Journal of Bone Oncology 19 (2019) 100268

Contents lists available at ScienceDirect

Journal of Bone Oncology

journal homepage: www.elsevier.com/locate/jbo

Research Paper

A screening-based approach identifies cell cycle regulators AURKA, CHK1 and PLK1 as targetable regulators of chondrosarcoma cell survival

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ARTICLE INFO

Keywords:
Chondrosarcoma
AURKA
PLK1
CHK1
Screen

ABSTRACT

Chondrosarcomas are malignant cartilage tumors that are relatively resistant towards conventional therapeutic approaches. Kinase inhibitors have been investigated and shown successful for several different cancer types. In this study we aimed at identifying kinase inhibitors that inhibit the survival of chondrosarcoma cells and thereby serve as new potential therapeutic strategies to treat chondrosarcoma patients.

An siRNA screen targeting 779 different kinases was conducted in JJ012 chondrosarcoma cells in parallel with a compound screen consisting of 273 kinase inhibitors in JJ012, SW1353 and CH2879 chondrosarcoma cell lines. AURKA, CHK1 and PLK1 were identified as most promising targets and validated further in a more comprehensive panel of chondrosarcoma cell lines. Dose response curves were performed using tyrosine kinase inhibitors: MK-5108 (AURKA), LY2603618 (CHK1) and Volasertib (PLK1) using viability assays and cell cycle analysis. Apoptosis was measured at 24 h after treatment using a caspase 3/7 assay. Finally, chondrosarcoma patient samples ($N = 34$) were used to examine the correlation between *AURKA, CHK1* and *PLK1* RNA expression and documented patient survival.

Dose dependent decreases in viability were observed in chondrosarcoma cell lines after treatment with MK-5108, LY2603618 and volasertib, with cell lines showing highest sensitivity to PLK1 inhibition. In addition increased sensitivity to conventional chemotherapy was observed after CHK1 inhibition in a subset of the cell lines. Interestingly, whereas *AURKA* and *CHK1* were both expressed in chondrosarcoma patient samples, *PLK1* expression was found to be low compared to normal cartilage. Analysis of patient samples revealed that high *CHK1* RNA expression correlated with a worse overall survival.

AURKA, CHK1 and PLK1 are identified as important survival genes in chondrosarcoma cell lines. Although further research is needed to validate these findings, inhibiting CHK1 seems to be the most promising potential therapeutic target for patients with chondrosarcoma.

1. Introduction

Chondrosarcomas account for 20% of primary bone tumors and are characterized by malignant cartilage producing cells [1]. Depending on the morphology and the location, chondrosarcoma can be subdivided into conventional chondrosarcoma and more rare subtypes; dedifferentiated chondrosarcoma, mesenchymal chondrosarcoma, clear cell chondrosarcoma and periosteal chondrosarcoma. Conventional chondrosarcoma accounts for 85% of all chondrosarcoma cases [1] and is further classified into central chondrosarcoma, located in the medulla of the bone, and peripheral chondrosarcoma, found at the surface of the bone [2]. These two different conventional chondrosarcoma subtypes show the same histological features: however a distinct molecular background is observed [1]. Conventional chondrosarcoma is classified into three different grades, which is the most important prognostic factor. Atypical cartilaginous tumors (ACT)/chondrosarcoma grade I show a low cellularity, a large amount of cartilage matrix, rarely metastasize, and have a relatively good prognosis. Grade II and grade III chondrosarcomas behave more aggressive and show a more cellular histology with reduced cartilage matrix and a poor prognosis. Patients

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https://doi.org/10.1016/j.jbo.2019.100268
Received 27 June 2019; Received in revised form 9 November 2019; Accepted 13 November 2019
Available online 17 November 2019
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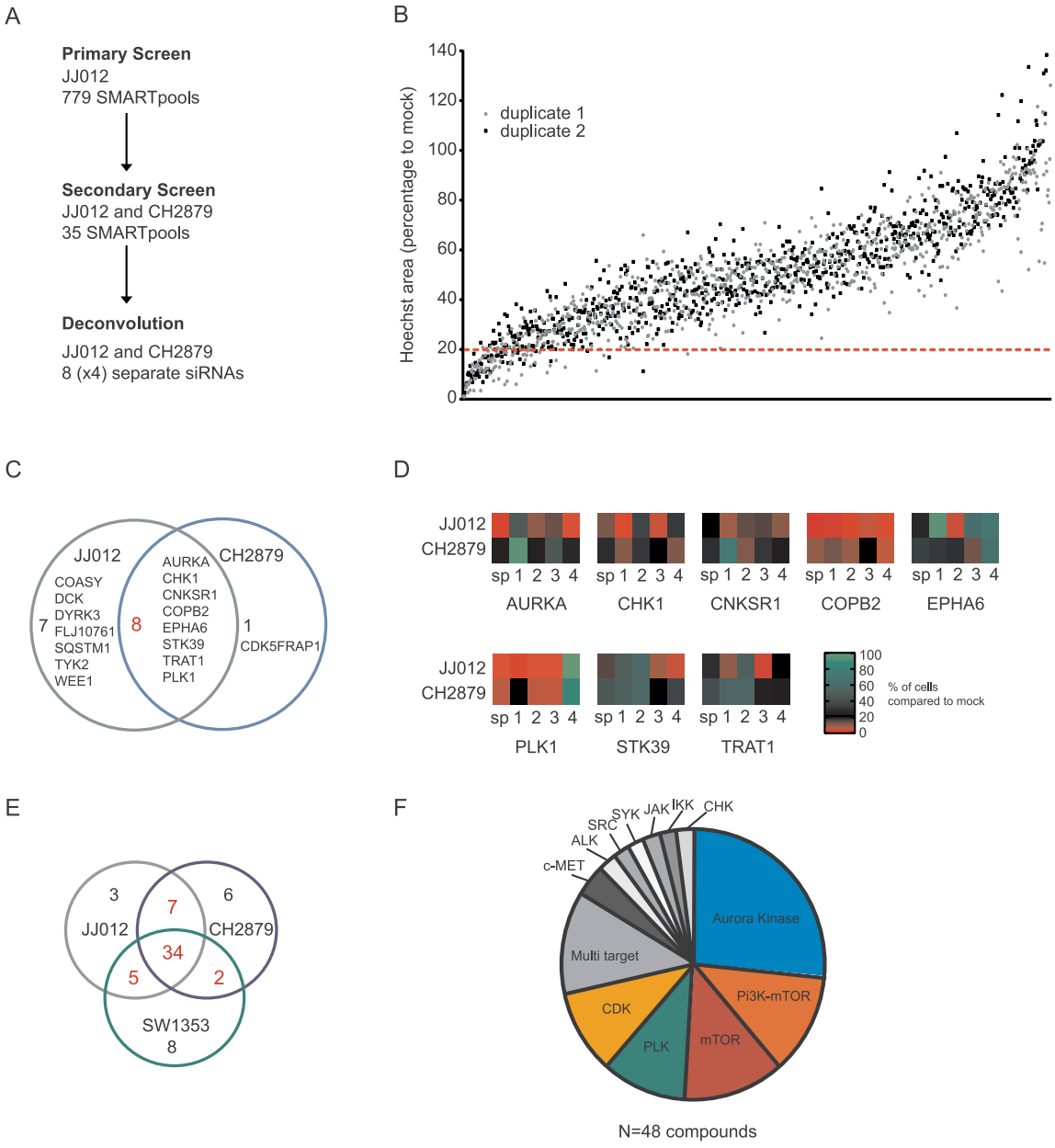

Fig. 1. siRNA screen and compound screen identify PLK1, AURKA and CHK1 as potentially important kinases for survival of chondrosarcoma cells. A. Set-up of siRNA screen. Primary screening was performed on 779 SMARTpools targeting kinases and kinase related genes. The secondary screen was performed in JJ012 and CH2879 cells and consisted of 35 SMARTpool siRNAs identified in the primary screen (decreased cell proliferation below 20% compared to mock conditions). Deconvolution consisted of 4 separate siRNAs and the SMARTpool targeting 9 different genes. B. Hoechst area as a percentage to mock for JJ012 cells. Each dot represents one SMARTpool targeting one Kinase or kinase related gene. Duplicates are shown for each gene and only when both screens showed a percentage below 20% it was considered as a hit. C. Kinases that showed cell killing in both JJ012 and CH2879 were selected for deconvolution (AURKA, CHK1, CNKSR1, COPB2, EPHA6, IRAK3, STK39, TRAT1, PLK1). D. Deconvolution results in JJ012 and CH2879 cells showing that AURKA, CHK1, COPB2, CNKSR1 and PLK1 are important for cell survival in both cell lines. E. Compound screen results in JJ012, CH2879 and SW1353 showing 35 hits in common in the top 50 compounds in each cell line. In addition, 8 compounds were found in JJ012 and CH2879, 6 in JJ012 and SW1353 and 2 in CH2879 and SW1353. F. Compounds that were identified in all three or two out of three cell lines were selected and showed that Aurora kinase, Pi3K-mTOR, mTOR, PLK, CDK and multi-target comprised the largest groups. In addition, compounds targeting c-MET, ALK, SRC, SYK, JAK, IKK and CHK were identified.

with grade II chondrosarcomas show 64% overall survival and patients with grade III chondrosarcomas show a very poor 10 years overall survival of only 29% [1,2]. As chondrosarcomas are resistant to conventional radio- and chemotherapy, the only treatment option to date for patients with chondrosarcoma is surgical removal. This is a major problem especially for patients with tumors in inoperable locations and patients with metastatic disease [2].

