Original paper

PARP-1 expression in CD34+ leukemic cells in childhood acute lymphoblastic leukemia: relation to response to initial therapy and other prognostic factors

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Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear protein that impacts DNA repair and apoptosis. Both experimental and ongoing clinical studies indicate that PARP-1 inhibitors are potent and promising anticancer agents. However, the outcome of treatment with PARP-1 inhibitors depends on the expression of PARP-1 protein in the tumor cells. This study aimed to assess PARP-1 expression in peripheral blood CD34+ leukemic cells before and after 12 hours of prednisone administration as well as the relation between PARP-1 expression and early treatment response to initial therapy and other prognostic factors (immunophenotype, age, initial peripheral blood white blood count [WBC], and risk factor group).
The study comprised 43 children with *de novo* ALL. Cytospins of peripheral blood were stained with mouse anti-CD34-FITC and anti-PARP-1 antibody followed by goat anti-mouse APC-conjugated antibody. DNA was counterstained with PI (propidium iodide). Cellular fluorescence was measured by a laser scanning cytometer.
Statistically significant differences in baseline PARP-1 expression with respect to early treatment response (good vs. poor), ALL immunophenotype (ALL B vs. ALL T), age (children $<$ 1 years and $>$ 6 years vs. children 1-6 years), initial WBC ($<$ 20 000/μl vs. $\geq$ 20 000/μl), and risk factor group (SR vs. IR vs. HR) were not found. PARP-1 expression was increased 12 hours after treatment in poor early treatment responders, whereas it remained statistically unchanged with respect to ALL immunophenotype, age, initial WBC, risk factor group and early treatment response. The overexpression of PARP-1 in poor early treatment responders suggests that it may contribute to treatment failure in this group of children with ALL. Our observation – if confirmed by other studies – may form the rationale for administration of PARP inhibitors in selected subsets of ALL children.

Key words: poly(ADP-ribose) polymerase-1, PARP-1, poly(ADP-ribose) polymerase-1 inhibitors, childhood acute lymphoblastic leukemia (ALL), early treatment response, prognostic factors.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common cancer in children [1, 2]. Despite the undeniable progress in ALL treatment, still 20-30% of patients die from relapse or side effects of chemotherapy [3, 4]. Commonly accepted prognostic factors, such as immunophenotype of leukemic cells, age, initial peripheral blood white blood cell count (WBC), chromosomal abnormalities, early treatment response as well as minimal residual disease (MRD) are not entirely sufficient to stratify patients into appropriate

risk groups [5, 6, 7]. Therefore, it seems that further improvement of the ALL treatment results is strongly associated with the necessity to include in the current prognostic classification factors related to individual sensitivity and resistance to cytotoxic drugs [8].

One of the mechanisms of drug resistance is the lack of balance between DNA repair and induction of apoptosis. Poly(ADP-ribose) polymerase-1 (PARP-1) encoded by the *PARP-1* gene is a 113 kDa nuclear protein essential for repair of strand DNA breaks caused by chemotherapy or radiotherapy. In response to DNA damage PARP-1 catalyzes the formation of long chains of poly(ADP-ribose) (PAR) that are covalently attached to PARP-1 itself (automodification) or other nuclear protein involved in DNA repair, e.g. histone H1, H2B, p53, and HMG proteins (heteromodification) [9, 10]. If the extent of DNA damage exceeds the repair capability, PARP-1 promotes apoptosis. Apoptosis-inducing factor (AIF) is the main effector of PARP-1-induced apoptosis being released by the excessive accumulation of PAR polymers in damaged cells [9]. The baseline activity of PARP-1 is low, but it is estimated that the DNA-damaging agents induce almost a 50-100-fold increase of *PARP-1* gene expression [9]. PARP-1 expression has been found in most of the normal human tissues including stem cells and various immune cells as well as in several cell lines such as Jurkat T-cell leukemia, K562 myeloid leukemia and the CCRF-CEM lymphoblastic leukemia cell line [11].

