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REVIEW PAPER

Doxorubicin-induced cell cycle arrest and apoptosis in HL-60 leukemia cells – a narrative review

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ABSTRACT

Introduction and aim. Doxorubicin is an anthracycline widely used in cancer therapy. Although the complete mechanism of action of doxorubicin is not fully understood, it is known to effectively destroy cancer cells by intercalating into DNA. This article aims to evaluate the anticancer effect of doxorubicin with particular emphasis on HL-60 leukemia cells and cell cycle/apoptosis.

Material and methods. This narrative review contains a collection and evaluation of peer-reviewed publications on the anticancer properties and therapeutic applications of doxorubicin. It focuses on doxorubicin-induced cell cycle arrest and apoptosis in HL-60 leukemia cells, with particular emphasis on toxicity and resistance. A literature review was conducted using the following databases: PubMed and Google Scholar.

Analysis of the literature. Doxorubicin exhibits potent anticancer activity against many cancer types. Unfortunately, due to serious side effects, primarily related to the cardiotoxicity of the drug, its potential cannot be fully exploited**.**

Conclusion. In recent years, many approaches have been developed to circumvent the limitations of doxorubicin. Identifying all the pathways of doxorubicin action is important for planning combination therapy, mitigating side effects, and developing new drugs. However, further clinical studies are needed to determine their long-term safety and efficacy.

Keywords. cancer, cell line, doxorubicin, HL-60 cells, leukemia

Introduction

HL-60 cells are a well-established model of human promyelocytic leukemia, widely used to study mechanisms of cell cycle control, differentiation, and apoptosis. Doxorubicin is one of the key chemotherapeutic agents used in the treatment of hematologic cancers. Together, this model allows researchers to investigate how leukemia cells respond to cytotoxic treatment, explore mechanisms of sensitivity and resistance, and identify pathways that may be targeted to improve therapeutic outcomes.

Characteristics of acute promyelocytic leukemia

The term leukemia refers to neoplastic diseases of the hematopoietic system characterized by abnormal proliferation, impaired maturation, and the release of white blood cells from the bone marrow and other tissues. These diseases are accompanied by organ infiltration and the presence of immature cell forms in the peripheral blood. Leukemia is divided into acute and chronic forms. In acute leukemia, it is mainly immature blast cells that have lost their ability to differentiate that proliferate.[1]

Acute promyelocytic leukemia is an aggressive subtype of acute myeloid leukemia. With a characteristic clinical course. It was first identified and described in 1957. Its characteristic feature is a translocation between chromosomes 15 and 17, resulting in the formation of the PML-RARA fusion protein (a fusion of the promyelocyt-

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ic leukemia (PML) gene and the retinoic acid receptor α (RARα)), which disrupts normal cell differentiation.[2]

Apart from this form of translocation (t(15;17)(q22;q21)-PML-RARA), there are other less common variants of translocation, but always involving the RARA gene. In addition to chromosomal translocation, there is an accumulation of abnormal promyelocytes in the blood and bone marrow, as well as coagulation disorders with features of disseminated intravascular coagulation and a tendency to bleed due to hypofibrinogenemia.[2–4]

Of all types of leukemia in adults, acute leukemia accounts for 40% of cases, the vast majority of which are acute myeloid leukemia. According to data from the Adult Acute Leukemia Registry, between 2004 and 2010, an average of over 600 cases per year were reported, mainly in people over 50 years of age.[5]

Treatment of leukemia

The treatment of acute promyelocytic leukemia is a complex process based on targeted therapy. It most often involves administering all-trans retinoic acid (ATRA) or arsenic trioxide (ATO) to patients, often in combination with chemotherapy.[6] High concentrations of ATRA in the cell increase the expression of typical retinoic acid receptors, which displace the PML-RARα protein. ATRA induces the differentiation of cancer cells and also limits their proliferation, as has been demonstrated, among others, in acute promyelocytic leukemia.[7,8] Arsenic trioxide, on the other hand, binds to the PML subunit, leading to degradation of the PML-RARα fusion protein and disrupting cancer cell self-renewal.[8] Therapy based on combining all-trans retinoic acid with anthracycline chemotherapy is a highly effective method of induction treatment for patients with acute promyelocytic leukemia. It allows for complete remission in up to 95% of patients and is associated with a lower recurrence rate than monotherapy.[8]

