Preclinical Characterization of Novel Chordoma Cell Systems and Their Targeting by Pharmacological Inhibitors of the CDK4/6 Cell-Cycle Pathway

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Abstract

Chordomas are tumors that arise at vertebral bodies and the base of the skull. Although rare in incidence, they are deadly owing to slow growth and a lack of effective therapeutic options. In this study, we addressed the need for chordoma cell systems that can be used to identify therapeutic targets and empower testing of candidate pharmacologic drugs. Eight human chordoma cell lines that we established exhibited cytology, genomics, mRNA, and protein profiles that were characteristic of primary chordomas. Candidate responder profiles were identified through an immunohistochemical analysis of a chordoma tissue bank of 43 patients. Genomic, mRNA, and protein expression analyses confirmed that the new cell systems were highly representative of chordoma tissues. Notably, all cells exhibited a loss of CDKN2A and p16, resulting in universal activation of the CDK4/6 and Rb pathways. Therefore, we investigated the CDK4/6 pathway and responses to the CDK4/6-specific inhibitor palbociclib. In the newly validated system, palbociclib treatment efficiently inhibited tumor cell growth in vitro and a drug responder versus nonresponder molecular signature was defined on the basis of immunohistochemical expression of CDKN46/pRb (S780). Overall, our work offers a valuable new tool for chordoma studies including the development of novel biomarkers and molecular targeting strategies. Cancer Res; 75(18); 1–9. ©2015 AACR.

Introduction

Chordomas are rare tumors considered to arise from persistent notochordal remnants along in the vertebral bodies (1). Because of their slow growth, there is no efficient standard chemotherapy: therapy of choice is surgery followed by radiotherapy. After surgical therapy of this orphan disease chordoma recurs in up to 50% of patients and metastasizes in up to 40% (1). Only a minority is cured by surgery, the disease-free survival is generally short (2–4). The median survival is 6 to 7 years after diagnosis, although the range varies from months to 25 years. High-dose radiotherapy is administered for residual or recurrent disease (5–10). Patients who have metastases and have inoperable recurrent disease are not amenable for further radiotherapy. Therefore, there is a strong unmet need for systemic pharmacologic therapy. Aggressive chemotherapy has been described to be effective in rare cases of dedifferentiated chordomas (11). Only limited response is seen by therapy with alkylating agents (ifosfamid), anthracyclines (doxorubicin), and cisplatin (12, 13). In the first prospective phase II study with 9-nitrocamptothecin, one patient out of 15 achieved a partial remission (14). Recently, new targeted therapy options using erlotinib, cetuximab, gefitinib, sunitinib, thalidomide, and lapatinib have been published (15–18). An activated platelet-derived growth factor receptor β (PDGFRB) was described as a basis for therapy with imatinib in patients with progressive disease (19, 20).

Because of the rarity of this tumor and to only a few well characterized chordoma cell lines there is limited experience with preclinical models (21–23). Lately, systematic pharmacologic screening of our first published two chordoma cell lines U-CH1 and U-CH2 has opened new insights for new potential targets (24).

Meanwhile, we have established and characterized 6 additional chordoma cell lines. We found that the tumor suppressor p16 (CDKN2A) is deleted and that p16 protein is not expressed in these cell lines. This deletion has been described in the majority of chordomas (25–27). The downstream machinery of p16 can be efficiently inhibited by CDK4/6 inhibitors such as palbociclib.
(28). Inhibition of this pathway by palbociclib is a defined mechanism to reduce the proliferation and growth of neoplastic cells (29, 30).

Because of the loss of p16 protein in chordoma cell lines, we investigated the downstream molecules of this pathway in the cell lines and in tissue comprising chordoma from 43 patients. We analyzed the effect of palbociclib on the growth of these cell lines and defined potential responder types.

Materials and Methods

Cell culture and establishment of cell lines

The establishment of human chordoma cell lines U-CH1 and U-CH2 was published by us in 2001 and 2010, respectively (31, 32). The new human chordoma cell lines (Nos.: U-CH3, U-CH6, U-CH7, U-CH10, U-CH11, U-CH12) were all established from sacrococcygeal chordomas using the same protocols (31, 32). The cell lines U-CH3, U-CH6, and U-CH7 were derived from patients’ tumors from the Royal National Orthopedic Hospital (Stanmore, Middlesex, United Kingdom), and released from the Stanmore Musculoskeletal Biobank (Cambridgeshire 2 Research Ethics Committee; reference 09/H0304/78). The cell lines U-CH1, U-CH2, U-CH10, U-CH11, and U-CH12 were derived from patients’ tumors from the Department of Trauma Surgery and the Department of General and Visceral Surgery, University of Ulm (Ulm, Germany). Approval for this procedure was obtained from the local ethics committee.

