by the possibility of changes in the patient population and treatment practices. Nevertheless, the 12-week PFS in our study was as high as, or higher than, those in recent studies of two agents commonly used as second-line treatment for soft tissue sarcoma—ifosfamide (PFS, 65%) and trabectedin (PFS, 40% to 56%).[15]

Prolonged stable disease and responses seem possible, but the onset of response may be late (74 weeks for the one partial response). Patients with WDLS as well as dedifferentiated tumors were able to achieve prolonged stable disease (Fig 3). However, objective tumor regression tended to occur in dedifferentiated tumors (partial response in Fig 3; Fig 4), suggesting the possibility of greater activity in dedifferentiated tumors.

A second phase II study is ongoing at our institution to evaluate PD0332991 at a different dose and schedule (125 mg once per day for 21 days, every 28 days), which may be associated with less hematologic toxicity. Tumor biopsies will also be performed to study mechanisms of acquired drug resistance in patients who have disease progression after initial benefit.

Targeting CDK4 in WDLS/DDLS has been of interest for several years. A previous clinical trial of the pan-CDK inhibitor flavopiridol in combination with doxorubicin demonstrated some clinical benefit in WDLS/DDLS (stable disease for $>$ 3 months in seven of 12 patients).[16] However, our study, with a more potent and selective CDK4/CDK6 inhibitor, is the first to our knowledge to show objective tumor regression and thus provides important proof of principle. Recent data show that the rare cases of WDLS/DDLS without *CDK4* amplification often involve other genetic abnormalities in the same pathway, such as amplification of the cyclin D1 gene (*CCND1*) or loss of the p16 gene (*CDKN2A*).[17] Thus, CDK4 inhibition may be effective in those tumors as well. WDLS/DDLS are complex tumors with multiple chromosomal abnormalities, so inhibition of a single oncogene would not be expected to effectively treat all patients. However, this study demonstrates that treatment with a selective CDK4 inhibitor is associated with favorable PFS and can lead to radiographic response, at least in a subset of patients with this disease, and provides an important foundation for future studies.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.
Employment or Leadership Position: None **Consultant or Advisory Role:** Mark A. Dickson, Pfizer (C); Samuel Singer, Pfizer (C); Gary K. Schwartz, Pfizer (C) **Stock Ownership:** None **Honoraria:** None **Research Funding:** None **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Mark A. Dickson, Cristina R. Antonescu, Li-Xuan Qin, Samuel Singer, Gary K. Schwartz
Administrative support: William D. Tap, Samuel Singer
Provision of study materials or patients: Mark A. Dickson, William D. Tap, Mary Louise Keohan, Sandra P. D'Angelo, Mrinal M. Gounder, Cristina R. Antonescu, Aimee M. Crago, Samuel Singer
Collection and assembly of data: Mark A. Dickson, Mary Louise Keohan, Sandra P. D'Angelo, Mrinal M. Gounder, Cristina R. Antonescu, Jonathan Landa, Dustin D. Rathbone, Mercedes M. Condy, Yelena Ustoyev, Gary K. Schwartz
Data analysis and interpretation: Mark A. Dickson, William D. Tap, Mary Louise Keohan, Sandra P. D'Angelo, Mrinal M. Gounder, Li-Xuan Qin, Dustin D. Rathbone, Aimee M. Crago, Gary K. Schwartz
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Conyers R, Young S, Thomas DM: Liposarcoma: Molecular genetics and therapeutics. Sarcoma [epub ahead of print on December 27, 2010]
2. Dalal KM, Kattan MW, Antonescu CR, et al: Subtype specific prognostic nomogram for patients with primary liposarcoma of the retroperitoneum, extremity, or trunk. Ann Surg 244:381-391, 2006
3. Crago AM, Singer S: Clinical and molecular approaches to well differentiated and dedifferentiated liposarcoma. Curr Opin Oncol 23:373-378, 2011
4. Binh MB, Sastre-Garau X, Guillou L, et al: MDM2 and CDK4 immunostainings are useful adjuncts in diagnosing well-differentiated and dedifferentiated liposarcoma subtypes: A comparative analysis of 559 soft tissue neoplasms with genetic data. Am J Surg Pathol 29:1340-1347, 2005
5. Sirvent N, Coindre JM, Maire G, et al: Detection of MDM2-CDK4 amplification by fluorescence in situ hybridization in 200 paraffin-embedded tumor samples: Utility in diagnosing adipocytic lesions and comparison with immunohistochemistry and real-time PCR. Am J Surg Pathol 31:1476-1489, 2007
6. Tap WD, Eilber FC, Ginther C, et al: Evaluation of well-differentiated/de-differentiated liposarcomas by high-resolution oligonucleotide array-based comparative genomic hybridization. Genes Chromosomes Cancer 50:95-112, 2011
7. 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
8. Barretina J, Taylor BS, Banerji S, et al: Subtype-specific genomic alterations define new targets for soft-tissue sarcoma therapy. Nat Genet 42:715-721, 2010
9. Fry DW, Harvey PJ, Keller PR, et al: Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. Mol Cancer Ther 3:1427-1438, 2004
10. Toogood PL, Harvey PJ, Repine JT, et al: Discovery of a potent and selective inhibitor of cyclin-dependent kinase 4/6. J Med Chem 48:2388-2406, 2005
11. Schwartz GK, LoRusso PM, Dickson MA, et al: Phase I study of PD 0332991, a cyclin-dependent kinase inhibitor, administered in 3-week cycles (Schedule 2/1). Br J Cancer 104:1862-1868, 2011
12. Eisenhauer EA, Therasse P, Bogaerts J, et al: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 45:228-247, 2009
13. Van Glabbeke M, Verweij J, Judson I, et al: Progression-free rate as the principal end-point for phase II trials in soft-tissue sarcomas. Eur J Cancer 38:543-549, 2002
14. Fleiss J: Statistical Methods for Rates and Proportions. New York, NY, Wiley, 1981, pp 13-15
15. Penel N, Van Glabbeke M, Marreaud S, et al: Testing new regimens in patients with advanced soft tissue sarcoma: Analysis of publications from the last 10 years. Ann Oncol 22:1266-1272, 2011
16. Luke JJ, D'Adamo DR, Dickson MA, et al: The cyclin-dependent kinase inhibitor flavopiridol potentiates doxorubicin efficacy in advanced sarcomas: Preclinical investigations and results of a phase I dose-escalation clinical trial. Clin Cancer Res 18:2638-2647, 2012
17. Louis-Brennetot C, Coindre JM, Ferreira C, et al: The CDKN2A/CDKN2B/CDK4/CCND1 pathway is pivotal in well-differentiated and dedifferentiated liposarcoma oncogenesis: An analysis of 104 tumors. Genes Chromosomes Cancer 50:896-907, 2011

Acknowledgment

We thank Margaret Leversha, PhD, from the Molecular Cytogenetic Core at Memorial Sloan-Kettering Cancer Center, for technical assistance with fluorescent in situ hybridization.