Clinical studies indicate that the level of PARP expression in tumor cells is related to outcome of treatment [10, 12, 13]. PARP inhibitors (PARPi) are evaluated in clinical trials as a part of anticancer therapy in various tumors: breast cancer, ovarian and fallopian tube cancer, prostate cancer, liver, pancreas and colorectal cancer, glioblastoma multiforme, lung cancer, multiple myeloma, and chronic lymphocytic leukemia [13, 14].

To the best of our knowledge, PARP-1 expression in clinical samples of childhood ALL is not fully assessed and has been mostly limited to cell line analysis [15, 16]. It has been reported that expression of PARP is decreased in children with drug-resistant ALL, but time-resolved changes have not been evaluated [15]. Therefore, the aim of this study was to reveal changes in PARP-1 expression in peripheral blood CD34+ leukemic cells collected before and after 12 hours of prednisone administration as well as the relation of PARP-1 expression and prognostic factors such as early response to treatment, immunophenotype of leukemic cells, age, WBC at diagnosis, and risk factor group.

Table I. Clinical characteristics of patients

Parameter	No. of patients (n = 43)
Age:	
< 1 and > 6 years	20
1-6 years	23
Gender:	
M	22
F	21
Risk group:	
standard risk (SR)	12
intermediate risk (IR)	27
high risk (HR)	4
WBC at diagnosis:	
< 20 000 cells/μl	14
≥ 20 000 cells/μl	29
Immunophenotype:	
ALL B	34
ALL T	9
Early response to treatment:	
good	36
poor	7

Material and methods

Patients

The study comprised 43 children (22 boys, 21 girls) aged 15-214 months (mean 194 months; median 57 months) with *de novo* ALL diagnosed between December 2004 and March 2012 at the Department of Pediatrics, Hematology and Oncology, Pomeranian Medical University, Szczecin. The diagnosis was based on morphologic examination and flow cytometry immunophenotyping of bone marrow cells. Leukemic cells in all studied cases reveal CD34 expression. The characteristics of patients are shown in Table I.

All children were treated according to one of the consecutive BFM protocols: ALL IC BFM 2002 or ALL IC BFM 2009. The initial therapy was the same for all children and consisted of oral administration of prednisone 60 mg/m^2/day in three divided doses plus one age-related dose of intrathecal methotrexate on the first day of treatment (6 mg < 1 years, 8 mg 1-2 years, 10 mg 2-3 years, 12 mg > 3 years). On day 1 of treatment three consecutive doses of prednisone were given at 6-hour intervals. Based on two criteria, i.e. prednisone response (as defined by the BFM group) and bone marrow leukemic cell rate on days 15 and 33, the patients were classified as good early

treatment responders (i.e. peripheral blood leukemic cell count < 1000/μl on day 8 and < 5% leukemic cells in bone marrow on days 15 and 33; n = 36) and poor early responders (peripheral blood leukemic cell count ≥ 1000/μl on day 8 and ≥ 5% leukemic cells in bone marrow on day 15 and/or 33; n = 7). Children aged less than 6 years and more than 1 year with an initial WBC count less than 20 000/μl, with no t(9;22) or t(4;11) translocation and with a good early treatment response were classified as standard risk patients. In turn, children regardless of age, with the presence of t(9;22) or t(4;11) translocation or with a poor early treatment response, were classified as high risk patients. The remaining children were included in the intermediate risk group. The follow-up time was 2-112 months (mean 57.9 months, median 58.5 months).

The study was approved by the Bioethics Committee of the Pomeranian Medical University.