Protein kinases are important for cellular processes and are often found deregulated in cancer [3]. Kinome profiling in chondrosarcoma cell cultures previously revealed that the AKT, Src and Ras/Raf/MEK pathways were most active in chondrosarcoma [4]. In addition, Src kinases were shown to be important for chemoresistance, as shown by sensitization for doxorubicin upon inhibition with dasatinib, as well as a high expression of Src kinase family members in chondrosarcoma patient tissues [5]. Using phospho-RTK arrays the phosphorylation status of 42 RTKs was investigated in chondrosarcoma cell lines, which led to the observation of a heterogeneous RTK activation pattern in these cells. P-S6 activation was found in 69% of conventional

chondrosarcoma and 44% of dedifferentiated chondrosarcoma indicating that the downstream PI3K/mTOR pathway might be an important therapeutic target [6]. Furthermore, we previously showed a role for mTORC1 and C2 as an important regulator of chondrosarcoma metabolism [7].

To further unravel the role of kinases in chondrosarcoma we chose a screening-based approach using siRNAs targeting 779 different kinases and kinase related genes. In addition a compound screen was performed consisting of 273 compounds targeting kinases implicated in survival pathways often deregulated in cancer. By comparing the hits we aim at identifying kinase regulated pathways that are important for chondrosarcoma survival.

2. Material and methods

2.1. Cell culture

Conventional chondrosarcoma cell lines SW1353 (ATCC), JJ012 [8], CH2879 [9], CH3573 [10] and L835, and dedifferentiated chondrosarcoma cell lines L3252B, L2975 [11] and NDCS1 [12] were cultured in RPMI-1640 (Gibco, Invitrogen Life-Technologies, Scotland, UK) supplemented with 10 or 20% Fetal Calf Serum (Gibco, Invitrogen Life-Technologies, Scotland, UK). Mesenchymal chondrosarcoma cell line MCS170 [13] was cultured in IMDM medium (Gibco, Invitrogen Life-Technologies, Scotland, UK) supplemented with 15% fetal Calf Serum. All cell lines were cultured at 37 °C in a humidified incubator (5% CO_2). Identity of cell lines was confirmed using the Cell ID Gene Print 10 system (Promega Benelux BV, Leiden, The Netherlands) before and after completion of the experiments. Mycoplasma tests were performed on a regular basis.

2.2. Compounds

MK-5108 (S2770), LY2603618 (S2626), Volasertib (S2235) and ABT-737 (S1002) (positive control apoptosis assay) were purchased from Selleckchem and dissolved in DMSO to a working stock of 10 mM according to the manufacturer's instructions. Z-VAD-FMK was obtained from BD biosciences (550377). Doxorubicin and Cisplatin were obtained in a solution of 0.9% NaCl from the inhouse pharmacy of the Leiden University Medical Centre.

2.3. siRNA screen

To identify critical genes for chondrosarcoma cell survival, a focused targeted siRNA screen was performed on the JJ012 central chondrosarcoma cell line targeting kinases and kinase related genes (Dharmacon, GE life sciences, Landsmeer, the Netherlands, G-003505). Hits were selected for further validation, when both duplicates showed a reduction in cell numbers of 80% or more. A secondary screen including 35 most promising hits was performed in JJ012 and CH2879 chondrosarcoma cell lines (Fig. 1A). Reverse transfection was performed using SMARTpools of 4 different siRNAs targeting the same gene in a final concentration of 50 nM DharmaFECT 3 (Thermo Fisher Scientific Inc. Waltham, MA USA, T-2003) was used as a transfection reagent according to the manufacturer's instructions. Deconvolution confirmation screens, including 9 hits identified in both cell lines were performed on JJ012 and CH2879 cells where each of the four individual siRNAs was transfected separately. A gene was considered as a hit when three out of four individual siRNAs mimicked the SMARTpool in both cell lines or when one cell line showed at least three out of four the other at least two out of four siRNAs mimicking the SMARTpool. Mock (no siRNA), *GFP,* and *GAPDH* siRNAs were used as a negative control and *KIF11* siRNA as a positive control. Transfection was performed using 7000 cells/well for JJ012 and 10,000 cells/well for CH2879 cells in μ-clear 96 well black clear bottom plates (Corning B.V. Life Sciences, Amsterdam, the Netherlands). 24 h after transfection the medium was replaced with medium containing either 1 μM doxorubicin, 5 μM cisplatin or PBS and after five days cells were fixed with formalin and stained with Hoechst. Imaging was performed using a BD-pathway microscope. To quantify the amount of nuclei the total Hoechst area was determined using Image Pro analyzer software and normalized to mock treated cells as described previously [14].

2.4. Compound screen

A compound screen was performed in JJ012, SW1353 and CH2879 cells using a kinase library from Selleckchem (2014, L1200) containing 273 compounds targeting different pathways. SW1353 and JJ012 were plated at an optimal density of 5000 cells/well and CH2879 cells were plated at a density of 7000 cells/well. The screen was performed in duplicate in μ-clear 96 well black clear bottom plates (Corning B.V. Life Sciences, Amsterdam, the Netherlands). After overnight attachment of the cells, compounds were added in a concentration of 1 μM as single treatment or in combination with 0.05 μM doxorubicin or 0.8 μM cisplatin. A high concentration of doxorubicin (5 μM) was used as a positive control. After 72 h of incubation cell viability was assessed using Presto Blue viability reagent (see next paragraph).

2.5. Viability assay

Optimal cell amounts for each cell line were seeded in triplicate in 96-well plates. After 24 h, increasing concentrations from 0 to 1000 nM of MK-5108 and Volasertib or 0–1250 nM LY2603618 were added to the appropriate wells and cells were incubated for an additional 72 h. After the incubation period, a Presto Blue assay (Thermo Fisher Scientific Inc. Waltham, MA USA, A13262) was carried out according to the manufacturer's instructions. After 1 h, viability results were measured by fluorescence at 590 nm on a fluorometer (Victor3V, 1420 multilabel counter, Perkin-Elmer, Groningen, the Netherlands). All experiments were performed in triplicate at least 3 times.

2.6. RNA isolation and quantitative real time PCR

RNA was isolated from fresh frozen tissue of 34 conventional chondrosarcoma primary tumor tissues and six cartilage control tissues: three growth plates, and three articular cartilage tissues (Supplementary Table 1). RNA was isolated using TRIzol (Invitrogen, Carlsbad CA) followed by RNA clean up using the RNeasy mini kit (Qiagen) according to the manufacturer's instructions. All samples were handled as approved by the LUMC ethical board (B17.021). AURKA, CHK1 and PLK1 expression was normalized towards housekeeping genes PPIA and CPSF6 as previously described [15]. Primer pairs are described in supplementary Table 2.

2.7. Cell cycle analysis

Optimal cell amounts for each cell line were seeded in 6-well plates and allowed to attach overnight. Cells were treated with IC_{50} concentrations of LY2603618, MK-5108 or Volasertib. After 24 h, cells were stained with Solution 18 Ao-DAPI (Catalog no. 910-3018, Chemometec, Denmark) according to the manufacturer's instructions and cells were counted using an automated cell analyzer (NucleoCounter NC-250, Chemometec, Denmark). Remaining cells were centrifuged for 5 min at 500 g at 4 °C. Supernatant was removed and the cells were washed with PBS (B. BraunMelsungen AG, Melsungen, Germany). Methanol fixation was carried out, after which cells were washed with PBS/Tw 0.05%. The samples were centrifuged using the same settings and were then washed with PBA/Tw 0.05% (PBS/1.0% BSA/Tw 0.05%). After the final centrifugation of the cells, cells were stained with10 μM DAPI in PBA/Tw 0.05%. The cells were stored at 4° and analysis was carried out next day using the NC-250 nucleoCounter. Results were analyzed using, Winlist 3D and ModFit LT

software (Verity software house). Five biological replicates were included for each sample.