As controls for the Western blot analysis, we used the cell lines SK-LMS-1, CaCo2, and HEK-293; to control the effects of palbociclib on cell proliferation in the MTS cell viability assay, MCF7 and CAL51 cells served as references (Leibniz-Institut DSMZ; Bochum, Germany; ref. 33).

Comparative genomic hybridization

For comparative genomic hybridization (CGH), the protocol as described by Lichter and colleagues (1995) was used. Image acquisition was performed with the image analysis system ISIS (Meta-Systems).

FISH analysis

FISH was performed according standard protocols using a commercial available Zytolight SPEC CDKN2A/CEN9 DUAL COLOR probe (Vysis/Abbott).

mRNA expression analysis


Western blot analysis

The following antibodies were used: brachyury (Santa Cruz Biotechnology Inc., 1:1,000, Sc-20109), CDK4 (Zytomed Systems, 1:1,000, 603-1840), CDK6 (Abcam, 1:1,000, Ab 54576), Rb (Cell Signaling Technology, 1:2,000, 9309), pRb (Ser780, Cell Signaling Technology, 1:1,000, 9307), p16 (Santa Cruz Biotechnology, 1:100, Sc-56330), cleaved caspase-3, and cleaved PARP (Cell Signaling Technology, 1:2,000, 9664 and 5625), β-actin (Sigma Aldrich, 1:1,000–1:10,000).

Palbociclib

Palbociclib (PD-0332991) is a selective cyclin-dependent kinase (CDK) inhibitor for CDK4 and CDK6, and inhibits the phosphorylation of the retinoblastoma protein (Rb). The reagent was purchased as PD 0332991 isethionate from Sigma-Aldrich Inc.. Palbociclib was dissolved in water and diluted in 10, 100, and 1,000 nmol/L concentrations as published. For establishment of the IC_{50} values, palbociclib was used in concentrations of in 3, 6, 10, 30, 60, 100, 300, 600, and 1,000 nmol/L.

Abemaciclib

Abemaciclib (Ly2835219) is an alternative selective cyclin-dependent kinase (CDK) inhibitor of CDK4 and CDK6 and was purchased from Selleckchem. Abemaciclib was dissolved in water and was used in concentrations of 3, 6, 10, 30, 60, 100, 300, 600, 1,000, 10,000 nmol/L.

Flow cytometry (FACS)

Cell lines U-CH1 and U-CH2 were subcultured and treated with palbociclib (1,000 nmol/L) for 3 days. For cell-cycle analysis, a protocol with propidium iodide (BD FACS Calibur) and Cell Quest as detection software (BD Biosciences) were used. All FACS analysis experiments were performed as triplicates.

Cell counting

An inverse microscope coupled to a digital camera adapter was used. The pictures were uploaded in the UTHSCSA Image Tool version 3.0 Final (http://compdnt.uthscsa.edu/dig/itdesc.html). The control and the treated cell lines were counted in 6 wells for calculation of the median with the SD.

Proliferation assay

The Alamar Blue Assay was used as outlined by the manufacturer using a 96-well plate reader (Spectramax 190).

Single tandem repeat analysis

For a genotyping of the cell lines U-CH1, U-CH2, U-CH3, U-CH6, U-CH7, and U-CH12, we performed a single tandem repeat (STR) analysis using the PowerPlex 16 and Power Plex ESX 17 kit (Promega). For the cell lines U-CH10 and U-CH11 the GenomeLab Human STR Primer Set Kit (Beckman Coulter) and the AmpliTaq GOLD DNA Polymerase (Life Technologies) was used, respectively. The analysis was performed according to the manufacturers’ handbook.