Preparation of peripheral blood cells for analysis

Five ml of peripheral blood was sampled before prednisone administration and 12 hours after the first dose of prednisone into sterile tubes containing 50 μl of heparin (Polfa Warszawa, Poland). The blood sample was mixed with an equal volume of PBS (phosphate buffered saline), pH 7.4, without Ca or Mg (Biomed, Lublin, Poland). The mixture was then placed on Ficoll (Gradisol L, Aqua-Media, Łódź, Poland) followed by centrifugation at 2200 rpm for 25 minutes (Eppendorf Centrifuge 5403, Germany). The mononuclear cell-containing layer was collected and rinsed twice in PBS each time at 1200 rpm for 5 minutes. The cell pellet was re-suspended in 1 ml PBS, fixed and stored in 80% ethanol at –20°C (1 : 10). To deposit cells on glass slides (Knittel manual/Glass), a cell suspension volume of 500 ml was centrifuged twice at 500 rpm for 5 minutes (Rotina 38, Hettich, Germany). Subsequently cells were permeabilized by immersing them in 1% paraformaldehyde solution in PBS at 4°C for 15 minutes.

Immunofluorescence

For simultaneous detection of CD34 and PARP-1 in leukemic cells, slides were rinsed in PBS twice, each time for 5 minutes, then 100 μl of 1 : 50 diluted monoclonal mouse anti-CD34 antibody conjugated with FITC (Santa Cruz Biotechnology, # sc-7324) and 100 μl of 1:50 diluted monoclonal mouse anti-PARP-1 antibody (Santa Cruz Biotechnology, # sc-8007) were added and the slides were incubated for 1 hour at room temperature (RT). Then the slides were rinsed in PBS for 5 minutes and 100 μl of 1 : 100 diluted goat anti-mouse IgG antibody conjugated with APC (Santa Cruz Biotechnology, # sc-3818) was added. The slides were incubated for 1 hour at RT, rinsed in PBS twice for 5 minutes, and then incubated for 30 minutes at RT with 100 μl of propidium iodide (PI; # P4170, Sigma Aldrich) in a concentration of 10 μg/ml in the presence of ribonuclease A (Ribonuclease A type I-AF, # R5503, Sigma Aldrich) in a concentration of 100 μg/ml. During all incubations cells were deposited in a humid chamber and protected from light. Slides prepared from the same blood sample and stained according to the described protocol but without primary antibodies served as negative controls.

Fluorescence measurements

Slides were mounted with coverslips and PARP-1-associated APC long red fluorescence (emission maximum 660 nm), CD34-associated FITC green fluorescence (emission maximum 520 nm) and DNA-associated PI red fluorescence (emission maximum 580 nm) excited by 633 nm helium-neon laser and 466 nm argon laser, respectively, were measured simultaneously by a laser scanning cytometer (LSC, CompuCyte Corporation, USA). DNA-associated PI red fluorescence was used as a contouring parameter (threshold contour).

Values of integrated PARP-1-associated APC long red fluorescence and CD34-associated FITC green fluorescence were measured over the whole leukemic cell and recorded as FCS 3.0 files by WinCyte 3.4 software.