2.8. Apoptosis assay

The caspase glo 3/7 assay (Promega, Madison, WI, USA) was used to detect apoptosis, according to the manufacturer's instructions. Cells were plated in a white 96-well plate and treated the next day with IC_{50} concentrations (obtained from dose response curves) of MK-5108, LY2603618, Volasertib for 24 h. ABT-737 and Doxorubicin were added to the cells as a positive control. Z-vad-FMK, a caspase inhibitor was used as a positive control. After treatment period, caspase glo 3/7 assay was added to cells, which were incubated for an additional 30 min at room temperature. Caspase activity was measured by luminescence using a luminometer (Victor3V, 1420 multilabel counter, Perkin-Elmer) according to the manufacturer's instructions. Experiments were performed three times in duplicate.

2.9. Western blotting

Protein expression of Chk1 (Cell signaling technology #2360) and p-Chk1(S345) (Cell signaling technology, #2348) was determined in JJ012, SW1353 and CH2879 in control conditions and after treatment for 2 or 24 h with IC50 concentrations of LY2603618 (JJ012 1 μM, SW1353 441 nM, CH2879 449 nM). In addition PARP cleavage (Cell signaling technology #9532) was assessed after treatment of JJ012, SW1353 and CH2879 for 2 or 24 h with IC50 concentrations of MK-5108, LY2603618 or Volastertib (MK-5108; SW1353: 1 μM, JJ012: 513 nM, CH2879: 847 nM, Volasertib: SW1353: 34 nM, JJ012 11 nM, CH2879: 24 nM, LY2603618 as described above). Lysates were obtained of cells grown until 70% confluence using hot-SDS buffer (1% SDS, 10 mM Tris/EDTA with complete inhibitor (Roche #11697498001) and phosSTOP (Roche #04906837001) as previously described [4]. Expression of gapdh (Cell signaling technology #5174) was determined as a loading control. A total of 10 μg was loaded on the gel for each sample and blocking was performed using 5% milk. Primary antibodies were diluted in 5% BSA (bovine serum albumin) and incubated overnight. Blotting was performed on PVDF membranes and detection was done using enhanced chemo-luminescence (west Pico Plus chemiluminescent Substrate, Thermo Fisher Scientific, Waltham, MA, USA) followed by visualization using the ChemiDoc imaging system of Biorad.

2.10. Statistical analysis

Dose response curves and IC_{50} values were determined using Prism 7 GraphPad software. Statistically significant differences were assessed by performing a 2-way ANOVA test, correcting for multiple comparisons using Turkey's test. To assess synergy access over Bliss was calculated and percentages above 12 were considered as synergistic [16,17]. Overall survival was determined using SPSS software by performing a Kaplan Meyer analysis and assessing significance using a Mantel Cox Log Rank test.

3. Results

3.1. siRNA screen identifies PLK1, AURKA, COPB2, CHEK1, and CNKSR1 as most important survival genes in chondrosarcoma cells

A siRNA screen targeting 779 kinases and kinase related genes identified 35 genes that decreased survival of JJ012 chondrosarcoma cells more than 80% upon inhibition in both duplicate measurements (see Fig. 1A and B and supplementary Table 3). No siRNAs were identified that could sensitize JJ012 cells to doxorubicin or cisplatin. Transfection with control *GFP* siRNAs led to a slight reduction in cell amounts compared to mock conditions in most plates, however silencing of *GAPDH* led to a large reduction in cell amounts, indicating that this cannot be used as a control for CS siRNA screens (supplementary figure 1). Knock down of *KIF11* led to a strong reduction in cell amounts, indicating successful knock down in all plates except plate 7 which was treated with doxorubicin or cisplatin. This did not influence the hit selection process. A second validation screen was performed in JJ012 and CH2879 cell lines including the 35 most promising siRNAs to select genes important for both cell lines. This reduced the number of candidates to eight that were found in both JJ012 and CH2879 cells (Fig. 1C). The remaining 19 targets, from the first JJ012 screen, could not be reproduced/identified in JJ012 and were absent as well in CH2879 cells. The eight hits identified in both JJ012 and CH2879 were selected for deconvolution and *PLK1, AURKA, CHK1, COPB2* and *CNKSR1* were confirmed (Fig. 1D).

3.2. Compound screening identifies cell cycle regulators as most promising targets in chondrosarcoma cell lines

In parallel with the siRNA screen a compound screen including 273 compounds targeting kinases and kinase related pathways was performed in three different chondrosarcoma cell lines. The top 50 compounds, that showed the highest reduction in cell numbers as compared to DMSO treated controls, of each cell line were compared and 34 compounds were found to be effective in all three cell lines (Fig. 1E), while in total 48 compounds were shared between at least 2 cell lines. The percentage of compounds targeting a specific pathway as compared to the total of 48 hit compounds are represented in Fig. 1F. Inhibitors of cell cycle regulators Aurora kinases (13/16), Polo like kinases (5/5) and Cyclin dependent kinases (4/12)represent a substantial portion (Fig. 1F), confirming the findings of the siRNA screen. Also, inhibitors of the Pi3K and mTOR pathway (12/25) are one of the major hits. Furthermore, inhibitors of cMET (2/13), ALK (1/2), SRC (1/3), SYK (1/5), JAK (1/13), IKK (1/2) and CHK (1/3) are also represented (see supplementary Table 4 for a list of selected compounds). Similar to the siRNA screen, no pathways were identified that upon inhibition clearly sensitized for either doxorubicin or cisplatin (supplementary Table 5). Based on the overlap between the siRNA and the kinase inhibitor screen we chose to continue with PLK1, AURKA and CHK1 as most promising targets. In addition, previous studies already showed that mTOR, CDK and Src are important therapeutic targets for patients or subsets of patients with chondrosarcoma [4,18,19].

3.3. Inhibition of AURKA, CHK1 and PLK1 in chondrosarcoma cells results in a dose dependent decrease in viability, which is not related to RNA expression levels

Dose dependent decreases in viability were observed when different chondrosarcoma cell lines were treated with inhibitors for AURKA (MK-5108), CHK1 (LY2603618) or PLK1 (Volasertib) (Fig. 2A). Lowest IC_{50} values were obtained when cell lines were treated with PLK1 inhibitor volasertib, however treatment with CHK1 inhibitor LY2603618 also led to low IC_{50} values (see Table 1). These values have previously been shown as clinically achievable in human plasma in phase I and phase II trials [20–22]. L835, L3252 and MCS170, which are slower growing *TP53* wildtype cell lines showed higher IC_{50} values for all compounds compared to the other faster growing cell lines. RNA expression analysis showed a variable expression pattern of *AURKA, CHK1* and *PLK1* across the cell lines, with highest expression of all three in CH2879 (Fig. 2B). Only few cell lines responded to inhibition of AURKA, which was not correlated to the level of *AURKA* expression. Likewise, no correlation was observed between sensitivity to CHK1 inhibition and *CHK1* expression levels. Interestingly, of the three lines showing low *PLK1* expression, L835 and MCS-170 showed a poor response to PLK1 inhibition, whereas L2975 exhibited a response, which although *PLK1* expression was lower, still resembled the response patterns of high *PLK1* expressors, but never reached 0% cell viability. Combination

