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

Adrian von Witzelben¹, Lukas T. Goerttler¹, Ralf Marienfeld¹, Holger Barth², André Lechel³, Kevin Mellert¹, Michael Böhm¹, Marko Kornmann⁴, Regine Mayer-Steinacker⁵, Alexandra von Baer⁶, Markus Schultheiss⁶, Adrienne M. Flanagan⁷, Peter Möller¹, Silke Brüderlein¹, and Thomas F.E. Barth¹

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 CDK4/6/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); 3823-31. ©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 (PDGFRβ) was described as a basis for therapy with imatinib in patients with progressive disease (19, 20).

Because of the rareness 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.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

A. von Witzelben and L.T. Goerttler contributed equally to this article.

Corresponding Author: Peter Möller, Institute of Pathology, M23, University of Ulm, Albert-Einstein-Allee 11, Ulm D-89081, Germany. Phone: 49-731-5630; Fax: 49-731-563562; E-mail: peter.moeller@uniklinik-ulm.de

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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 (Stamnege, 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 cell line were used. All FACS analysis experiments were performed as triplicates.

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).

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 FACSCalibur) 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://compdent.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 PowerPlex ESX 17 kit (Promega). For the cell lines U-CH10 and U-CH11 the Genome-Lab 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.

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 IC50 values, palbociclib was used in concentrations of 3, 6, 10, 30, 60, 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 used in concentrations of 3, 6, 10, 30, 60, 100, 300, 600, 1,000, 10,000 nmol/L.

FISH analysis

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

mRNA expression analysis

Analysis was performed according to the standard protocol (Agilent Technologies) using the Whole Human Genome Microarray (Design ID: 014850).


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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 bad 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, Z0331), 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), CDK4 (Zytomed Systems, 1:100, 603-1840), CDK6 (Abcam, 1:500, Ab 54576), Rb (Cell Signaling Technology, 1:100, 9309), pRb (Ser780; Cell Signaling Technology, 1:100, 9309), Rb (Cell Signaling Technology, 1:100, 9307), p16 (Santa Cruz Biotechnology, 1:100, Sc-56330), Cyclin D1 (SP4, DCS, Cl677C01), 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</th>
<th>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>P&lt;0.05</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>F&lt;0.05</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>P&lt;0.05</td>
<td>8 d</td>
</tr>
<tr>
<td>U-CH 4</td>
<td>Sacrum</td>
<td>P</td>
<td>F</td>
<td>74</td>
<td>8</td>
<td>NOS</td>
<td>9/2012</td>
<td>P&lt;0.05</td>
<td>10 d</td>
</tr>
<tr>
<td>U-CH 5</td>
<td>Sacrum</td>
<td>M</td>
<td>M</td>
<td>50</td>
<td>1/2013</td>
<td>NOS</td>
<td></td>
<td></td>
<td></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.
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; Supplementary Fig. S6). 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 G1 phase and decrease in S and G2 phase. To rule out increased apoptosis rates, we analyzed expression of cleaved PARP and cleaved caspase-3.

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 (IC50 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%
negative for p16 protein, most probable due to a loss of CDKN2A and controlling the activity of CDK4 and CDK6 with respect to the cell cycle. In all chordoma cell lines, we found recurrent losses of CDKN2A in a high percentage of chordoma cells. In contrast, the downstream molecules CDK4/CDK6 and pRb are expressed in the cell lines. Inhibition of the chordoma cells leads to a consistent reduction of growth when inhibited by the specific CDK4/CDK6 inhibitor palbociclib or an alternative CDK4/CDK6 inhibitor LY2835219 (abemaciclib). We show that in a cohort of chordoma tissue samples more than 80% of the patients have a potential responder phenotype characterized by protein expression of CDK4/6 and pRb.

Chordoma, being an orphan disease, is not treated uniformly when it comes to chemotherapy. However, due to screening on large scale by Xia and colleagues (24), there is increasing evidence that chemotherapy targeting molecules like tyrosine kinases (e.g., EGFR; refs. 15, 16, 42) may be an additional therapeutic option. The first xenograft experiments using the chordoma cell line U-CH1 showed that the cells preserve the typical cytology and immunology, thus enabling further analysis of relevant pathways in situ (22).