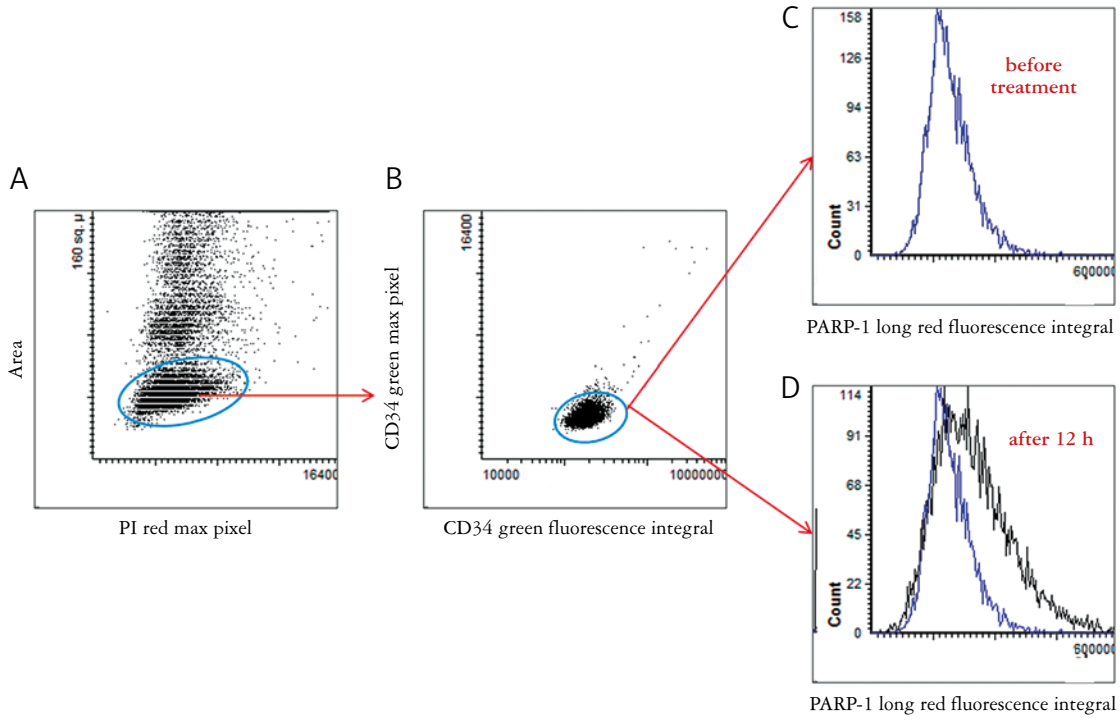
To measure integrated PARP-1-associated APC long red fluorescence and CD34-associated FITC green fluorescence over the whole cell, the integration contour was set at +4 pixels from the threshold, whereas peripheral inner and outer contours were introduced at +4 and +8 pixels from the threshold contour. The integrated fluorescence was calculated by the software as the sum of fluorescence of all pixels within the integration fluorescence contour. Integrated fluorescence was assessed in arbitrary units as a mean value. Background fluorescence was automatically subtracted by the software. In each slide at least 1000 cells were measured. Principles of evaluating PARP-1-associated APC long red fluorescence and CD34-associated FITC green fluorescence are presented in Fig. 1.

Statistical analysis

PARP expression was assessed as a mean ±SD of the APC-associated long red fluorescence integral. The Wilcoxon test, Mann-Whitney U test and ANOVA Kruskal-Wallis test were used for statistical analysis. P-values equal to or less than 0.05 were considered as statistically significant.

Results

PARP expression in CD34+ cells was found in all studied cases and ranged from 105 198 to 1 014