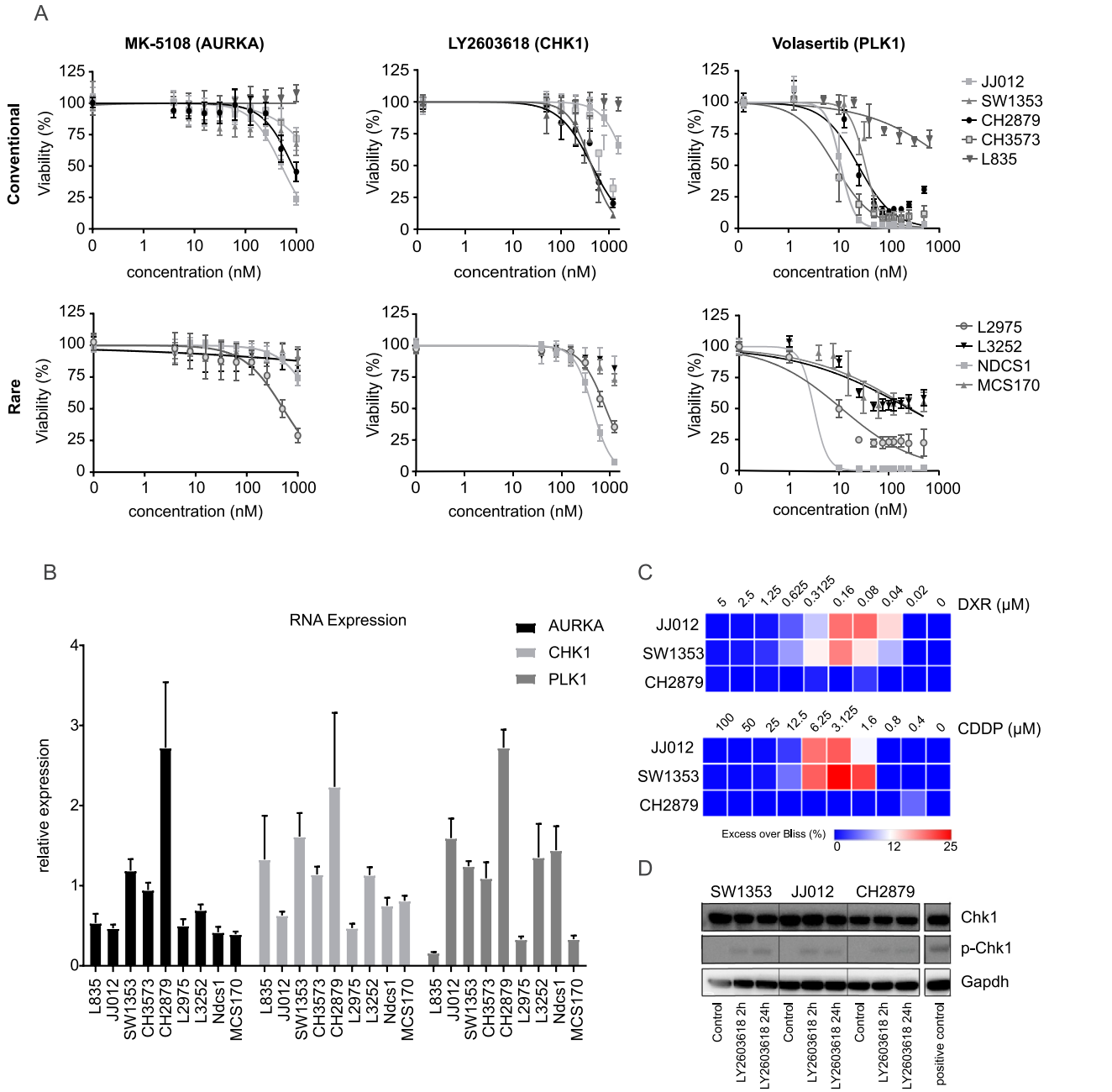

Fig.. 2. Chondrosarcoma cell lines are sensitive for compounds targeting AURKA, CHK1 and PLK1. A. Dose response curves showing viability measured after 72 h using presto blue viability reagent for 9 chondrosarcoma cell lines targeting AURKA (MK-5108), CHK1 (LY2603618) or PLK1 (volasertib). The top three panels represent the conventional chondrosarcoma cell lines and the bottom panel the rare chondrosarcoma cell lines including three dedifferentiated cell lines (L2975, L3252 and NDCS1) and one mesenchymal chondrosarcoma cell line (MCS-170). Highest sensitivity is observed after inhibition with Volasertib. Experiments were performed in triplicate at least three times. B. RNA expression in chondrosarcoma cell lines for *AURKA, CHK1* and *PLK1*. No correlation between expression and sensitivity for the different inhibitors is observed. C. Excess over Bliss percentages of combination treatment of 100 nM LY2603618 and doxorubicin (DXR) or cisplatin (CDDP) showing that JJ012 and SW1353 can be sensitized to conventional chemotherapy after Chk1 inhibition. D. Western blot showing CHK1 and P-CHK1 (S345) expression after treatment for 2 or 24 h with IC50 concentrations of LY2603618. Gapdh expression is assessed as a loading control. Hela cells treated with Hydroxyurea have been used as a positive control for p-CHK1 expression.

treatment with doxorubicin or cisplatin and MK-5108 and LY2603618 was performed in three conventional chondrosarcoma cell lines and results showed a synergistic effect between doxorubicin and cisplatin with the CHK1 inhibitor LY2603618 in JJ012 and SW1353 cell lines as shown by more than 12% increase in Excess over Bliss score (Fig. 2C). This shows that chondrosarcoma cells are more sensitive towards treatment with chemotherapy when CHK1 is inhibited. Increased phosphorylation of CHK1 on Ser345 was observed after treatment with LY2603618 in JJ012, SW1353 and CH2879, indicating that inhibiting CHK1 under these conditions leads to activation of ATM/ATR (Fig. 2D). No basic CHK1 phosphorylation was observed on Ser345.

3.4. Cell cycle analysis reveals a block in G2 after AURKA inhibition

Cell cycle analysis was carried out after 24 h of treatment with MK-5108, LY2603618 or Volasertib and showed that inhibition of AURKA with MK-5108 caused a block in G2/M in both JJ012 ($p < 0.0001$) and CH2879 ($p < 0.0001$) cell lines (Fig. 3A, Supplementary figure 2).

Table 1
IC50 values and 95% confidence intervals for nine different chondrosarcoma cell lines treated with inhibitors for AURKA (MK-5108), CHK1 (LY2603618) or PLK1 (Volasertib).

	MK-5108	LY2603618	Volasertib
JJ012	513.4 (464.2–570.1)	> 1000	10.98 (10.31–11.73)
SW1353	> 1000	441.2 (386.6–502.8)	33.67 (31.76–35.66)
CH2879	847.6 (723.3–109)	449.3 (401–04.6)	24.28 (20.69–28.18)
CH3573	> 1000	815.2 (730.2–918.40	8.3829 (7.083–9.809)
L835	> 1000	> 1000	> 1000
L2975	520.8 (440.5–631)	826.1 (781.3–875.8)	11.33 (9.109–13.92)
Ndcsi	> 1000	442.6 (420.5–465.8)	3.166 (2.54–4.003)
L3252	> 1000	> 1000	252.4 (187.1–363.9)
MCS170	> 1000	> 1000	276.1 (181.2–482)

Treatment with CHK1 inhibitor LY2603618 did result in an increase in S-phase ($p = =0.0003$) and debris ($p = =0.0004$) in JJ012, but not in CH2879. Thus, while inhibiting AURKA clearly showed a G2/M arrest in both cell lines, only JJ012 showed a clear S-phase arrest after CHK1 inhibition. To assess whether this cell cycle arrest led to apoptotic cell death, caspase 3/7 dependent apoptosis was evaluated after 24 h of treatment with MK-5108, LY2603618 or Volasertib (Fig. 3B). Results were variable, but small increases were observed after treatment with MK-5108 or Volasertib in SW1353. PARP cleavage was assessed to determine general cell death and showed a small amount of PARP cleavage, especially in MK-5108 treated JJ012 and CH2879 cells, but no clear differences were observed (Fig. 3C). These results indicate that even though the cell cycle inhibitors successfully caused cell cycle arrest in both cell lines, this did not directly lead to an increase in cell death after 24 h.

3.5. High CHK1 RNA expression in chondrosarcoma tissue samples is correlated with a worse overall survival

Expression of *AURKA, CHK1* and *PLK1* was investigated in a panel of chondrosarcoma tissue samples and revealed that both *AURKA* and *CHK1* showed higher RNA expression compared to cartilage in a subset of chondrosarcoma patients (Fig. 4A). *PLK1* expression was lower compared to normal cartilage in all samples tested, but high in chondrosarcoma cell lines. Most samples that showed higher *AURKA* or *CHK1* expression compared to normal cartilage were in the high-grade group, but this was not significantly different. Samples were divided