MTS cell proliferation assay

CellTiter 96Aqueous One Solution Cell Proliferation Assay from Promega was used to determine the cell viability. Therefore, the chordoma cell lines as well as MCF7 and CAL51 cell line were cultivated in 96-well plates and incubated with increasing concentrations of palbociclib (0–1,000 nmol/L) in the medium. MTS was added after 3 days of incubation at 37°C to the medium and the absorption was measured at 490 nm using a microtiter plate reader as described earlier (34).
Chordoma tissue bank

Chordoma samples from 43 patients were available from the Ulm tissue bank (median age: 69 years; range: 17–84 years; 26 male, 17 female). Tumor size varied between 0.5 and 50 cm. Ten patients had metastatic disease and 21 had recurrences. The follow-up data are available from 40 patients: 27 are alive (survey range from 0.5 to 234 months). The diagnosis was based on histologic subtyping (WHO 2013) and revealed in most instances a NOS (not otherwise specified) subtype, including chordomas with focal chondroid, renal cell cancer like, and hepatoid differentiation (Supplementary Table S3).

Chordoma patient samples were pseudonymized to comply with the German law for correct usage of archival tissue for clinical research (35). Approval for this procedure was obtained from the local ethics committee.

Immunomorphology

Immunohistochemistry were performed on formalin-fixed, paraffin-embedded tissue sections as described (36, 37). The proportion of chordoma cells showing a positive immunoreactivity were categorized as follows: "no immunoreactivity detected" (−); "immunoreactivity in up to 30%" (+); immunoreactivity in more than 30% and up to 70%" (+++) and "immunoreactivity in more than 70%" (++++) of the total number of chordoma cells analyzed (Fig. 4A and B).

The following antibodies were used: polyclonal antibody against S-100 protein (Dako; 1:1,000, Z0334), monoclonal antibodies against cytokeratin (AE1/AE3, Dako, 1:100, M3515), epithelial membrane antigen (EMA; E29, Dako, 1:500, M0613), vimentin (3B4, Dako, 1:300, M7020), brachyury (Santa Cruz Biotechnology, 1:100, Sc-20109), CD9 (Zytomed Systems, 1:100, 603-1840), CD6 (Dako, 1:100, M0720), Rb (Cell Signaling Technology, 1:100, 9309), pRb (Ser780; Cell Signaling Technology, 1:100, 9307), p16 (Santa Cruz Biotechnology, 1:100, Sc-6530), Cyclin D1 (SP4, DCS, C677C01), and Ki-67 (clone MIB-1; Dianova, 1:200, M7240).

Statistical analysis

All statistical tests were two-sided ANOVA and P values were regarded as significant if they were lower than 0.05 (GraphPad Prism software, Version 6).

Results

Characterization of chordoma cell lines

We established 8 stable cell lines using standard protocols (Table 1, for typical cytology see Supplementary Fig. S2; refs. 31, 32). All cell lines express the chordoma marker brachyury (Supplementary Fig. S3). STR analyses were performed of the parental chordomas and the corresponding cell lines in June 2015; these analyses confirmed the origin of the cell lines (Supplementary Table S5).

Additional information about these cell lines U-CH1, U-CH2, U-CH10, and U-CH11 can be requested via the Chordoma Foundation homepage (http://www.chordomafoundation.org/).

CGH data showed a highly overlapping pattern of recurrent aberrations and are thus regarded as representative on the genomic level with recurrent gains on chromosome 7q (75%) and losses on chromosome 3 (75%), 9p and 10p (60% each; Supplementary Fig. S1; Supplementary Table S1). By FISH analysis, we found that all cell lines showed losses of CDKN2A ranging from 50% to 86%, including a biallelic complete deletion of CDKN2A for U-CH10. Additional FISH analyses revealed that this loss of CDKN2A was already present in the parental tumor of the chordoma cell lines in a high percentage of cells analyzed (Supplementary Table S2).

All cell lines showed high mRNA expression levels of typical chordomas markers such as brachyury, collagen 2A1, and CD24 (Supplementary Fig. S4A–S4C). The expression data sets were clustered in an unsupervised approach [hierarchical clustering (nonaveraged) under Euclidean conditions in a centroid linkage rule] with the data of the NCI-60 panel of cancer cell lines. This is a public available expression data set from the GEO database of NCBI. These cell lines have been analyzed using a similar platform as for the chordoma cell lines. The chordoma cell lines (GEO accession number: GSE68497) clustered together against the background of all cell lines.