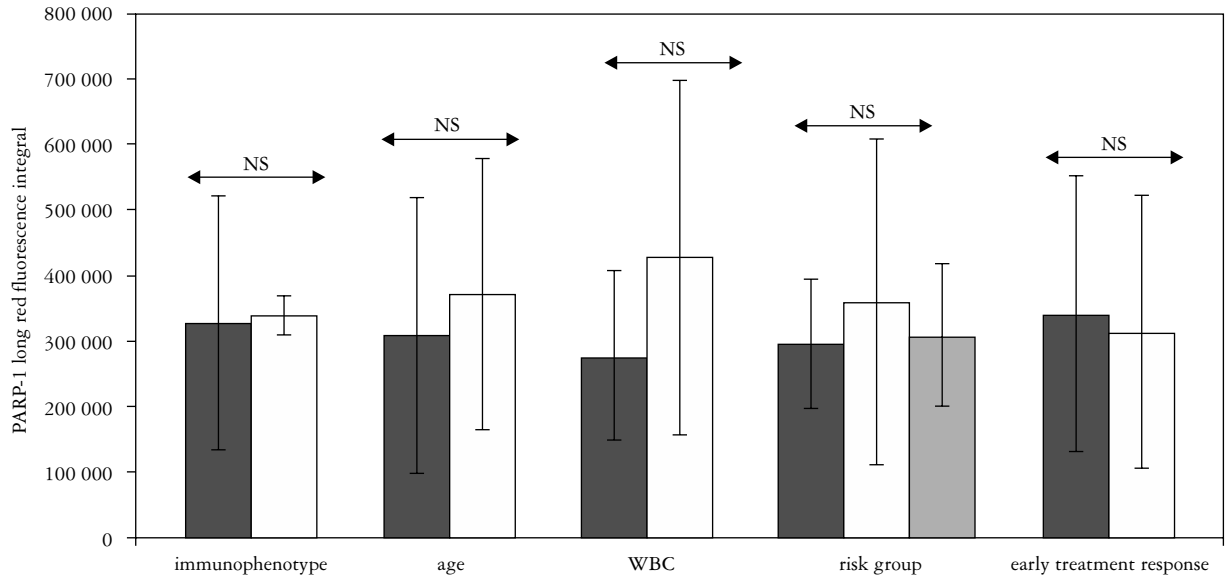


Based on two parameters, DNA-associated PI red fluorescence maximal pixel and cell area cells were selected for further analysis (dot-plot A). PARP-1 expression was assessed in peripheral blood mononuclear CD34 positive leukemic cells (dot-plot B). Distribution and time resolved changes (before and 12 hours after treatment) of PARP-1 long red fluorescence in a given patient are presented in histograms C and D respectively.

Fig. 1. Principles of measurements of PARP-1 expression in childhood ALL

653 arbitrary units (mean 336 393 ±207732 before treatment and 352 993 ±236190 after 12 hours of treatment). There were no statistically significant differences of baseline mean values of PARP-1 long red fluorescence with respect to early treatment response (good vs. poor), ALL immunophenotype (ALL B vs. ALL T), age (children < 1 years and > 6 years vs. children 1-6 years), initial WBC (< 20 000/μl vs. ≥ 20 000/μl), or risk factor group (SR vs. IR vs. HR) (Fig. 2).

Statistically significant differences between mean values of PARP-1 long red fluorescence measured be-

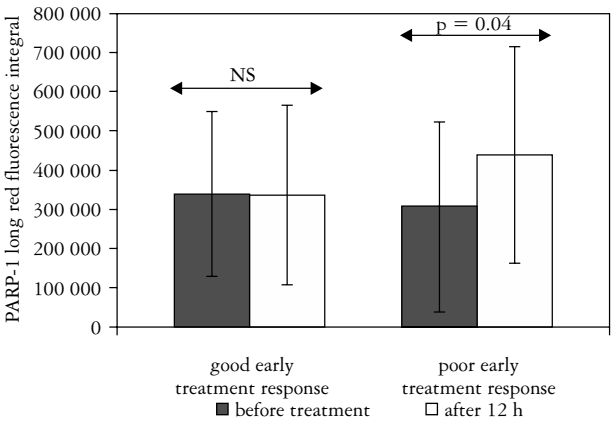


PARP-1 long red fluorescence integral measured in peripheral blood CD34+ leukemic cells before treatment (mean ±SD) with respect to ALL immunophenotype, age, initial WBC, risk factor group, and early treatment response.
Dark grey bars represent: ALL B, age < 1 years and > 6 years, WBC < 20 000/μl, standard risk group, good early treatment response; white bars represent: ALL T, age 1-6 years, WBC ≥ 20 000/μl, intermediate risk group, poor early treatment response; medium grey bar represents high risk group).

Fig. 2. PARP-1 expression in childhood ALL with respect to risk factors measured before treatment

fore prednisone administration compared to 12 hours after the first dose of prednisone with respect to ALL immunophenotype, initial WBC, age, group risk, and good early treatment response were not found (data not shown).

In the group of poor early treatment responders, a statistically significant rise of PARP-1 long red fluorescence was observed 12 hours after prednisone administration (312 599 vs. 438 340; p = 0.04), whereas it remained unchanged at the same time point in the group of good early treatment responders (Fig. 3).



Changes in PARP-1 long red fluorescence integral measured in peripheral blood mononuclear CD34+ leukemic cells before treatment and 12 hours after treatment in the group of good and poor early treatment responders (mean +/– SD).