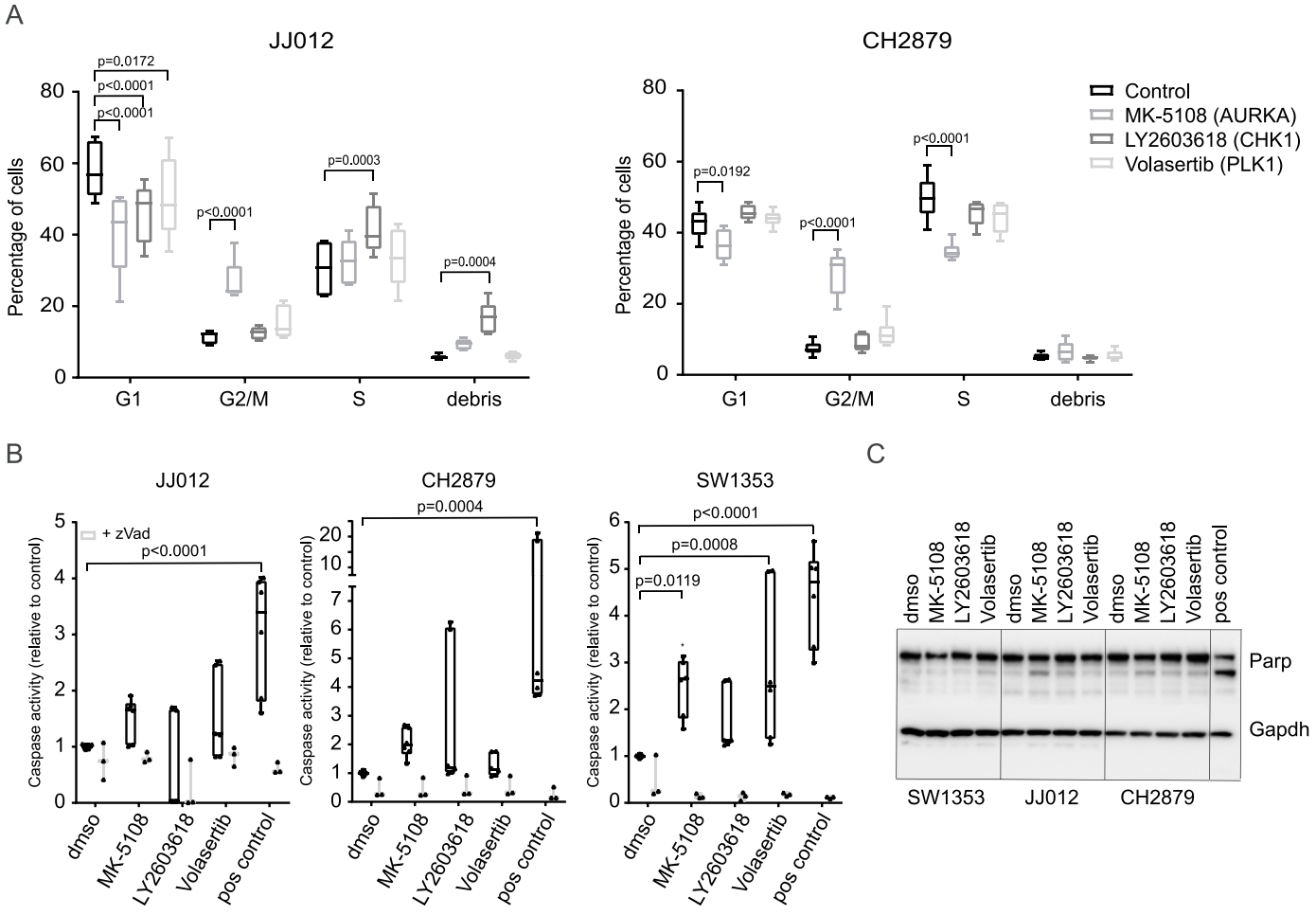

Fig.. 3. Cell cycle and cell death analysis after AURKA, CHK1 or PLK1 inhibition. A. Cell cycle analysis after 24 h of treatment with MK-5108, LY2603618 or Volasertib in JJ012 or CH2879 cells. Both cell lines show a decrease in G1 and an increase in G2 phase after treatment with MK-5108. In addition, CH2879 cells show a decrease in S phase after treatment with MK-5108. JJ012 cells show a decrease in G1 after inhibition with either MK-5108, LY2603618 or Volasertib and an increase in S-phase and debris after LY2603618 treatment. B. Apoptosis induction measured using the caspase-glo 3/7 kit in JJ012, CH2879 and SW1353 after treatment for 24 h with IC_{50} concentrations of MK5108, LY2603618 or Volasertib. Z-vad was added as a control. Only the positive control showed significant caspase induction in all cell lines. SW1353 showed significant upregulated caspase activity after treatment with MK-5108 and Volasertib compared to dmso treated controls. For both cell cycle and apoptosis experiments mean values are shown of three experiments performed in duplicate. *P*-values were calculated using a 2way ANOVA test, correcting for multiple comparisons using Tukeys test. C PARP cleavage assessed after 24 h of treatment using IC50 concentrations of MK-5108, LY2603618 and Volasertib. As a positive control CH2879 cells treated with the combination of ABT-737 and doxorubicin has been taken along.

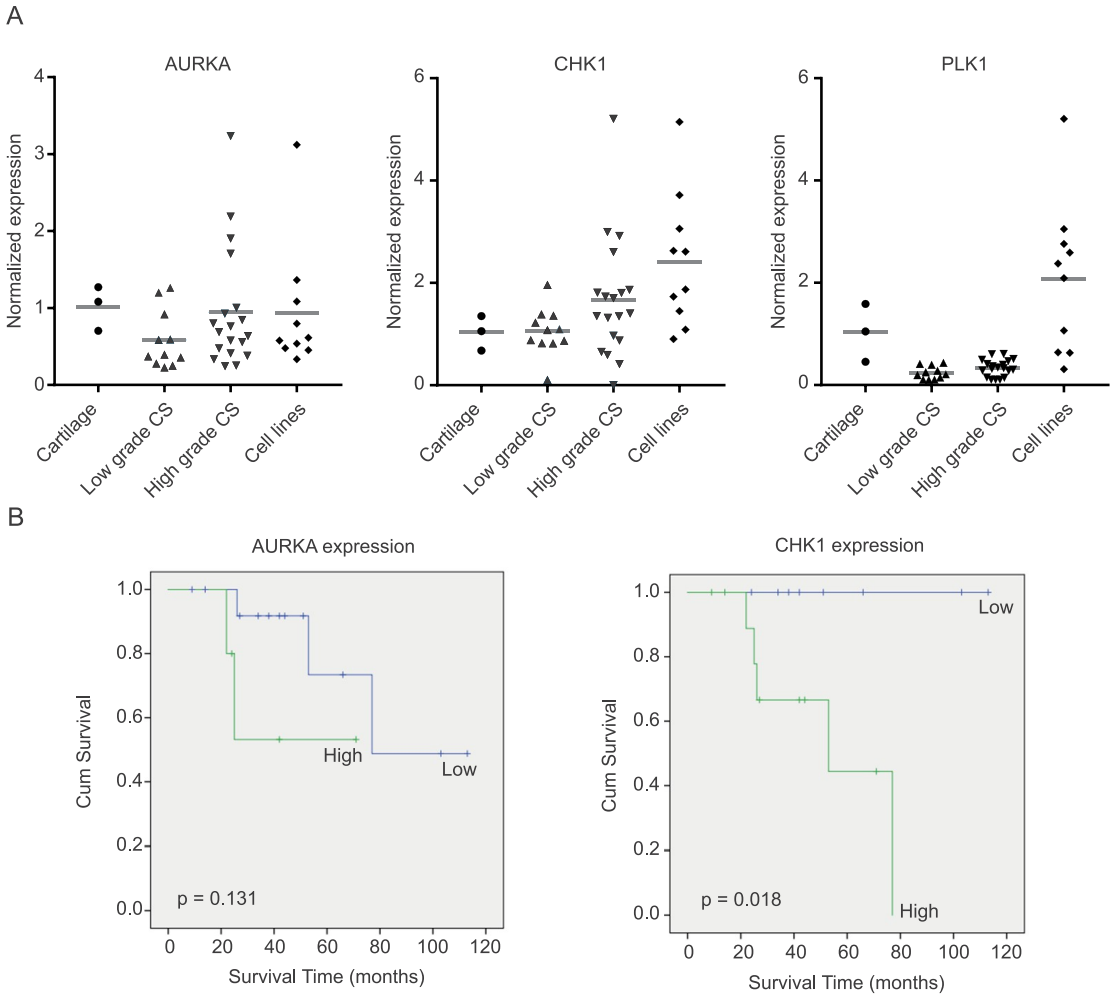

Fig. 4. Gene expression analysis shows that high expression of *CHK1* is correlated to a worse survival in chondrosarcoma patients. A.RNA expression of *AURKA, CHK1* and *PLK1* in chondrosarcoma patient samples and chondrosarcoma cell lines compared to expression in normal articular cartilage samples. Each dot represents one sample. B. Kaplan Meyer analysis of patient samples with low and high expression of *AURKA* and *CHK1*. High *CHK1* expression shows a significant correlation with a worse overall survival in chondrosarcoma patients. Samples with < 1 expression were considered as 'low' expression and samples > 1 expression were considered as high expression. *P* values were calculated using a mantel cox log rank test.

into high and low expression based on expression compared to normal cartilage (> 1 = high expression, < 1 = low expression) and survival analysis was performed (Fig. 4B). No significant difference in survival was observed between low or high expression of *AURKA* ($p = =0.131$), but high *CHK1* expression was significantly correlated with a worse overall survival compared to low *CHK1* expression ($p = =0.018$). These results show that *CHK1* expression is correlated towards a poor prognosis in chondrosarcoma patients.