We further included our own expression data of a nonchordoma cartilaginous cell line (brachyury-negative), which features cartilage differentiation and from tissue of a vertebral disk. These data were analyzed with our chordoma cell lines data as well as expression data from chondrocytes, liposarcomas, and pleomorphic sarcoma cell lines. In this analysis, the chordoma cell lines cluster together in one group and the cartilaginous cell line and the sample from the vertebral tissue cluster together with chondrocytes and the sarcoma cell lines; furthermore, the cluster of the chordoma cell lines intermingle with the data of the GEO expression data and therefore support the cluster analyses (Supplementary Fig. S5A and S5B).

A list of differentially expressed genes in correlation to the NCI-60 panel is available as Supplementary Table S4 (P < 10<−4 and fold change more than 10).

Table 1. Summarized data of the patients from which tissue the cell lines were established.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Localization</th>
<th>Primary/recurrences</th>
<th>Gender</th>
<th>Age at diagnosis in years</th>
<th>Size in cm</th>
<th>Histology primary tumor</th>
<th>Tissue acquisition</th>
<th>Kinetics doubling time in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-CH 1</td>
<td>Sacrum</td>
<td>R</td>
<td>M</td>
<td>45</td>
<td>20</td>
<td>NOS/renal</td>
<td>8/1998</td>
<td>2 d</td>
</tr>
<tr>
<td>U-CH 2</td>
<td>Sacrum</td>
<td>P</td>
<td>F</td>
<td>70</td>
<td>13.6</td>
<td>NOS/renal</td>
<td>9/2000</td>
<td>4 d</td>
</tr>
<tr>
<td>U-CH 3</td>
<td>Sacrum</td>
<td>P</td>
<td>F</td>
<td>65</td>
<td>11</td>
<td>NOS</td>
<td>6/2010</td>
<td>8 d</td>
</tr>
<tr>
<td>U-CH 6</td>
<td>Sacrum</td>
<td>Metastasis (skin)</td>
<td>M</td>
<td>Not known</td>
<td>9</td>
<td>NOS</td>
<td>5/2011</td>
<td>7 d</td>
</tr>
<tr>
<td>U-CH 10</td>
<td>Sacrum</td>
<td>R</td>
<td>F</td>
<td>74</td>
<td>13</td>
<td>NOS/renal</td>
<td>7/2000</td>
<td>14 d</td>
</tr>
<tr>
<td>U-CH 11</td>
<td>Sacrum</td>
<td>P</td>
<td>M</td>
<td>71</td>
<td>8</td>
<td>NOS</td>
<td>9/2012</td>
<td>10 d</td>
</tr>
<tr>
<td>U-CH 12</td>
<td>Sacrum</td>
<td>P</td>
<td>M</td>
<td>83</td>
<td>50</td>
<td>NOS</td>
<td>1/2013</td>
<td>7 d</td>
</tr>
</tbody>
</table>

NOTE: U-CH3, U-CH6, U-CH7 were established from tissue samples from A. Flanagan, UCL, London, United Kingdom. Abbreviation: NOS, Not otherwise specified.
Analysis of the CDK4/6/phospho-Rb pathway in chordoma cell lines

By FISH analyses, all chordoma cell lines and the parental tumors of the cell lines we found a loss of CDKN2A in a high percentage of cells (Supplementary Table S2). We conclude that this finding in the cell lines is not a secondary finding due to cell culture. We therefore focused our analysis on further components of the CDK4/6 pathway. In mRNA expression analysis, the cell-cycle molecules CDK4/6 and Rb are expressed on the mRNA level (data not shown) while we found a low mRNA expression of CDKN2A. All analyzed cell lines are p16 protein negative, while CDK4 and for CDK6 are protein positive (Fig. 1A).

Effect of palbociclib treatment on chordoma cell lines

We assumed that the CDK4/6 pathway is active in the cell lines and performed cell growth inhibition experiments with palbociclib. The pRb (S780) served as a marker molecule for the cell growth inhibition experiments.