Fig. 3. Changes in PARP-1 expression in the group of good and poor early treatment responders in childhood ALL

Discussion

PARP-1 is overexpressed in a variety of human cancers, and its expression has been associated with poor overall prognosis, especially in breast cancer [17]. Studies evaluating expression of PARP-1 protein in clinical samples of leukemia are limited and mainly restricted to acute leukemia in adults [8].

Our study revealed that the expression of PARP-1 in peripheral blood CD34 positive leukemic cells was present in all children diagnosed with ALL with high variation of data values. Baseline mean values of PARP-1-long red fluorescence with respect to early treatment response, ALL immunophenotype, age, initial WBC, and risk factor group were not statistically significant. We compared our results with the work of Holleman *et al*. and Pournazari *et al*., since PARP expression in clinical samples of childhood ALL was reported in these two papers only [8, 18]. Pornazari *et al*. reported that PARP-1 was differentially expressed, ranging from no to a very high degree of expression. PARP-1 protein was overexpressed in about two-thirds (61%) of patients with B-ALL [18]. The PARP-1 protein showed no correlation with specific chromosomal abnormalities associated with prognosis in B-ALL, as defined by the WHO [18]. Holleman *et al*. reported that PARP expression was present in all B-lineage samples, but absent in 4 of 15 T-lineage ALL samples [8]. They found that blasts of T-cell ALL had 7-fold decreased baseline expression of PARP-1 compared to blasts of B-cell ALL in children, which in their opinion was associated with increased drug resistance in T-cell ALL [8]. Our results do not confirm these observations, since a statistically significant difference in the baseline expression of PARP-1 between T and B cell ALL was not found. Differences in the percentage of positive ALL cases may stem from the fact that different methods, i.e. flow cytometry, immunocytochemistry or laser scanning cytometry, were used to quantify the expression of PARP-1 protein.

Prednisone alone is a powerful antileukemic compound. It has been implemented in almost all treatment protocols for children with ALL. In experimental as well as clinical settings it induces apoptosis of leukemic cells [19]. The selection of the blood sampling time points before treatment and at 12 hours after treatment was based on the assumptions that 1) peak plasma level of prednisolone is noted 3-4 hours after oral drug administration, and 2) in experimental models, DNA strand breaks and/or p89 PARP fragments were detected between 1-2 hours and 36 hours of incubation with a compound used to induce apoptosis [19]. Statistically significant differences between mean values of PARP-1 long red fluorescence measured before prednisone administration compared to 12 hours after the first dose of prednisone dose with respect to ALL immunophenotype, initial WBC, age, group risk, and good early treatment response were not found. However, in the group of poor early treatment responders, a statistically significant rise of PARP-1 long red fluorescence was observed 12 hours after prednisone administration, whereas it remained unchanged at the same time point in the group of good early treatment responders. These results may suggest that in children with a poor early response to treatment, increased expression of PARP-1 in leukemic cells within the first 12 hours of prednisone treatment is associated with activation of DNA repair and inhibition of apoptosis. Both pathways are most likely to play a role in *in vitro* resistance to prednisone in childhood ALL.

Resistance of leukemic cells to chemotherapeutic agents is associated with an unfavorable outcome in pediatric ALL [15]. Other studies reported that depletion of PARP, either by gene disruption, antisense RNA, or pharmacologic inhibitors, resulted in decreased drug-induced apoptosis [8]. Various explanations have been proposed for the requirement of PARP during apoptosis, including depletion of cellular nicotinamide-adenine nucleotide (NAD)+ and adenosine triphosphate (ATP) pools, modifica-