4. Discussion

Chondrosarcoma patients suffer from limited treatment options due to relative chemo- and radio resistance. In this study we sought to identify new targetable pathways in chondrosarcoma cells by performing a kinase focused siRNA and compound screen. By comparing the hits that were identified in both screens we identified AURKA, PLK1 and CHK1 as important survival regulators in chondrosarcoma cells. AURKA, PLK1 and CHK1 are all involved in cell cycle regulation and often deregulated in cancer cells [23].

Previous studies in chondrosarcoma already revealed that Src kinases could be a potential therapeutic target in chondrosarcoma [5]. In addition mTOR was shown to be active in a large portion of chondrosarcoma patient samples, as well as an important regulator of chondrosarcoma metabolism [6,7]. These hits were also confirmed in our compound screen data.

Cell cycle progression is tightly regulated and controlled by cyclin dependent kinases (CDKs). Activity of CDKs is induced by mitogenic signals but can be inhibited by cell cycle checkpoints in response to DNA damage. Proteins that function in regulating the cell cycle are often deregulated in cancer and can function as possible therapeutic targets as mono therapy or combination with chemotherapy [24]. In this study we show a possible role for targeting the cell cycle in chondrosarcoma.

AURKA and PLK1 are involved in G2 to M phase progression and are essential during mitosis and cytokinesis. AURKA phosphorylates PLK1 to activate Cyclin B-CDK1 complexes leading to progression from G2 to M-phase. During mitosis AURKA and PLK1 form several complexes with other proteins that regulate the maturation and separation of centrosomes and the assembly of the bipolar spindle. Furthermore PLK1 is a member of the chromosomal passenger complex (CPC) that regulates chromosome dynamics and cohesion, kinetochore microtubule attachments, spindle assembly checkpoint and cytokinesis [25]. Overexpression of both AURKA and PLK1 has been shown in a variety of different tumors [26,27].

A subset of chondrosarcoma cell lines showed a dose dependent decrease in viability after inhibiting AURKA using MK-5108, which was not related towards *AURKA* RNA expression. A clear block in G2/M phase of the cell cycle was observed after 24 h of treatment with MK-

5108. We did not find any correlation between expression of *AURKA* and survival, in contrast to a study from 2012 by Liang et al. in which they showed a correlation between high AURKA expression and a worse overall survival in chondrosarcoma patients [28]. A clinical study including 6 chondrosarcoma patients investigating the efficacy of Aurora Kinase A inhibition using Alisertib showed a partial response in one patient with dedifferentiated chondrosarcoma [29]. However a phase III study in lymphoma was discontinued because of lack of response compared to the other study arm [30]. Different studies testing combination strategies show different adverse effects and maximum tolerated dose, which indicates that results might vary between tumor types and patients [21,31–33]. Recently AURKA inhibitors have been identified as synthetic lethal with defective RB1 [34]. In chondrosarcoma 33% of tumors show a defect in the RB1 pathway [35], meaning that in these tumors treatment with AURKA inhibitors might be good treatment option.

Chondrosarcoma cell lines were sensitive for inhibition of PLK1 using Volasertib, but no clear cell cycle effects were observed after 24 h of treatment. This is surprising because PLK1, just like AURKA, is important for entry in M-phase, although multiple other non-cell cycle related functions have been proposed as well, for example PLK1 can regulate mTORC1 activity [36,37]. This can influence the effects that we detect on the cell cycle. Expression of PLK1 in chondrosarcoma tissue samples was low, indicating that targeting PLK1 might not be a good therapeutic strategy for chondrosarcoma patients.

CHK1 is activated by ATM or ATR after the occurrence of DNA damage. Its activation will cause the cell to halt cell proliferation in S or G2 and allows the cell to repair DNA damage. When CHK1 is inhibited DNA damage will accumulate and the cell will die during mitosis. Like AURKA and PLK1, CHK1 is also overexpressed in different types of cancer. Like AURKA and PLK1, CHK1 is also overexpressed in different types of cancer. Using LY2603618 CHK1 was inhibited in a panel of chondrosarcoma cell lines, which resulted in an increased phosphorylation of CHK1 at position S345, which is in agreement with previous reports [38]. Possibly single CHK1 inhibition already leads to activation of the DNA damage response, and IC_{50} concentrations are not high enough to completely prevent phosphorylation by ATM/ATR. A subset of chondrosarcoma cell lines was responding to the treatment, and an increase in S phase was observed in JJ012 cells, but not in CH2879 cells treated with LY2603618. In addition, JJ012 and SW1353 could be sensitized for chemotherapy using CHK1 inhibition. Previous studies already showed the importance of CHK1 in Ewing, Osteo- and soft tissue sarcomas [39–41] and its use in single and combination treatment in pre-clinical models. In addition, we also found a correlation between overall survival and *CHK1* RNA expression in chondrosarcoma patient tissues, indicating that more aggressive chondrosarcomas show higher expression of *CHK1*. Second generation CHK1 inhibitors, showing less toxicity compared to first generation inhibitors are currently tested in the clinic in combination with chemotherapy in advanced cancers [42–44].

Cells with defective P53 protein function have been shown to be more sensitive to inhibitors for CHK1 [45] as well as AURKA and PLK1 compared to cells with intact P53 [46,47]. Inactivation of P53 will lead to a compromised G1 checkpoint, which makes mutated cells more dependent on the G2 checkpoint to be able to repair DNA damage. In our study we do observe a difference in sensitivity between *TP53* wildtype and *TP53* mutant chondrosarcoma cell lines; cell lines with intact P53 are less sensitive to inhibition of AURKA, CHK1 or PLK1. However, these cells also grow slower compared to the other cell lines, which could confound the observed difference in response. Mutations in *TP53* have been observed in 20% of chondrosarcomas [35], indicating that inhibitors of cell cycle regulators might be of interest especially for these patients.

In conclusion, we performed a kinase focused siRNA and compound screen and identified cell cycle regulators AURKA, CHK1 and PLK1 as interesting targetable proteins for follow up studies. RNA expression analysis revealed expression of *AURKA* and *CHK1* in a subset of chondrosarcoma patients, while *PLK1* expression was minimal, compared to normal cartilage tissue. In addition high *CHK1* expression was correlated towards a decrease in survival time. Also inhibition of CHK1 could sensitize a subset of chondrosarcoma cells towards chemotherapy. Future studies should determine the role of cell cycle proteins in chondrosarcoma, however based on our results CHK1 seems to be a promising therapeutic candidate for patients with (*TP53* mutated) chondrosarcoma, especially in combination strategies.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Financial support

This work was financially supported by Dutch Cancer Society (UL2010-4873 and UL2013-6103) and performed in the context of EuroSARC, a collaborative project within the EC's 7th Framework programme under grant agreement 278742.

Acknowledgments

We are grateful to Dr JA Block (Rush University Medical Centre, Chicago, IL, USA), who provided us with the JJ012 cell line and Professor A Llombart Bosch (University of Valencia, Spain) for the CH2879 and CH3573 cell lines. Also we would like to thank M Namba (Okayama University Medical School, Shikata, Japan) for the OUMS27 cell line, Professor J.A. Fletcher for the MCS-170 cell line and Dr T Ariizumi (Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan) for the cell line NDCS-1.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jbo.2019.100268.

