Inhibiting Polo-like kinase 1 causes growth reduction and apoptosis in pediatric acute lymphoblastic leukemia cells

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ABSTRACT

This study investigated Polo-like kinase 1, a mitotic regulator often over-expressed in solid tumors and adult hematopoietic malignancies, as a potential new target in the treatment of pediatric acute lymphoblastic leukemia. Polo-like kinase 1 protein and Thr210 phosphorylation levels were higher in pediatric acute lymphoblastic leukemia (n=172) than in normal bone marrow mononuclear cells (n=10) ($P<0.0001$). High Polo-like kinase 1 protein phosphorylation, but not expression, was associated with a lower probability of event-free survival ($P=0.042$) and was a borderline significant prognostic factor ($P=0.065$) in a multivariate analysis including age and initial white blood cell count. Polo-like kinase 1 was necessary for leukemic cell survival, since short hairpin-mediated Polo-like kinase 1 knockdown in acute lymphoblastic leukemia cell lines inhibited cell proliferation by G2/M cell cycle arrest and induced apoptosis through caspase-3 and poly (ADP-ribose) polymerase cleavage. Primary patient cells with a high Polo-like kinase 1 protein expression were sensitive to the Polo-like kinase 1-specific inhibitor NMS-P937 *in vitro*, whereas cells with a low expression and normal bone marrow cells were resistant. This sensitivity was likely not caused by Polo-like kinase 1 mutations, since only one new mutation (Ser335Arg) was found by 454-sequencing of 38 pediatric acute lymphoblastic leukemia cases. This mutation did not affect Polo-like kinase 1 expression or NMS-P937 sensitivity. Together, these results indicate a pivotal role for Polo-like kinase 1 in pediatric acute lymphoblastic leukemia and show potential for Polo-like kinase 1-inhibiting drugs as an addition to current treatment strategies for cases expressing high Polo-like kinase 1 levels.

Introduction

Polo-like kinase 1 (PLK1) is the best characterized of five currently known members of the mammalian Polo-like kinase family, so named because of their homology to the Polo kinase first discovered in Drosophila.[1] The Polo-like kinases have an N-terminal kinase domain and one or several C-terminal protein-binding polo-box domains. The main functions of PLK1 are related to the regulation of mitotis through interaction and cross-talk with a large number of cell cycle mediators. Accordingly, its expression and activity is regulated throughout the cell cycle, increasing during G2 and peaking at M phase.[2,3] During this, PLK1 is involved in centrosome maturation, spindle formation, chromosome alignment and cytokinesis together with Aurora kinases A and B.[4] In addition, PLK1 is able to bind p53 and inhibit its pro-apoptotic function,[5] whereas, in turn, expression of PLK1 is transcriptionally repressed by p53 upon DNA damage, indicating a role for PLK1 in the G2/M checkpoint.[6]

Although these functions illustrate an important role for PLK1 in normal cell cycle progression, *in vivo* studies on PLK1 involvement in carcinogenesis are not in agreement as to whether PLK1 must be regarded as an oncogene or tumor suppressor. On the one hand, constitutive expression of murine PLK1 in NIH 3T3 cells enhances proliferation and leads to tumor formation when injected into nude mice.[7] On the other hand, $PLK^{+/-}$ mice display a 3-fold higher tumor incidence than wild-type mice.[8] This suggests that any aberration in PLK1 function, be it overexpression or loss of function, leads to dysregulated cell proliferation and could potentially result in carcinogenesis.

In a large number of human cancer types, PLK1 expression is up-regulated and high PLK1 is a predictor for poor prognosis.[9–20] PLK1 has been extensively studied in solid tumors, but more recent reports show elevated PLK1 expression in adult leukemias.[21–23] The PLK1/PLK3 inhibitor GW843682X displayed *in vitro* toxicity to a panel of pediatric cancer cell lines,[24] but no PLK1-specific inhibitor has yet entered clinical trials in pediatric malignancies. Identification of a common tumor-driving gene may allow the development of therapeutics that are applicable to different types of cancer patients. Here we show that PLK1 is essential for proliferation and survival of pediatric ALL cells of different genetic subtypes, and test the efficacy of the PLK1-selective inhibitor NMS-P937 (or NMS-1286937) in ALL cell lines and primary ALL samples. Whereas many PLK1 inhibitors also target PLK2 and PLK3, NMS-P937 is a newly developed ATP-competitive small-molecule

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inhibitor highly selective for PLK1 and the first orally administered selective PLK1 inhibitor entering phase I clinical trials[25] (*clinicaltrials.gov identifier: NCT01014429*). This study shows the potential of PLK1-targeting therapeutics in the treatment of childhood ALL, and indicates their value for further clinical evaluation.

Methods

Cell lines

Cell lines (DSMZ, Braunschweig, Germany) were cultured in RPMI+Glutamax with pen-strep, fungizone (Life Technologies, Bleiswijk, The Netherlands) and 10% or 20% fetal calf serum (Integro, Zaandam, The Netherlands), at 37°C and 5% CO_2.