After incubation of all cell lines with 100 nmol/L and 1,000 nmol/L palbociclib for 3 days, they showed a strong decrease of pRb (S780) expression with increase of palbociclib concentration (Fig. 1B). These data are supported by the immunocytological reactivity of U-CH1 with a reduction of the Ki-67 rating and reduction of pRb (S780) expression with increase of palbociclib concentration (Supplementary Fig. S6). Prompted by these results, we investigated the inhibitory effect of palbociclib on the growth of the chordoma cell lines in more detail by analyzing the amount of viable cells after palbociclib treatment with the MTS assay. Cells were incubated for 3 days with increasing concentrations (0, 3, 6, 10, 30, 60, 100, 300, 600, 1,000 nmol/L) of palbociclib and the amount of viable cells was measured. For the analyzed cell lines U-CH2, U-CH3, U-CH6 and U-CH7, the IC_{50} value for palbociclib was between 50 and 100 nmol/L (33), U-CH11 responded at higher concentrations (IC_{50} between 300 and 400 nmol/L). In this test, MCF7 cells and CAL51 cells served as established positive and negative control, respectively (38) (Fig. 3).

The inhibitory effect of palbociclib on the chordoma lines was confirmed by an alternative approach. The effect of a 6-day treatment with 10, 100, and 1,000 nmol/L palbociclib on the growth of U-CH1 and U-CH7, U-CH10, and U-CH11 was analyzed by cell counting (Fig. 2A). Moreover, the Alamar Blue assay confirmed the concentration-dependent growth inhibition after palbociclib treatment of U-CH2, U-CH3, U-CH6, and U-CH10 (Supplementary Fig. S7). All these lines responded to palbociclib with an up to 18.5% decreased cell growth. Taken together, our findings demonstrate using different methods that palbociclib inhibits the growth of the 8 chordoma lines, however, the cell lines showed different sensitivity toward palbociclib regarding growth inhibition.

The cell lines U-CH1 and U-CH2 were then used to analyze the cell cycle after treatment with 1,000 nmol/L of palbociclib for 3 days. Figure 2B and C show the increase in the G_1 phase and decrease in S and G_2 phase. To rule out increased apoptosis rates, we analyzed expression of cleaved PARP and cleaved caspase-3. We could not detect any cleaved PARP or cleaved caspase-3 (Supplementary Fig. S8A and S8B), suggesting that in these chordoma lines palbociclib inhibits cell proliferation rather than inducing apoptosis.

In addition to palbociclib, we investigated the effects of an alternative CDK4/6 inhibitor on the growth of the chordoma cells and treated U-CH1 and U-CH6 cells with the established compound LY2835219 (abemaciclib; ref. 39). We used these two chordoma cell lines as these lines represent paradigmatically the chordoma cell lines regarding growth and morphology. The results indicated that treatment of these cells with LY2835219 also resulted in reduced cell viability (Supplementary Fig. S9). Taken together, the results indicate that two different pharmacologic inhibitors, which both interfere with CDK4/6 in cells in vitro, inhibited the growth of the chordoma cell lines, implicating that this inhibition is due to the block of the CDK4/6 pathway.

Defining a potential responder versus potential nonresponder status

The protein expression profiles of the cell lines and the samples obtained from the tissue bank with respect to expression of CDK4, CDK6, p16, Rb, pRb, Cyclin D1, and Ki-67 index were grouped to define potential responders. Basis for this scoring was the response to palbociclib of the chordoma cell lines (IC_{50} values and growth inhibition) as compared with the quantitative Western blot ratio of pRb (S780)/β-actin and a corresponding expression of pRb in the chordoma samples. The potential responder type was stratified into groups (38, 40, 41). Every tumor tissue sample was assessed for the expression of p16, CDK4, CDK6, Rb, pRb, Cyclin D1, and Ki-67 index: 66% are negative for p16, 70% are positive for CDK4, 85% are positive for CDK6, and over 90% are positive for Rb and pRb (S780). Ki-67 index varied between 1% and 50%
null
division cycle progression (26), a loss of p16 will lead to a tonic activation of this pathway and thus is correlated negatively with prognosis, for example, in melanoma (44, 45). In line with this observation, the transcripts of CDK4 and CDK6 are upregulated on the mRNA level in the chordoma cell lines, and CDK4 and CDK6 proteins are consistently expressed in all our chordoma cell lines.