Doxorubicin as an anticancer drug

Doxorubicin is an anthracycline antibiotic. It was isolated from the bacterium *Streptomyces peucetius*, which was modified to find a substitute for another anthracycline discovered earlier – daunorubicin (also anticancer, but more cardiotoxic). It is a widely used substance in anticancer therapies, primarily for the treatment of leukemia, breast cancer, bladder cancer, lung cancer, and Hodgkin's lymphoma.[9,10] Although the complete mechanism of action of doxorubicin is not fully understood, it is known to intercalate into DNA, causing double-strand breaks and inhibiting nucleic acid biosynthesis (both DNA and RNA). In addition, it inhibits topoisomerase II and (in combination with iron) generates free radicals, which also initiate DNA damage.[10] According to Ye et al., doxorubicin may affect proliferation by proteolytically activating the transcription factor CREB3L1 at the endoplasmic reticulum membrane.[11] CREB3L1 is a protein with one end located in the lumen of the endoplasmic reticulum and the other (terminated with an NH2 group) in the cytoplasm of the cell. Through a ceramide-mediated mechanism, the drug triggers regulated intramembrane proteolysis (RIP). This results in a two-step cleavage of the molecule, allowing the N-terminal domain of the protein to enter the cell nucleus, where it drives the transcription of genes that inhibit cell proliferation.[11,12] Fig. 1. illustrates the molecular mechanisms of action of doxorubicin at the cellular level.

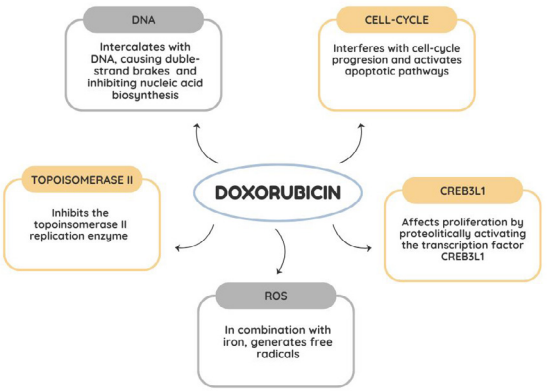

Fig. 1. Mechanisms of doxorubicin action

Aim

The aim of this review is to summarize current knowledge on the effects of doxorubicin on HL-60 leukemia cells, with a focus on its influence on cell cycle regulation, induction of apoptosis, and the key mechanisms that shape therapeutic response and limitations. By integrating findings from recent in vitro studies, the review seeks to highlight the molecular pathways involved and identify areas that may guide future research on improving the efficacy and safety of doxorubicin-based treatments.

Material and methods

This article is a narrative literature review focusing on acute promyelocytic leukemia, the HL-60 cell line, and the biological effects of doxorubicin. A literature search was conducted using the PubMed and Google Scholar databases to identify relevant publications published between 2000 and 2024. The selected time frame was chosen to include both classical studies describing leukemia biology, cell cycle regulation, and apoptosis, as well as more recent research addressing molecular mechanisms of doxorubicin action, multidrug resistance, and treatment-related toxicity. The search strategy involved the use of predefined keywords and their combinations, including "acute promyelocytic leukemia," "HL-60," "cell line," "doxorubicin," "anthracyclines," "apoptosis," "cell cycle," "DNA damage," and "multidrug resistance." Boolean operators (AND/OR) were used to broaden or narrow the search scope. The reference lists of selected articles were manually reviewed to identify additional

relevant publications. Inclusion criteria included original research articles, review articles, reports, and textbook chapters that addressed: (i) the epidemiology, pathogenesis, and molecular characteristics of acute leukemias; (ii) the mechanisms of action of doxorubicin in cancer cells; (iii) in vitro studies using the human promyelocytic leukemia cell line HL-60; and (iv) cellular processes relevant to this review, including apoptosis, cell cycle regulation, responses to DNA damage, and mechanisms of drug resistance. Exclusion criteria included studies unrelated to leukemia or anthracycline-based therapy, publications of no biological relevance to the topic, and conference abstracts. After selection, 37 publications were included in the final narrative review.