Patients

Written informed consent was obtained to use excess diagnostic material for research purposes, as approved by the institutional review boards. Bone marrow or peripheral blood from children with newly diagnosed ALL and from children without hematologic disorder (normal bone marrow; nBM) were processed as previously described.[26] Genetic subtypes of precursor B-ALL (hyperdiploid, MLL/11q23-rearranged, ETV6-RUNX1/t(12;21)(p13;q22), TCF3/19p13-rearranged, BCR-ABL1/t(9;22)(q34;q11), and 'B-other' (negative for aforementioned features) and T-ALL were identified as described before.[27] Two consecutive German Co-operative Study Group for Childhood ALL (COALL) trials (06-97 and 07-03) were combined for survival analyses and referred to as COALL-97/03.

Reverse phase protein arrays

Reverse phase protein arrays (RPPA) were performed with 172 pediatric ALL samples (*Online Supplementary Table S1*) and 10 nBM samples as previously described,[28-30] in collaboration with E. Petricoin (George Mason University, Manassas, VA, USA). Antibodies were from Cell Signaling Technology (Danvers, MA, USA) (PLK1, #4535; Aurora B, #3094), BD Pharmingen (Breda, The Netherlands) (Thr210-phosphorylated PLK1, #558400), and AbD Serotec (Kidlington, UK) (Aurora A, MCA2249).

Lentiviral infection

HEK293T cells were transfected as described before[31] with psPAX2 (Addgene plasmid 12260) and pMD2.G (Addgene plasmid 12259), and a pLKO.1 Mission® vector (Sigma-Aldrich, Zwijndrecht, The Netherlands) containing a puromycin selection marker and a short hairpin RNA (shRNA) against PLK1 (TRCN0000121222) or eGFP (SHC005) as a non-targeting control (NTC). After 24 h, transduced cells were selected on puromycin (Sigma-Aldrich). Cell viability was determined by trypan blue exclusion.

Western blot

Western blots were performed as previously described.[31] Antibodies were from Cell Signaling Technology (PLK1 (208G4), #4513; cleaved Poly (ADP-ribose) polymerase (PARP), #9541; Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), #2118) and Abcam (Cambridge, UK) (β-actin, ab6276).

MTS drug sensitivity assay

Cell viability after NMS-P937 (Nerviano Medical Sciences, Nerviano, Italy) exposure was determined by MTS assay as described previously,[31] and calculated as a percentage of untreated controls. GI50 values represent drug doses inhibiting cell growth by 50% within 72 h.

Sequencing

ALL patient (n=38) and cell line (n=4) DNA was screened by 454 sequencing of twelve PCR products covering PLK1 exons 1-7, encompassing the kinase domain(s) (*Online Supplementary Table S2*). The average number of sequence reads achieved per PCR product was 161. The sequencing success rate for PCR products (defined as >30 reads) based on all samples was 92% (*Online Supplementary Table S3*). Mutations were confirmed by Sanger sequencing.

Statistical analysis

Mann-Whitney U and Spearman's rank correlation (r_s) tests were performed in IBM SPSS v20.0.0.1. Univariate and multivariate analyses of event-free survival (EFS) of the COALL-97/03 cohort (n=123) (*Online Supplementary Table S4*) were performed with Cox's proportional hazards model, stratified for treatment arms. An event was defined as relapse, non-response to therapy, secondary malignancy or death. The cumulative incidence of relapse (CIR) was analyzed in 'R' v2.14.0 with the method of Fine and Gray and death as competing risk event.

Further details of Design and Methods are available in the *Online Supplementary Appendix*.

Results

PLK1 protein is over-expressed in ALL patients

PLK1 protein expression was analyzed by reverse phase protein array (RPPA) in 172 ALL patient samples and in 10 nBM cases as an estimate for expression in normal hematopoietic cells. PLK1 protein levels were 2.6-fold higher in ALL than in nBM cases (P<0.0001) (Figure 1A). There was no significant difference between B- and T-lineage ALL, but within the precursor B-ALL group TCF3-rearranged patient samples had a 1.3-fold higher expression than cells without this translocation (P<0.0001). This differential expression was not observed at the mRNA level in a cohort of 859 primary ALL and 8 nBM cases (*Online Supplementary Figure S1*). We also investigated phosphorylation levels of PLK1 Thr210, the major activating phosphorylation site of PLK1.[32] ALL samples contained 1.8-fold more Thr210 phosphorylated PLK1 than nBM (P<0.0001) (Figure 1B). In contrast to the higher total PLK1 expression in TCF3-rearranged cases, the phosphorylation level of PLK1 did not differ between TCF3-rearranged and other ALL cases. Neither total protein nor Thr210 phosphorylation levels of PLK1 were significantly associated with gender, age or white blood cell (WBC) count at diagnosis (*Online Supplementary Figure S2*).