tion of proteins involved in apoptosis such as p53, facilitation of oligonucleosomal DNA fragmentation, and up-regulation of P-glycoprotein in PARP-depleted cells [8]. In another study the same authors suggested that caspase-3 activation and PARP inactivation may play a role in prednisolone- and l-asparaginase-induced apoptosis, but are not essential to vincristine- and daunorubicin-induced apoptosis [15]. Kannan *et al.* reported that Notch 1-related transcription factors stimulate higher expression of PARP-1 messenger RNA and thus induce apoptosis in B-ALL cell lines, an effect not seen in T-ALL cell lines [20]. Pottier *et al.* stated that lower SMARCB1 expression increased prednisolone resistance in childhood ALL. SMARCB1 is a core member of the SWI/SNF complex, which promotes glucocorticoid effects through nucleosome remodeling [16]. Chemotherapy-induced overexpression of PARP-1 in children with a poor early response to treatment may serve as evidence for an association between PARP-1 expression and resistance to prednisone, and hence poor response to treatment.

PARP inhibitors, namely olaparib, niraparib and veliparib, have been evaluated in clinical trials either as single agents or in combination with DNA-damaging therapies [21, 22, 23, 24]. These agents have raised considerable interest because of their potential clinical activity for patients whose tumors have defects in the homologous recombination (HR) pathway as in patients with *BRCA1* mutation [17]. PARP inhibitors imitate the nicotinamide moiety of nicotinamide adenine dinucleotide, and bind to the enzyme's catalytic domain, inhibiting automodification and subsequent release of the enzyme from the site of DNA damage. By doing so, PARP inhibitors also prevent access of other repair proteins to the site of DNA cleavage [17]. Initial evidence suggests that low PARP levels (and activity) reduce the response to PARP inhibitors. Decreased PARP protein might be selectively helpful to resist the "poisoning" activity of drug-induced DNA-PARP aggregates. This hypothesis is in agreement with results from insertional mutagenesis screens that identify PARP-1-depleted cells as up to 100-fold more resistant to olaparib as compared with wild type cells. Therefore, PARP-1-depleted cells may experience loss of cell viability (through SSB repair inhibition) but also resistance to PARP inhibition (through preclusion of PARP-trapping cytotoxic effect) [23].

Wang *et al*. reported that RUNX1, RUNX2 double knockout mice had impairment in DNA repair. The *RUNX1* gene is one of the most frequently mutated in human leukemia [25]. RUNX proteins play a critical role in the FANCD2 response and recruitment following DNA damage. Loss and dysfunction of RUNX proteins leads to DNA repair defects. The authors treated human HEK293T, HCT116, and HeLa-S3 cell lines with dysfunctional RUNX1 and RUNX3 with PARP inhibitors to observe the effect of synthetic lethality.

The cells were sensitive to treatment with PARP inhibitors, and the response to treatment was better if the PARP inhibitor was combined with the chemotherapy agent mitomycin c. This shows that the synthetic lethal effects of PARP inhibition and mitomycin c exposure could potentially be considered as a new treatment option for targeting RUNX-associated leukemia [25].

We conclude that the overexpression of PARP-1 in poor early treatment responders suggests that it may contribute to treatment failure in this group of children with ALL. Our observation – if confirmed by other studies – may form the rationale for administration of PARP inhibitors in selected subsets of ALL children.

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The authors declare no conflict of interest.