References

[1] P.C.W. Hogendoorn, J.V.M.G. Bovee, G.P. Nielsen, Chondrosarcoma (grades I-III), including primary and secondary variants and periosteal chondrosarcoma, in: C.D. M. Fletcher, J.A. Bridge, P.C.W. Hogendoorn, F. Mertens (Eds.), WHO Classification of Tumours of Soft Tissue and Bone, 2013, pp. 264–268.
[2] H. Gelderblom, P.C.W. Hogendoorn, S.D. Dijkstra, C.S. van Rijswijk, A.D. Krol, A.H. Taminiau, J.V. Bovee, The clinical approach towards chondrosarcoma, Oncologist 13 (3) (2008) 320–329.
[3] E.D. Fleuren, L. Zhang, J. Wu, R.J. Daly, The kinome 'at large' in cancer, Nat. Rev. Cancer 16 (2) (2016) 83–98.
[4] Y.M. Schrage, I.H. Briaire-de Bruijn, N.F. de Miranda, O.J. van, A.H. Taminiau, W.T. van, P.C.W. Hogendoorn, J.V.M.G. Bovee, Kinome profiling of chondrosarcoma reveals SRC-pathway activity and dasatinib as option for treatment, Cancer Res. 69 (15) (2009) 6216–6222.
[5] J.G. van Oosterwijk, M.A. van Ruler, I.H. Briaire-de Bruijn, B. Herpers, H. Gelderblom, B. van de Water, J.V.M.G. Bovee, Src kinases in chondrosarcoma chemoresistance and migration: dasatinib sensitises to doxorubicin in TP53 mutant cells, Br. J. Cancer 109 (5) (2013) 1214–1222.
[6] Y.X. Zhang, J.G. van Oosterwijk, E. Sicinska, S. Moss, S.P. Remillard, W.T. van, C. Buehnemann, A.B. Hassan, G.D. Demetri, J.V. Bovee, A.J. Wagner, Functional profiling of receptor tyrosine kinases and downstream signaling in human chondrosarcomas identifies pathways for rational targeted therapy, Clin. Cancer Res. (2013).
[7] R.D. Addie, Y. de Jong, G. Alberti, A.B. Kruisselbrink, I. Que, H. Baelde, J. Bovee, Exploration of the chondrosarcoma metabolome; the mTOR pathway as an important pro-survival pathway, J. Bone Oncol. 15 (2019) 100222.
[8] S.P. Scully, K.R. Berend, A. Toth, W.N. Qi, Z. Qi, J.A. Block, M.U. Award, Interstitial collagenase gene expression correlates with in vitro invasion in human chondrosarcoma, Clin. Orthop. Relat. Res. (376) (2000) 291–303.
[9] R. Gil-Benso, C. Lopez-Gines, J.A. Lopez-Guerrero, C. Carda, R.C. Callaghan, S. Navarro, J. Ferrer, A. Pellin, A. Llombart-Bosch, Establishment and characterization of a continuous human chondrosarcoma cell line, ch-2879: comparative histologic and genetic studies with its tumor of origin, Lab Invest. 83 (6) (2003) 877–887.
[10] S. Calabuig-Farinas, R.G. Benso, K. Szuhai, I. Machado, J.A. Lopez-Guerrero, J.D. de, A. Peydro, M.T. San, L. Navarro, A. Pellin, A. Llombart-Bosch,