PLK1 is known to interact and share mitotic functions and pathways with Aurora kinases A and B. We previously observed that Aurora kinase B expression was elevated in TCF3-rearranged patients,[31] which prompted us to investigate whether there is a correlation between PLK1 and Aurora kinase expression. In our patient cohort, PLK1 protein expression was indeed correlated to expression of Aurora kinase B (r_s=0.53 and P<0.0001), and to a lesser extent to Aurora kinase A (r_s=0.32 and P<0.0001) (Figure 1C and D, respectively).

High Thr210 phosphorylation levels of PLK1 are associated with poor outcome

Next, we investigated the prognostic value of PLK1 expression by determining the probability of event-free survival (EFS) and cumulative incidence of relapse (CIR)

for patients treated according to one treatment protocol, i.e. the German COALL-97/03 protocol (n=123). Cases were classified into high and low PLK1 protein expression (Figure 2A) or Thr210 phosphorylation level (Figure 2B). The cut offs for 'high' PLK1 expression and phosphorylation were based on the highest tertile when ranking the complete RPPA patient cohort from low to high PLK1 protein expression or Thr210 phosphorylation. Cox's univariate analysis of EFS, stratified for treatment arms, revealed that Thr210 pPLK1 levels (*P*=0.042; hazard ratio 2.01, 95% confidence interval (CI) 1.03-3.92), but not total PLK1 protein levels (*P*=0.59), gender (*P*=0.56), WBC count at diagnosis (*P*=0.14), age (*P*=0.26) or genetic subtype (*P*=0.31), were predictive for an unfavorable outcome in pediatric ALL (Figure 2C and D). Multivariate analysis including age and WBC count revealed that high PLK1 Thr210 phosphorylation level was a borderline significant independent prognostic factor (*P*=0.065; hazard ratio 1.89, 95%CI: 0.96-3.71) (*Online Supplementary Table S5*).

High PLK1 protein and Thr210-phosphorylation levels were not significantly associated with a higher CIR (*P*=0.42 and *P*=0.10, respectively; *Online Supplementary Figure S3*).

PLK1 knockdown inhibits cell growth and induces apoptosis in leukemic cell lines

To test whether PLK1 is required for leukemic cell proliferation or survival, we silenced PLK1 protein expression using lentivirally-delivered shRNAs in a T-ALL cell line (JURKAT) and three precursor B-ALL cell lines with different genetic aberrations (SEM, REH and 697). Knockdown was confirmed by Western blot: PLK1 protein expression was decreased by at least 50% within 48 h after transduction (Figure 3A). Within 48-96 h, transfection of the PLK1 short hairpin (shPLK1) decreased proliferation (Figure 3B) and induced apoptosis, as indicated by the cleavage of