Analysis of the literature

Characteristics of the HL-60 cell line

HL-60 is a human leukemia cell line established in 1977 from lymphocytes from the blood of a patient with acute promyelocytic leukemia. It is a suspension cell line with lymphoblastic morphology and a doubling time of up to 48 hours. The cells proliferate continuously and are capable of spontaneous and induced differentiation. HL-60 cells are cultured in RPMI 1640 medium containing 2 mM glutamine and 10-20% FBS. However, the cells can also proliferate in the absence of serum, provided that insulin and transferrin are present.[13,14]

Most HL-60 cells have a variety of surface antigens characteristic of immature myeloid cells. Many karyotypic changes, such as chromosomal translocations and alterations in chromosome number, are observed in HL-60 populations. One of the distinct genetic changes in HL-60 is increased amplification of DNA sequences encoding the *c-myc* gene, which encodes a transcription factor that plays an essential role in regulating the cell cycle, proliferation, apoptosis, and metabolism. Although the amplification magnitude varies between sublines, the relative abundance of c-myc RNA in cells of each subline is proportional to the number of gene copies.[14,15]

HL-60 cells constitute a population arrested at the stage of immature myeloid precursors. However, in each culture, approximately 10% of cells exhibit the characteristics of mature myeloid cells and are therefore capable of spontaneous differentiation. However, some factors can increase the proportion of differentiating cells in the population to as much as 90%. Among the known factors inducing HL-60 differentiation are phorbol 12-myristate 13-acetate (PMA), dimethyl sulfoxide (DMSO), vitamin D3, and retinoic acid.[13,14]

Characteristics of the processes most frequently analyzed in response to anticancer treatment

Cell cycle control systems

The cell cycle is a strictly ordered set of events throughout the life of a cell. Cell division is an integral and crucial part of this cycle. This process maintains the continuity of life in both prokaryotes and eukaryotes. The division of a single cell, during which it passes on its genetic material, allows the formation of new daughter cells that are separate organisms and the development and repair of entire tissue structures in multicellular organisms.[16] For the entire cell cycle to proceed correctly, the events of each phase must be completed before the cell enters the next phase; therefore, mechanisms that regulate the cycle are necessary. This role is performed by cycle checkpoints, the three main ones being located in the G1 phase and at the transition between the G2 and M phases.[16,17] They prevent the progression of mutated or damaged G0 cells (which are in a dormant state). When cell abnormalities are recognized by checkpoints, either the error is repaired or the cell cycle is arrested. The integrity of the cell genome is monitored by the p53 transcription factor, which halts the cell cycle in response to DNA damage. The p53 factor regulates the transcription of the cyclin-dependent kinase inhibitor (CDKI) p21, an active inhibitor of CDK4, 6, and 2, thereby preventing retinoblastoma protein phosphorylation and keeping the cell in G1.[18,19] Another highly sensitive marker of DNA damage in cells caused by ionizing radiation, oxidative stress, or genotoxic stress is histone H2A.X, whose phosphorylated form accumulates at sites of damage.[20]

Cancer proliferation

During the cell cycle, the number of cells increases over time through division, i.e., proliferation. Mammalian cells divide approximately every 24 hours, with the S phase being the longest stage. As mentioned earlier, this process is strictly regulated in healthy cells, but in cancer, it becomes uncontrolled. Although proliferation is not accelerated in every case of cancer, it is always chronic.[21]

In clinical practice, several techniques are used to assess cancer cell proliferation, including methods based on the percentage of cells in the S phase, the mitotic index, and proliferation markers (cyclins, proliferating cell nuclear antigen (PCNA), nuclear protein Ki67). Based on cyclin A expression, it is possible to distinguish DNA-synthesizing cells. Cyclin B is a regulator of the mitotic phase. An increase in the percentage of cells expressing cyclins A, B, and E indicates rapid tumor proliferation. Similarly, the expression of Ki67 and PCNA proteins provides information about the intensity of proliferation in the population.[22]

Apoptotic death

Apoptosis is a strictly controlled, orderly mechanism of programmed cell death. This process is controlled by a series of signaling pathways in the cell, which can be initiated by signals from both inside and outside the cell.