References

1. Matysiak M. Childhood leukemias [Article in Polish]. Pediatria po Dyplomie 2014; 18: 23-26.
2. Belson M, Kingsley B, Holmes A. Risk factors for acute leukemia in children: A review. Environ Health Perspect 2007; 115: 138-45.
3. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukemia. Lancet 2013; 381: 1943-1955.
4. Hunger SP, Lu X, Devidas M, et al. Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: A report from the Children's Oncology Group. J Clin Oncol 2012; 30: 1663-1669.
5. Jeha S, Pui CH. Risk-adapted treatment of pediatric acute lymphoblastic leukemia. Hematol Oncol Clin North Am 2009; 23: 973-990.
6. Schrappe M. Prognostic factors in childhood acute lymphoblastic leukemia. Indian J Pediatr 2003; 70: 817-824.
7. Friedmann AM, Weinstein HJ. The role of prognostic features in the treatment of childhood acute lymphoblastic leukemia. Oncologist 2000; 5: 321-328.
8. Holleman A, den Boer ML, Kazemier KM, et al. Resistance to different classes of drugs is associated with impaired apoptosis in childhood acute lymphoblastic leukemia. Blood 2003; 102: 4541-4546.
9. Kiliańska ZM, Żołnierczyk J, Węgierska-Gądek J. Biological activity of poly(ADP-ribose)polymerase-1[Article in Polish]. Postepy Hig Med Dosw (Online) 2010; 64: 344-363.
10. Javle M, Curtin NJ. The potential for poly(ADP-ribose) polymerase inhibitors in cancer therapy. Ther Adv Med Oncol 2011; 3: 257-267.
11. Gaymes TJ, Shall S, MacPherson LJ, et al. Inhibitors of poly ADP-ribose polymerase (PARP) induce apoptosis of myeloid leukemic cells: potential for therapy of myeloid leukemia and myelodysplastic syndromes. Haematologica 2009; 94: 638-646.
12. Nosho K, Yamamoto H, Miakmi M, et al. Overexpression of poly(ADP-ribose polymerase-1 (PARP-1) in the early stage of colorectal carcinogenesis. Eur J Cancer 2006; 42: 2374-2381.
13. Domagała P, Huzarski T, Lubiński J, et al. PARP-1 expression in breast cancer including BRCA1-associated, triple negative

and basal-like tumors: possible implications for PARP-1 inhibitor therapy. Breast Cancer Res Treat 2011; 127: 861-869.
14. https://clinicaltrials.gov/ct2/results?term=Parp+inhibitor&-Search=Search
15. Holleman A, den Boer ML, Kazemier KM, et al. Decreased PARP and procaspase-2 protein levels are associated with cellular drug resistance in childhood acute lymphoblastic leukemia. Blood 2005; 106: 1817-1812.
16. Pottier N, Cheok MH, Yang W, et al. Expression of SMARCB1 modulates steroid sensitivity in human lymphoblastoid cells: identification of a promoter snp that alters PARP1binding and SMARCB1 expression. Hum Mol Genet 2007; 16: 2261-2271.
17. Rojo F, García-Parra J, Zazo S, et al. Nuclear PARP-1 protein overexpression is associated with poor overall survival in early breast cancer. Ann Oncol 2012; 23: 1156-1164.
18. Pournazari P, Padmore RF, Kosari F, et al. B-lymphoblastic leukemia/lymphoma: overexpression of nuclear DNA repair protein PARP-1 correlates with antiapoptotic protein Bcl-2 and complex chromosomal abnormalities. Hum Pathol 2014; 45: 1582-1587.
19. Urasiński T, Urasińska E, Grabarek J, et al. Good early treatment response in childhood acute lymphoblastic leukemia is associated with Bax nuclear accumulation and PARP cleavage. Med Sci Monit 2009; 15: CR294-CR301.
20. Kannan S, Sutphin RM, Hall MG, et al. Notch activation inhibits AML growth and survival: a potential therapeutic approach. J Exp Med 2013; 210: 321-337.
21. Kummar S, Chen A, Parchment RE, et al. Advances in using PARP inhibitors to treat cancer. BMC Med 2012; 10: 25.
22. Underhill C, Toulmonde M, Bonnefoi H. A review of PARP inhibitors: from bench to bedside. Ann Oncol 2011; 22: 268-279.
23. Lupo B, Trusolino L. Inhibition of poly(ADP-ribosyl)ation in cancer: old and new paradigms revisited. Biochim Biophys Acta 2014; 1846: 201-215.
24. Benafif S, Hall M. An update on PARP inhibitors for the treatment of cancer. Onco Targets Ther 2015; 8: 519-528.
25. Wang CQ, Krishnan V, Tay LS, et al. Disruption of Runx1 and Runx3 leads to bone marrow failure and leukemia predisposition due to transcriptional and DNA repair defects. Cell Rep 2014; 8: 767-782.

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