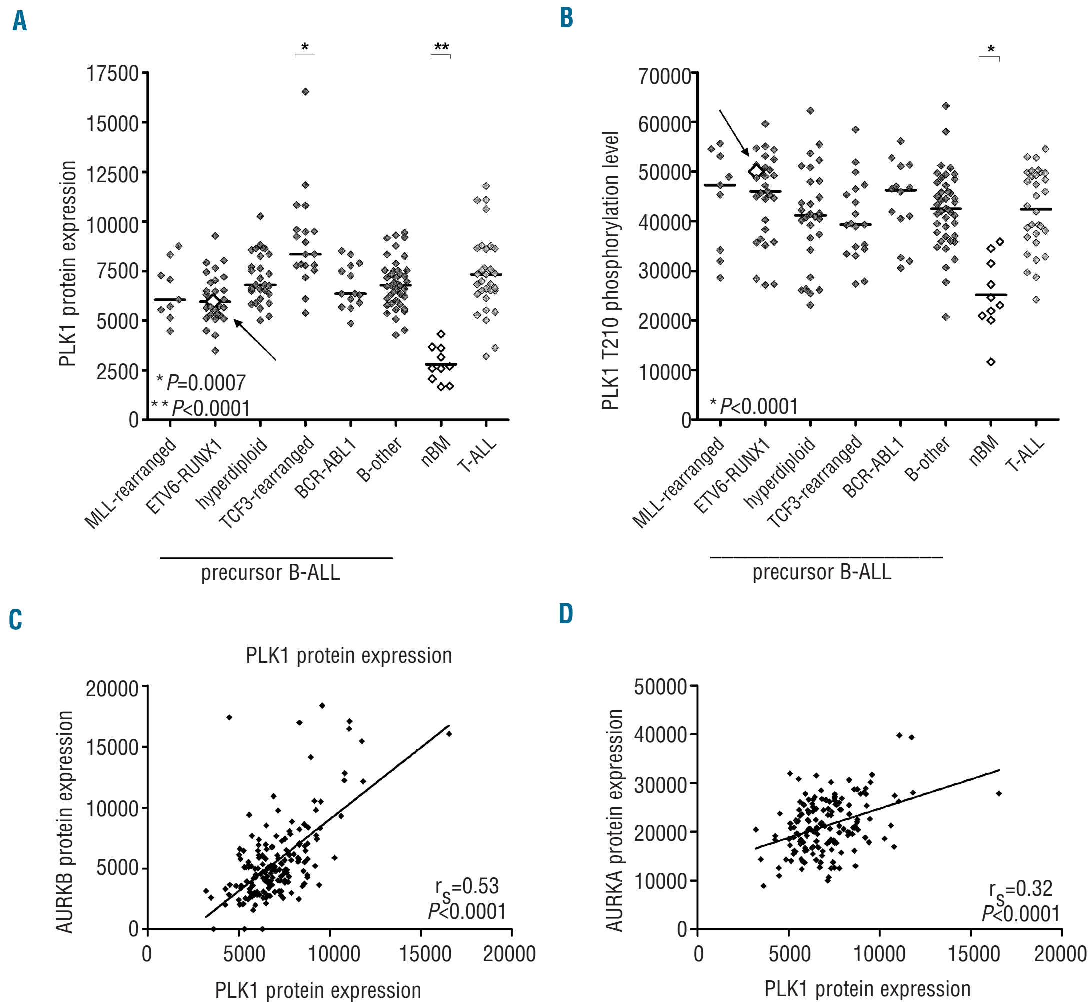

Figure 1. PLK1 protein expression and phosphorylation levels are elevated in pediatric ALL patients. PLK1 total protein levels (A) and PLK1 Thr210 phosphorylation levels (B) were analyzed by RPPA in different subtypes of ALL patients (n=172) and nBM mononuclear cells (n=10). Horizontal bars indicate group medians. Asterisks indicate statistical significance for nBM *versus* leukemic samples, and for TCF3-rearranged cases *versus* other precursor B-ALL cases. Arrows point towards ◇, representing a patient harboring a Ser335Arg mutation in exon 5 of PLK1. C and D) Correlations of PLK1 and AURKB (C) or AURKA (D) protein expression levels determined by RPPA in 172 or 162 patients, respectively. Diagonal lines represent the best fit. The correlation coefficient and its significance were determined by the non-parametric Spearman's rank (r_s) test. All expression levels are presented in normalized fluorescence units.

PARP (Figure 3A), in all cell lines compared to transfection of a non-targeting control short hairpin. It is worthy of note that antibiotic (puromycin) selection contributed to PARP cleavage in NTC-treated cell lines with a low transfection efficiency, i.e. 697. Further analysis of the mechanism of cell death showed that apoptosis was caspase-3-dependent and was preceded by a G2/M cell cycle arrest (*Online Supplementary Figure S4*).

ALL cell lines and patients are sensitive to PLK1 inhibitor NMS-P937

Fifteen ALL cell lines were exposed to a serial dilution (1.5nM-3.3μM) of the PLK1 small-molecule inhibitor NMS-P937 and cell viability was assessed after 72 h. GI50 values ranged from 0.023 to 0.23μM (Figure 4A). Sensitivity was not associated with a specific subtype of ALL.

To determine the sensitivity of primary ALL cells to NMS-P937, 15 ALL patient samples, ranging from low to high PLK1 expression or Thr210 phosphorylation level, were cultured in the presence of 0.12μM NMS-P937 and cell viability was measured after 96 h of exposure. Leukemic cell survival was significantly correlated with total PLK1 protein expression (r_s=-0.58 and *P*=0.02), with high-expressing samples being more sensitive to NMS-P937 (Figure 4B). There was no correlation between PLK1 phosphorylation level and sensitivity to NMS-P937 (Figure 4C).

We also tested the toxicity of NMS-P937 to healthy bone marrow by exposing nBM mononuclear cells to a concentration range of the inhibitor. The cells were resistant to NMS-P937 up to a concentration of at least 3.3μM (Figure 4D).