Characterization of a new human cell line (CH-3573) derived from a grade II chondrosarcoma with matrix production, Pathol. Oncol. Res. 18 (4) (2012) 793–802.
[11] J.G. van Oosterwijk, J.D. de, M.A. van Ruler, P.C. Hogendoorn, P.D. Dijkstra, C.S. van Rijswijk, I. Machado, A. Llombart-Bosch, K. Szuhai, J.V. Bovee, Three new chondrosarcoma cell lines: one grade III conventional central chondrosarcoma and two dedifferentiated chondrosarcomas of bone, BMC. Cancer 12 (2012) 375.
[12] N. Kudo, A. Ogose, T. Hotta, H. Kawashima, W. Gu, H. Umezu, T. Toyama, N. Endo, Establishment of novel human dedifferentiated chondrosarcoma cell line with osteoblastic differentiation, Virchows Arch. 451 (3) (2007) 691–699.
[13] Y. de Jong, A.M. van Maldegem, A. Marino-Enriquez, D. de Jong, J. Suijker, I.H. Briaire-de Bruijn, A.B. Kruisselbrink, A.M. Cleton-Jansen, K. Szuhai, H. Gelderblom, J.A. Fletcher, J.V. Bovee, Inhibition of Bcl-2 family members sensitizes mesenchymal chondrosarcoma to conventional chemotherapy: report on a novel mesenchymal chondrosarcoma cell line, Lab Invest. 96 (10) (2016) 1128–1137.
[14] J.C. Puigvert, H. de Bont, B. van de Water, E.H. Danen, High-throughput live cell imaging of apoptosis, Curr Protoc. Cell Biol. Chapter 18 (2010) 1–13 Unit 18 10.
[15] L. Hameetman, L.B. Rozeman, M. Lombaerts, J. Oosting, A.H. Taminiau, A.M. Cleton-Jansen, J.V.M.G. Bovee, P.C.W. Hogendoorn, Peripheral chondrosarcoma progression is accompanied by decreased Indian Hedgehog signalling, J. Pathol. 209 (4) (2006) 501–511.
[16] A.A. Borisy, P.J. Elliott, N.W. Hurst, M.S. Lee, J. Lehar, E.R. Price, G. Serbedzija, G.R. Zimmermann, M.A. Foley, B.R. Stockwell, C.T. Keith, Systematic discovery of multicomponent therapeutics, Proc. Natl. Acad. Sci. U.S.A 100 (13) (2003) 7977–7982.
[17] W.R. Greco, G. Bravo, J.C. Parsons, The search for synergy: a critical review from a response surface perspective, Pharmacol. Rev. 47 (2) (1995) 331–385.
[18] J. Perez, A.V. Decouvelaere, T. Pointecouteau, D. Pissaloux, J.P. Michot, A. Besse, J.Y. Blay, A. Dutour, Inhibition of chondrosarcoma growth by mTOR inhibitor in an in vivo syngeneic rat model, PLoS One 7 (6) (2012) e32458.
[19] Y.M. Schrage, S. Lam, A.G. Jochemsen, A.M. Cleton-Jansen, A.H. Taminiau, P.C. Hogendoorn, J.V. Bovee, 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 (9A) (2009) 2843–2852.
[20] P.M. Ellis, N.B. Leighl, V. Hirsh, M.N. Reaume, N. Blais, R. Wierzbicki, B. Sadrolhefazi, Y. Gu, D. Liu, K. Pilz, Q. Chu, A randomized, open-label phase II trial of volasertib as monotherapy and in combination with standard-dose pemetrexed compared with pemetrexed monotherapy in second-line treatment for non-small-cell lung cancer, Clin. Lung Cancer 16 (6) (2015) 457–465.
[21] M. Amin, S.E. Minton, P.M. LoRusso, S.S. Krishnamurthi, C.A. Pickett, J. Lunceford, D. Hille, D. Mauro, M.N. Stein, A. Wang-Gillam, L. Trull, A.C. Lockhart, A phase I study of MK-5108, an oral aurora a kinase inhibitor, administered both as monotherapy and in combination with docetaxel, in patients with advanced or refractory solid tumors, Invest. New Drugs 34 (1) (2016) 84–95.
[22] B. Laquente, J. Lopez-Martin, D. Richards, G. Illerhaus, D.Z. Chang, G. Kim, P. Stella, D. Richel, C. Szcylik, S. Cascinu, G.L. Frassineti, T. Ciuleanu, K. Hurt, S. Hynes, J. Lin, A.B. Lin, D. Von Hoff, E. Calvo, A phase II study to evaluate LY2603618 in combination with gemcitabine in pancreatic cancer patients, BMC Cancer 17 (1) (2017) 137.
[23] T. Otto, P. Sicinski, Cell cycle proteins as promising targets in cancer therapy, Nat. Rev. Cancer 17 (2) (2017) 93–115.
[24] C.C. Mills, E.A. Kolb, V.B. Sampson, Development of chemotherapy with cell-cycle inhibitors for adult and pediatric cancer therapy, Cancer Res. 78 (2) (2018) 320–325.
[25] V. Joukov, A. De Nicolo, Aurora-PLK1 cascades as key signaling modules in the regulation of mitosis, Sci. Signal 11 (543) (2018).
[26] Z. Liu, Q. Sun, X. WangPLK1, A potential target for cancer therapy, Transl. Oncol. 10 (1) (2017) 22–32.
[27] E. Willems, M. Dedobbeleer, M. Digregorio, A. Lombard, P.N. Lumapat, B. Rogister, The functional diversity of Aurora kinases: a comprehensive review, Cell Div. 13 (2018) 7.
[28] X. Liang, D. Wang, Y. Wang, Z. Zhou, J. Zhang, J. Li, Expression of aurora kinase A and B in chondrosarcoma and its relationship with the prognosis, Diagn. Pathol. 7 (2012) 84.
[29] M.A. Dickson, M.R. Mahoney, W.D. Tap, S.P. D'Angelo, M.L. Keohan, B.A. Van Tine, M. Agulnik, L.E. Horvath, J.S. Nair, G.K. Schwartz, Phase II study of MLN8237 (Alisertib) in advanced/metastatic sarcoma, Ann. Oncol. 27 (10) (2016) 1855–1860.
[30] O.A. O'Connor, M. Ozcan, E.D. Jacobsen, J.M. Roncero, J. Trotman, J. Demeter, T. Masszi, J. Pereira, R. Ramchandren, A. Beaven, D. Caballero, S.M. Horwitz, A. Lennard, M. Turgut, N. Hamerschlak, F.A. d'Amore, F. Foss, W.S. Kim, J.P. Leonard, P.L. Zinzani, C.S. Chiattone, E.D. Hsi, L. Trumper, H. Liu, E. Sheldon-Waniga, C.D. Ullmann, K. Venkatakrishnan, E.J. Leonard, A.R. Shustov, I. Lumiere Study, Randomized phase III study of Alisertib or investigator's choice (Selected single agent) in patients with relapsed or refractory peripheral T-Cell lymphoma, J Clin Oncol 37 (8) (2019) 613–623.
[31] S.G. DuBois, Y.P. Mosse, E. Fox, R.A. Kudgus, J.M. Reid, R. McGovern, S. Groshen, R. Bagatell, J.M. Maris, C.J. Twist, K. Goldsmith, M.M. Granger, B. Weiss, J.R. Park, M.E. Macy, S.L. Cohn, G. Yanik, L.M. Wagner, R. Hawkins, J. Courtier, H. Lai, F. Goodarzian, H. Shimada, N. Boucher, S. Czarnecki, C. Luo, D. Tsao-Wei, K.K. Matthay, A. Marachelian, Phase II trial of alisertib in combination with irinotecan and temozolomide for patients with relapsed or refractory neuroblastoma, Clin. Cancer Res. 24 (24) (2018) 6142–6149.
[32] G. Falchook, R.L. Coleman, A. Roszak, K. Behbakht, U. Matulonis, I. Ray-Coquard, P. Sawrycki, L.R. Duska, W. Tew, S. Ghamande, A. Lesoin, P.E. Schwartz, J. Buscema, M. Fabbro, A. Lortholary, B. Goff, R. Kurzrock, L.P. Martin, H.J. Gray, S. Fu, E. Sheldon-Waniga, H.M. Lin, K. Venkatakrishnan, X. Zhou, E.J. Leonard, R.J. Schilder, Alisertib in combination with weekly paclitaxel in patients with advanced breast cancer or recurrent ovarian cancer: a randomized clinical trial, JAMA Oncol. 5 (1) (2019) e183773.
[33] A.T. Fathi, S.A. Wander, T.M. Blonquist, A.M. Brunner, P.C. Amrein, J. Supko, N.M. Hermance, A.L. Manning, H. Sadrzadeh, K.K. Ballen, E.C. Attar, T.A. Graubert, G. Hobbs, C. Joseph, A.M. Perry, M. Burke, R. Silver, J. Foster, M. Bergeron, A.Y. Ramos, T.T. Som, K.M. Fishman, K.L. McGregor, C. Connolly, D.S. Neuberg, Y.B. Chen, Phase I study of the aurora a kinase inhibitor alisertib with induction chemotherapy in patients with acute myeloid leukemia, Haematologica 102 (4) (2017) 719–727.
[34] X. Gong, J. Du, S.H. Parsons, F.F. Merzoug, Y. Webster, P.W. Iversen, L.C. Chio, R.D. Van Horn, X. Lin, W. Blosser, B. Han, S. Jin, S. Yao, H. Bian, C. Ficklin, L. Fan, A. Kapoor, S. Antonysamy, A.M. Mc Nulty, K. Froning, D. Manglicmot, A. Pustilnik, K. Weichert, S.R. Wasserman, M. Dowless, C. Marugan, C. Baquero, M.J. Lallena, S.W. Eastman, Y.H. Hui, M.Z. Dieter, T. Doman, S. Chu, H.R. Qian, X.S. Ye, D.A. Barda, G.D. Plowman, C. Reinhard, R.M. Campbell, J.R. Henry, S.G. Buchanan, Aurora a kinase inhibition is synthetic lethal with loss of the RB1 tumor suppressor gene, Cancer Discov. 9 (2) (2019) 248–263.
[35] P.S. Tarpey, S. Behjati, S.L. Cooke, L.P. Van, D.C. Wedge, N. Pillay, J. Marshall, S. O'Meara, H. Davies, S. Nik-Zainal, D. Beare, A. Butler, J. Gamble, C. Hardy, J. Hinton, M.M. Jia, A. Jayakumar, D. Jones, C. Latimer, M. Maddison, S. Martin, S. McLaren, A. Menzies, L. Mudie, K. Raine, J.W. Teague, J.M. Tubio, D. Halai, R. Tirabosco, F. Amary, P.J. Campbell, M.R. Stratton, A.M. Flanagan, P.A. Futreal, Frequent mutation of the major cartilage collagen gene COL2A1 in chondrosarcoma, Nat. Genet. 45 (8) (2013) 923–926.
[36] Z. Li, Y. Kong, L. Song, Q. Luo, J. Liu, C. Shao, X. Hou, X. Liu, Plk1-mediated phosphorylation of TSC1 enhances the efficacy of rapamycin, Cancer Res. 78 (11) (2018) 2864–2875.
[37] S. Ruf, A.M. Heberle, M. Langelaar-Makkinje, S. Gelino, D. Wilkinson, C. Gerbeth, J.J. Schwarz, B. Holzwarth, B. Warscheid, C. Meisinger, M.A. van Vugt, R. Baumeister, M. Hansen, K. Thedieck, PLK1, (polo like kinase 1) inhibits mTOR complex 1 and promotes autophagy, Autophagy 13 (3) (2017) 486–505.
[38] F.Z. Wang, H.R. Fei, Y.J. Cui, Y.K. Sun, Z.M. Li, X.Y. Wang, X.Y. Yang, J.G. Zhang, B.L. Sun, The checkpoint 1 kinase inhibitor LY2603618 induces cell cycle arrest, DNA damage response and autophagy in cancer cells, Apoptosis 19 (9) (2014) 1389–1398.
[39] Z. Baranski, T.H. Booij, A.M. Cleton-Jansen, L.S. Price, B. van de Water, J.V. Bovee, P.C. Hogendoorn, E.H. Danen, Aven-mediated checkpoint kinase control regulates proliferation and resistance to chemotherapy in conventional osteosarcoma, J Pathol 236 (3) (2015) 348–359.
[40] S.L. Koppenhafer, K.L. Goss, W.W. Terry, D.J. Gordon, mTORC1/2 and protein translation regulate levels of CHK1 and the sensitivity to CHK1 inhibitors in Ewing sarcoma cells, Mol. Cancer Ther. 17 (12) (2018) 2676–2688.
[41] A. Laroche-Clary, C. Lucchesi, C. Rey, S. Verbeke, A. Bourdon, V. Chaire, M.P. Algeo, S. Cousin, M. Toulmonde, V. Velasco, J. Shutzman, A. Savina, F. Le Loarer, A. Italiano, CHK1 inhibition in soft-tissue sarcomas: biological and clinical implications, Ann. Oncol. 29 (4) (2018) 1023–1029.
[42] D.S. Hong, K. Moore, M. Patel, S.C. Grant, H.A. Burris 3rd, W.N. William Jr., S. Jones, F. Meric-Bernstam, J. Infante, L. Golden, W. Zhang, R. Martinez, S. Wijayawardana, R. Beckmann, A.B. Lin, C. Eng, J. Bendell, Evaluation of prexasertib, a checkpoint kinase 1 inhibitor, in a phase Ib study of patients with squamous cell carcinoma, Clin Cancer Res 24 (14) (2018) 3263–3272.
[43] D. Hong, J. Infante, F. Janku, S. Jones, L.M. Nguyen, H. Burris, A. Naing, T.M. Bauer, S. Piha-Paul, F.M. Johnson, R. Kurzrock, L. Golden, S. Hynes, J. Lin, A.B. Lin, J. Bendell, Phase I study of LY2606368, a checkpoint kinase 1 inhibitor, in patients with advanced cancer, J Clin Oncol 34 (15) (2016) 1764–1771.
[44] P.G. Pilie, C. Tang, G.B. Mills, T.A. Yap, State-of-the-art strategies for targeting the DNA damage response in cancer, Nat. Rev. Clin. Oncol. 16 (2) (2019) 81–104.
[45] A.A. Levesque, A. Eastman, p53-based cancer therapies: is defective p53 the Achilles heel of the tumor? Carcinogenesis 28 (1) (2007) 13–20.
[46] M. Marxer, H.T. Ma, W.Y. Man, R.Y. Poon, p53 deficiency enhances mitotic arrest and slippage induced by pharmacological inhibition of Aurora kinases, Oncogene 33 (27) (2014) 3550–3560.
[47] S. Sur, R. Pagliarini, F. Bunz, C. Rago, L.A. Diaz, J. Kinzler K.W., B. Vogelstein, N. Papadopoulos, A panel of isogenic human cancer cells suggests a therapeutic approach for cancers with inactivated p53, Proc. Natl. Acad. Sci. U S A 106 (10) (2009) 3964–3969.