The next downstream molecule in the p16 pathway is the Rb protein, which is phosphorylated by CDK4/6. In Western blot analysis, all 8 chordoma cell lines expressed the Rb protein. These cyclin-dependent kinases phosphorylate the Rb protein at Serine 780 (33). The cell lines were all positive for pRb serine 780. Taken together, the CDK4/6 pathway is active in all chordoma cell lines and loss of Rb is correlated with ineffectiveness to inhibition (40, 46). In this setting, we investigated whether inhibition of the p16 pathway would result in decreased pRb protein in the chordoma cell lines. We found that this protein was reduced in a dose-dependent manner after palbociclib treatment in the chordoma cell lines. In the inhibition experiments performed in the current study, the expression levels of pRb directly correlated with cell proliferation. In these experiments the IC50 values for four chordoma cell lines were below 100 nmol/L of palbociclib and thus correspond to IC50 values reported for various other cancer cell lines (33). We further could show that an alternative CDK4/CDK6 inhibitor (Ly2835219) has a similar inhibitory effect on the chordoma cell lines. Therefore, two biochemically distinct inhibitors acting on CDK4/6 inhibited the growth of the chordoma cell lines, strongly suggesting that this inhibition is due to blocking the CDK4/6 pathway in these cells (28).

Figure 3. Effect of palbociclib on the growth of chordoma lines U-CH2, 3, 6, 7, and 11 as well as Cal51. Cells were grown in 96-well plates that were incubated for 3 days at 37°C with increasing concentrations of palbociclib (3, 6, 10, 30, 60, 100, 300, 600, 1,000 nmol/L) in the medium or left untreated for control. The amount of viable cells was determined by using the MTS cell viability test (GraphPad Prism software, Version 6).
We argued that growth inhibition of the chordoma cell would have an effect on the cell cycle as the cascade might directly lead to a block at the G1 phase checkpoint. We show that this is the case with a consequent increase of cells in G1 phase and a decrease of cells in S and G2 phase. The sub-G1 phase was only slightly increased. These effects of palbociclib on the chordoma cells are not due to increased apoptotic rates as shown by the absence of an increase of cleaved caspase-3 or cleaved PARP. Therefore, we

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conclude that the consequence of palbociclib on the chordoma cell lines is predominantly a growth inhibitory effect.

To dissect a potential responder phenotype from our tissue sample, we analyzed our tissue sample for the expression of p16, CDK4, CDK6, Rb, pRb, and cyclin D1 as compared with the responding cell lines. By immunohistochemical typing, we stratified our patient’s samples in various groups, leading to the definition of potential nonresponders and potential responders to a treatment with palbociclib. The potential nonresponder group was defined as p16-positive, and absence of CDK4 or CDK6 or Rb/pRb. Vice versa, we established a potential responder phenotype characterized by the absence of p16 protein but detectable CDK4/CDK6 and pRb (38, 40, 41).

More than 75% of chordoma samples were completely negative for p16 pointing to a defect in this pathway in more than two thirds of chordomas analyzed. Furthermore, immunoreactivity for CDK4 and CDK6 in our chordoma tissue banked samples showed that 56% of patients revealed coexpression of CDK4 and CDK6, while 35% either expressed CDK4 or CDK6, and only 9% were completely negative for these molecules. In addition, more than 90% of tissue samples were Rb- and pRb-positive.

Taken together, the analysis of more than 40 chordoma samples led us to a ascribe chordoma patients to potential responder, who might benefit from a treatment with palbociclib. We show that a higher Ki-67 index significantly correlated with higher pRb expression. Therefore, these molecules are potential biomarkers for tissue testing to identify a potential responder versus a potential nonresponder status in chordoma tissue.

There is a number of clinical trials and studies with palbociclib for various kinds of cancer such as squamous cell carcinoma of head and neck, liver, ovarian, prostate, and breast cancer in phase II/III, melanoma in phase I–II, gastrointestinal stromal tumors, mantle cell lymphoma, and a phase II study for dedifferentiated liposarcoma, which all show favorable effects on the progression-free survival and only moderate side effects (29, 41, 47–49). Our data strongly argue for an extension of palbociclib therapy to chordoma patients with a potential responder phenotype as has been defined by our analysis. Therefore, the initiation of a prospective study including chordoma patients expressing the defined biomarkers of the CDK4/6 pathway by immunohistologic profiling to define a potential responder phenotype, as has been done for substances like tyrosine kinase inhibitors, has to be considered as a promising option for future therapy of chordomas in prospective trials.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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