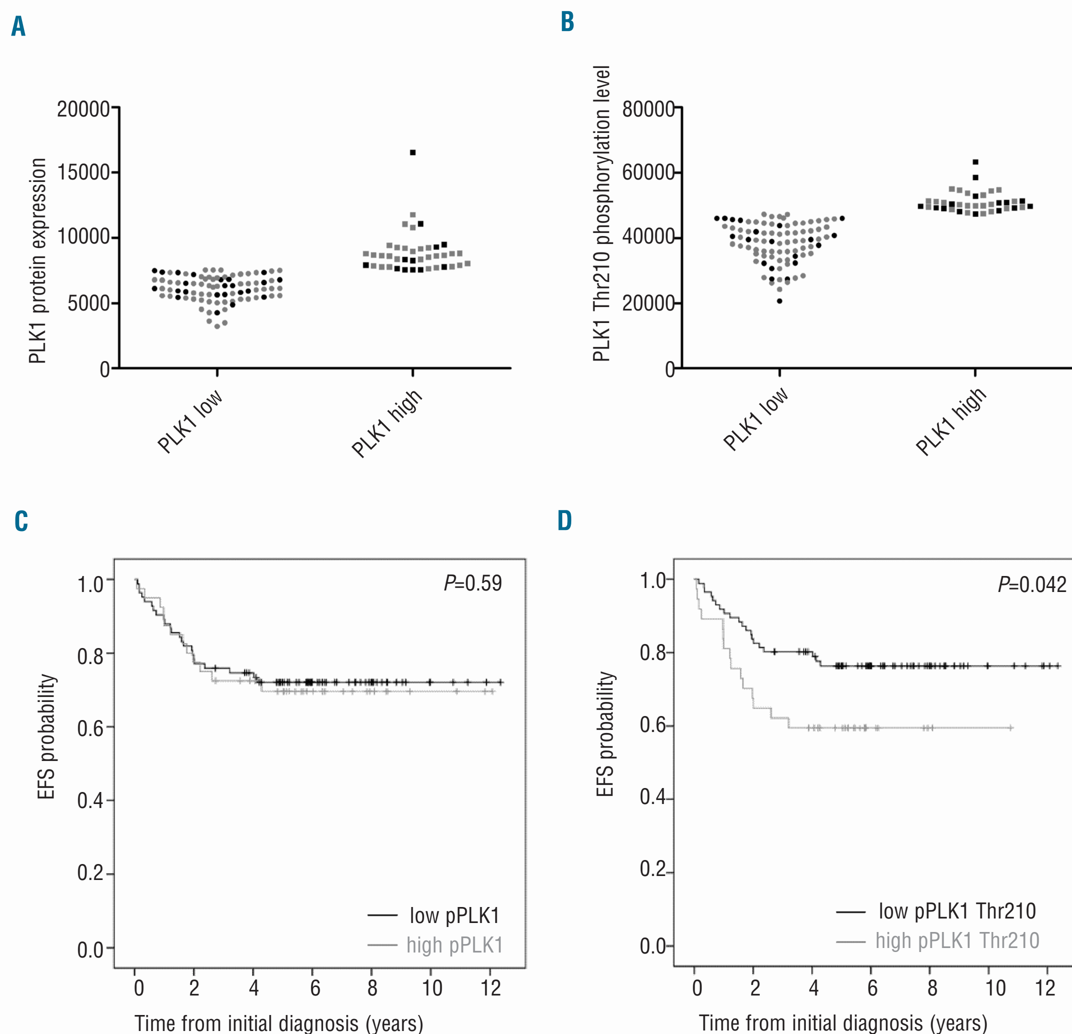

Figure 2. Event-free survival of pediatric ALL cases with high and low PLK1 expression and phosphorylation. Patients treated according to one treatment protocol, i.e. the German COALL-97/03 protocol, were used to analyze the prognostic value of (phospho-)PLK1. Patients were divided into groups with high (n=40) and low (n=83) PLK1 protein expression (A), and high (n=37) and low (n=86) Thr210 phosphorylation (B). The cut offs for the categories high and low were determined by the highest tertile of 172 patients when ranking from low to high PLK1 protein expression and phosphorylation level, respectively. Patients with an event (relapse, non-response to therapy, secondary malignancy or death) are depicted in black. The event-free survival (EFS) probability was determined for patients with high and low PLK1 protein expression (C) and Thr210 phosphorylation level (D). Vertical lines indicate censoring of a patient at the end of follow up or time of death. *P*-values are stratified for treatment arms based on patient risk classification.

PLK1 mutation Ser335Arg does not affect PLK1 expression and phosphorylation levels or NMS-P937 sensitivity

The 454 sequencing of the mutational hot spots of the PLK1 kinase domains (exons 1-7) of 38 primary ALL samples and 4 ALL cell lines revealed a single non-synonymous coding sequence variant (A1003C; Ser335Arg) in exon 5 of the PLK1 gene in a primary ETV6-RUNX1-positive case (sample ID #100 in *Online Supplementary Table S3*). This mutation was subsequently validated in a second phase of 454 sequencing that confirmed this variant at a frequency of 43% in 149 forward reads and 41% in 116 reverse reads. This was independently confirmed as a heterozygous mutation by further Sanger sequencing. Control-matched non-leukemic blood was not available to confirm mutation status; however, this variant was not found in SNP variant (dbSNP, etc.) or COSMIC databases, or in 158 non-leukemic samples screened.

This A1003C mutation did not lead to aberrant PLK1 expression or phosphorylation levels, since total PLK1 protein expression corresponded to the median expression for ETV6-RUNX1-positive patients and its phosphorylation level was only slightly above the median for this group (see arrows in Figure 1A and B). Furthermore, the mutation did not sensitize to NMS-P937 when comparing sensitivity of this sample and a wild-type (WT) patient sample with comparable PLK1 expression (Figure 4D).

Discussion

Improvement of existing therapies as well as development of new treatment options have improved the outcome for children diagnosed with ALL over the past decades. At present, the clinical outcome for children with ALL has reached a plateau of approximately 80% 5-year event-free survival.[33] Identification of new specific targets for therapy and development of more specific agents may help further improve the prognosis and reduce the late effects of treatment. This study aimed to validate PLK1 as a possible therapeutic target in pediatric ALL. Findings revealed that PLK1 is essential to the leukemic cell, and that PLK1 knockdown or inhibition leads to apoptosis and growth inhibition in leukemic cell lines and a subset of ALL patients. In addition, we identified a not previously described PLK1 Ser335Arg mutation with no apparent effect on PLK1 expression, phosphorylation level or sensitivity to inhibition by NMS-P937.