The mRNA expression profiling of our 8 cell lines revealed high mRNA levels of typical chordoma markers, including brachyury, collagen 2A1, and CD24 (32, 43). When compared with the NCI-60 cancer cell line panel and other public available mRNA expression data the chordoma cell lines clustered together confirming the characteristic profile of these tumors.

CDKN2A loss is a common genomic finding in chordoma (26, 27). We found recurrent losses of CDKN2A in all chordoma cell lines analyzed in a high percentage of cells. What is more, this finding was also present in the analyzed parental tumor, therefore excluding a secondary in vitro effect. The p16 protein was completely absent in all our chordoma cell lines.

As the tumor suppressor p16 (CDKN2A) plays a central role in controlling the activity of CDK4 and CDK6 with respect to the cell cycle (Fig. 4B and C). On the basis of these results, we defined four types of chordomas by scoring the expression profiles from 0 to 3 (38, 40, 41). The potential nonresponder type (type 0) was defined by p16 expression in over 10% of the tumor cells. As Rb and pRb are essential components of this pathway, lack of the molecules corresponds to a potential nonresponder. The absence of both CDK4 and CDK6 protein is not compatible with the function of this pathway and therefore also classified as a potential nonresponder status. The best assumed responder profile is characterized by expression of pRb (S780) in more than 70% of cells (++++).

The other categories describe immunoreactivity for pRb(S780) in increasing percentages of positive cells: 0 = p16 > 10% positive; Rb or pRb negative; CDK4 and CDK6 negative; 1 = pRb is +; 2 = pRb is ++; 3 = pRb is +++.

Applying the above categories to our cohort, we identified 5 of 42 chordoma samples as potential nonresponders (type 0). The potential responder profiles of 41 samples were stratified as follows, 10 (type 1), 13 (type 2), and 14 (type 3; Fig. 4A and B). Furthermore, on correlating the potential responder type regarding the pRb expression with the Ki-67 index, we found a significant positive correlation ($P = 0.0216$). i.e., higher proliferating chordomas have a higher pRb expression. For the chordoma cell lines, we found a trend correlating higher expression of pRb with a higher Ki-67 index ($P = 0.08$).

**Discussion**

In the current study we characterized 6 newly generated chordoma cell lines in direct comparison with two recently described chordoma lines. All these chordoma cell lines are negative for p16 protein, most probable due to a loss of CDKN2A in a high percentage of chordoma cells. In contrast, the downstream molecules CDK4/CDK6 and pRb are expressed in the cell lines. Inhibition of the chordoma cells leads to a consistent reduction of growth when inhibited by the specific CDK4/CDK6 inhibitor palbociclib or an alternative CDK4/CDK6 inhibitor LY2835219 (abemaciclib). We show that in a cohort of chordoma tissue samples more than 80% of the patients have a potential responder phenotype characterized by protein expression of CDK4/6 and pRb.

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As the tumor suppressor p16 (CDKN2A) plays a central role in controlling the activity of CDK4 and CDK6 with respect to the cell
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 at 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 IC_{50} values for four chordoma cell lines were below 100 nmol/L of palbociclib and thus correspond to IC_{50} 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).
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 patients' 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 of this pathway in more than two thirds of chordomas analyzed. Furthermore, immunonegativity 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 outcomes [29, 41, 47–49]. Our data strongly argue for an extensive trial 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.

Authors’ Contributions

Conception and design: A. von Witzleben, R. Marienfeld, H. Barth, M. Schultheiss, P. Möller, T.F.E. Barth


Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. von Witzleben, L.T. Goettler, H. Barth, A. Lechel, M. Kornmann, R. Mayer-Steinacker, A. von Baer, M. Schultheiss, A.M. Flanagan, P. Möller, T.F.E. Barth

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. von Witzleben, L.T. Goettler, H. Barth, K. Mellert, M. Kornmann, P. Möller, T.F.E. Barth

Writing, review, and/or revision of the manuscript: A. von Witzleben, L.T. Goettler, R. Marienfeld, H. Barth, K. Mellert, M. Kornmann, R. Mayer-Steinacker, A. von Baer, M. Schultheiss, A.M. Flanagan, P. Möller, T.F.E. Barth

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. von Witzleben, P. Möller, T.F.E. Barth

Study supervision: P. Möller, S. Brüderlein, T.F.E. Barth

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Adrian von Witzleben, Lukas T. Goerttler, Ralf Marienfeld, et al.


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