Although we did not observe overexpression of PLK1 in pediatric ALL on the mRNA level, in contrast to what had previously been shown in adult ALL,[22] we found that PLK1 protein expression was up-regulated in pediatric ALL cases compared with healthy bone marrow mononuclear cells. This is not surprising given the fact that PLK1 protein seems to be especially expressed and activated in proliferating cells.[34] However, *ex vivo* leukemic cells stop proliferating, and hence the elevated PLK1 protein expression in primary patients' cells outside the body is remarkable. This adds pediatric ALL to the list of cancer types that over-express PLK1. The expression of PLK1 seems important for cell survival and growth, as our study shows that PLK1 knockdown reduced proliferation and induced apoptosis in ALL cell lines, an effect that was also observed in solid tumor and AML cell lines.[21-23,35-40] The TCF3-translocated 697 cell line did not display the strongest phenotypic effect upon PLK1 knockdown, despite having the highest PLK1 expression. However, PLK1 expression was relatively high in all cell lines (*data not shown*), probably due to their proliferative capacity, and factors like transfection efficiency and rate of protein knockdown differ between

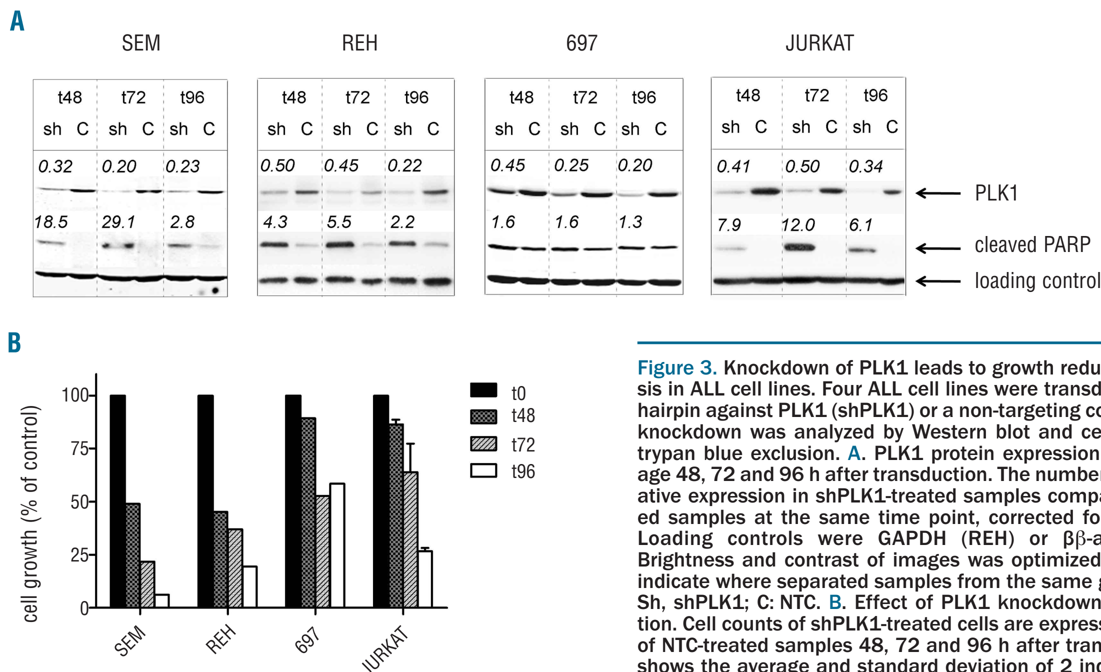

Figure 3. Knockdown of PLK1 leads to growth reduction and apoptosis in ALL cell lines. Four ALL cell lines were transduced with a short hairpin against PLK1 (shPLK1) or a non-targeting control (NTC). PLK1 knockdown was analyzed by Western blot and cell proliferation by trypan blue exclusion. A. PLK1 protein expression and PARP cleavage 48, 72 and 96 h after transduction. The numbers indicate the relative expression in shPLK1-treated samples compared to NTC-treated samples at the same time point, corrected for protein loading. Loading controls were GAPDH (REH) or ββ-actin expression. Brightness and contrast of images was optimized and dotted lines indicate where separated samples from the same gel were grouped. Sh, shPLK1; C: NTC. B. Effect of PLK1 knockdown on cell proliferation. Cell counts of shPLK1-treated cells are expressed in percentage of NTC-treated samples 48, 72 and 96 h after transduction. JURKAT shows the average and standard deviation of 2 independent experiments.

cell lines and influence the strength of shRNA-mediated effects.

We have previously shown that AURKB protein expression is up-regulated in pediatric ALL and correlates with sensitivity to an AURKB inhibitor.[31] It has been shown that PLK1 is required for activation of AURKB and *vice versa*,[41] but this does not explain the correlation between PLK1 and AURKB protein expression that we observed in ALL patients. Rather than a direct connection between the two, the cross-talk between PLK1 and AURKB and their shared functions in mitotic processes make it more likely that there is a common factor regulating the expression of PLK1, AURKB and possibly other proteins involved in the same pathway. The fact that both PLK1 and AURKB seem to be more highly expressed in ALL patients with a TCF3 translocation than in patients with other subtypes of ALL could suggest a role for TCF3 fusion products (transcription factors involved in maturation and proliferation) in the regulation of expression of these proteins. Combination of PLK1 inhibitor volasertib (BI 6727) and pan-Aurora kinase inhibitor AT9283 was shown to synergistically induce cell death in leukemia cell lines.[42] Taken together, combined PLK1/AURKB-targeted therapy may, therefore, be of benefit to TCF3-rearranged ALL patients as well as to other patients with a high expression of both proteins. This is an issue worth addressing in future studies.

High PLK1 expression has been associated with an unfavorable prognosis in a number of solid tumors, including non-small cell lung cancer, esophageal carcinoma, melanoma, colon carcinoma, gastric carcinoma, diffuse large B-cell lymphoma, neuroblastoma and bladder cancer.[13–20] Whether PLK1 expression has prognostic value in acute leukemia has not been reported. Here, we found that high Thr210 phosphorylation, but not total PLK1 protein level, was associated with a lower event-free survival and showed a trend towards being an independent adverse prognostic factor in pediatric ALL. This trend may be confirmed in a larger patient cohort. CIR analysis did not reveal a significantly increased incidence of relapse,

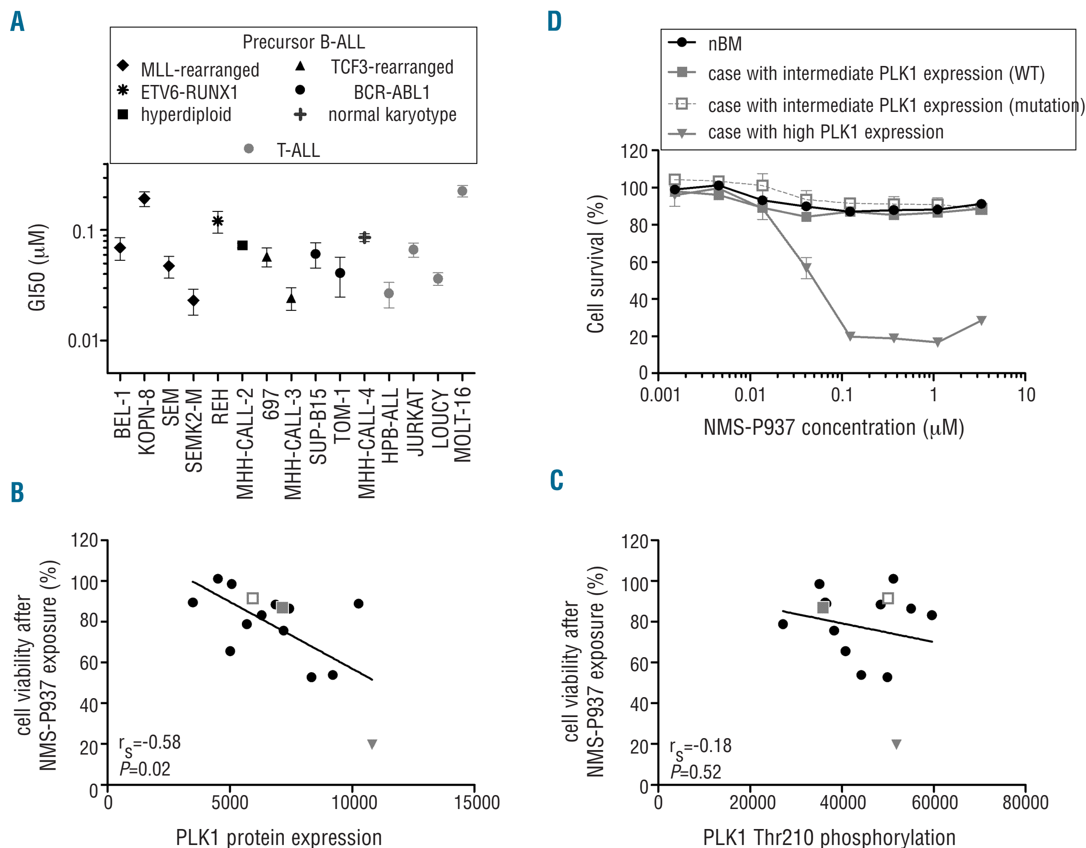

Figure 4. ALL patients with a high PLK1 expression are more sensitive to NMS-P937. ALL cell lines and primary samples were exposed to NMS-P937 and the effect on cell viability was determined with an MTS assay. A) GI50 values for ALL cell lines after 72 h of exposure to a concentration range of NMS-P937. Data presents the mean and standard error of the mean of 3 independent experiments. (A, B, C) Primary ALL samples (n=15) were exposed to 0.12 μM NMS-P937 and cell viability was determined after 96 h. Cell survival is expressed in percentage of untreated control cells. Graphs present correlation plots of cell survival and PLK1 protein expression (B) or Thr210 phosphorylation level (C), expressed in normalized fluorescence units. Cases indicated with a gray symbol correspond with those presented in Figure 4D. Diagonal lines represent the best fit. The correlation coefficient and its significance were determined by the non-parametric Spearman's rank (r_s) test. (D). Comparison of survival curves of nBM mononuclear cells, a wild-type (WT) and a PLK1-mutated (Ser335Arg) ALL case with intermediate PLK1 expression, and an ALL case with high PLK1 expression, after exposure to a concentration range of NMS-P937 for 96 h. Survival was calculated by comparing cell viability to untreated control cells.

indicating that high PLK1 Thr210 phosphorylation may be especially associated with leukemia-related death and toxicity of treatment. Since we also observed that primary leukemic cells with a high PLK1 expression are more sensitive to NMS-P937, the use of PLK1 inhibitors in pediatric ALL is of clinical interest.

NMS-P937 is an ATP-competitive compound that binds the ATP-pocket of PLK1, not including the Thr210 phosphorylation site.[25] The inhibitory effect of the compound is unlinked to the Thr210 phosphorylation level of PLK1, as demonstrated by the initial increase of Thr210 phosphorylation levels in response to NMS-P937[43] and our observation that PLK1 phosphorylation levels, in contrast to total PLK1 protein levels, are not correlated to sensitivity to NMS-P937. In line with the effective concentrations found in this study, Valsasina *et al*. showed that NMS-P937 inhibited proliferation of a range of solid tumor and leukemia cell lines at mid-nanomolar concentrations.[43] PLK1/PLK3 inhibitor GW843682X (also called compound 1 or tiophene 1)[44] was proven to be toxic to several high-risk pediatric cancer cell lines including acute leukemia. Interestingly, these studies also suggest that traditional leukemic drugs combined with a PLK1 inhibitor may enhance current treatment results. Combination therapy of cytarabine and NMS-P937 markedly increased survival of mice first injected with primary AML cells.[24,43] Also, a combination of vincristine, which was shown to increase PLK1 protein levels in an AML cell line, and GW843682X synergistically inhibited growth and induced apoptosis in leukemia cells *in vitro*.[22] These findings encourage further exploration of the efficacy of both PLK1-targeting monotherapy and combination therapy in a clinical setting.

Another issue that should be addressed in clinical trials is the potential toxicity of PLK1-targeting drugs to normal proliferating cells, since PLK1 is also required for normal cell division.[3] To diminish side-effects, a PLK1 inhibitor should preferentially be more specific for leukemic cells than their normal counterparts. We observed that primary ALL cases were indeed more sensitive to PLK1 inhibition by NMS-P937 than nBM cells. Renner *et al*.[23] showed that PLK1-targeting small interfering RNAs (siRNAs) and inhibitor BI 2635 did not affect the clonogenic potential of normal CD34$^+$ cells as much as primary AML cells. PLK1 silencing also markedly inhibited growth of several types of cancer cells, especially p53-deficient types, while barely affecting the growth of normal cells like human umbilical vein endothelial cells, fibroblasts and keratinocytes.[35,43,45] In addition, PLK1-depleted mouse models showed that even very low remaining levels of PLK1 were sufficient to sustain normal hematopoiesis, spermatogenesis and normal cell proliferation.[35] These are all indications that cancer cells may be more dependent on PLK1 expression and more sensitive to PLK1 inhibition than normal cells, offering a therapeutic window for PLK1-targeting drugs.

Phase I clinical trials with PLK1 inhibitors in adult malignancies have achieved promising results. BI 2536,[46] BI 6727[47] and GSK461364[48] showed antitumor activity and stabilized disease in a subset of patients with advanced solid tumors, with manageable and reversible toxicity. Based on our results, it is plausible that PLK1-targeting drugs may also have therapeutic potential in childhood ALL. Due to its high specificity for PLK1, NMS-P937 may be preferred over less specific compounds like GW843682X, BI 2536 and BI 6727, which are known to also target PLK2/3 and other kinases.[44,49,50]

A phase I trial with NMS-P937 in adults with advanced solid tumors has recently been completed (*clinicaltrials.gov identifier: NCT01014429*).

Our findings, as well as those of Ikezoe *et al*., indicating potential for PLK1 inhibition in generally poor prognostic adult ALL,[22] could be an incentive for the initiation of clinical trials with NMS-P937 in childhood and adult ALL.

In conclusion, this study has identified PLK1 as a possible therapeutic target in the treatment of children with ALL, especially cases with a high PLK1 expression, and supports the clinical evaluation of PLK1-targeting drug efficacy in these patients.

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Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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