Table 1. Table summarizing key studies on HL-60 cells treated with doxorubicin

Author, year	Doxorubicin concentration	Analyzed markers	Main effect	Detailed effects
Żuryń et al., 2007	0.5–10 µmol/L	cyclin A	dose-dependent increase in apoptosis; altered cyclin A expression and subcellular localization; G2/M cell cycle arrest	1) the expression of cyclin A in HL-60 cells increases in a dose-dependent manner following exposure to doxorubicin 2) higher concentrations of doxorubicin increase the proportion of apoptotic cells and induce the cytoplasmic translocation of cyclin A 3) doxorubicin modulates cyclin A expression, inducing apoptosis and morphological changes
Suzuki et al., 2005	0.5–16 µmol/L	caspase-3, caspase-8, caspase-9	caspase activation and DNA damage	1) doxorubicin exhibits higher cytotoxicity in cancer cell lines than in healthy cells 2) activation of caspases is observed, predominantly caspase-3 3) doxorubicin induces DNA fragmentation in HL-60 cells
Al-Abbas and Shaer, 2021	100 ng/mL (with 100–1000 µg/mL coumarin)	annexin V	induction of apoptosis	1) combined treatment with coumarin and doxorubicin increases apoptotic cell death in HL-60 and HL-60/ADR cells in a coumarin dose-dependent manner

During apoptosis, DNA and organelles are destroyed, and the cell's contents are packaged into vesicles (apoptotic bodies), which are then digested. Apoptosis, as a controlled process of cell destruction, protects normal cells by preventing inflammation in their vicinity.[16] It is also involved in cell replacement in healthy, mature tissues and in the focal elimination of cells during normal embryo development. Its mechanism has been relatively well understood thanks to research on *Caenorhabditis elegans,* where three proteins key to apoptosis have been identified: Ced-3, Ced-4, and Ced-9. Ced-3 and Ced-4 are present in the cell in an inactive form, but when the cell receives a death signal, they activate, triggering a cascade that activates nucleases and other proteases. Ced-9 is a protein in the outer mitochondrial membrane and, in a state where the cell does not receive a programmed death signal, it inhibits apoptosis, while Ced-3 and Ced-4 remain inactive. In mammalian cells, this process is more complex, but proteins very similar to those mentioned above are involved.[16,23]

Apoptosis pathways and signals

There are two interconnected pathways in programmed cell death: one that uses cellular death receptors (the exogenous pathway) and the other that uses mitochondria (the endogenous pathway). The exogenous pathway begins with the binding of a ligand (most often from the tumor necrosis factor (TNF) family) to the appropriate receptor on the cell surface. This is followed by the activation of adaptor proteins and the caspase-8 mechanism, which, when activated, can directly initiate the apoptotic program.[24] The intrinsic pathway is triggered by cell-damaging factors such as oxidative stress, DNA damage, or growth factor deficiency. During this pathway, the mitochondria undergo disintegration, leading to the release of proapoptotic factors such as cytochrome C into the cytoplasm. The described pathways converge in the execution phase. Effector caspases degrade some nuclear and cytoskeletal proteins.[24] Caspase-3 plays a significant role here, inducing the disintegration of the cell into apoptotic bodies. As the cell dies, phosphatidylserine is exposed on its cell membrane, which is later recognized by phagocytes. Annexin V, a protein commonly used as a marker of apoptosis, binds strongly to phosphatidylserine.[24]

Fundamental processes such as cell cycle progression and DNA integrity are crucial for proper cell function; therefore, disturbances in these processes directly affect cell survival and proliferative potential. As summarized in Table 1, studies investigating the effects of doxorubicin on HL-60 cells demonstrate concentration-dependent alterations in apoptosis and cell cycle regulation.

In a study examining cyclin A expression in HL-60 cells under the influence of doxorubicin, Żuryń et al. showed that, with increasing doxorubicin dose (0–5 µmol/L) and the proportion of apoptotic cells in the population, the number of cells expressing cyclin A decreased.[25] The cyclin A2/Cdk1 complex initiates the transition from the G2 phase to the M phase. In contrast, the cyclin A2/Cdk2 complex initiates the transition from the G1 phase to the S phase, whereby the cyclin A level reflects proliferative activity.[22,26] As reported by Denard et al., doxorubicin inhibits cancer cell proliferation by activating proteolytic cleavage of CREB3L1.[11] It stimulates the proteolytic cleavage of CREB3L1, a membrane-associated transcription factor, allowing the NH2-terminal domain of CREB3L1 to enter the cell nucleus, where it activates transcription of genes encoding cell cycle inhibitors, thereby limiting cell proliferation.[11]

Doxorubicin has a toxic effect not only on target cancer cells but also on healthy human cells with normal physiology and morphology, albeit to a noticeably lesser extent. In a 2005 study, Suzuki et al. compared the cytotoxic activity of doxorubicin against cancer cells using the HL-60, HSC-2, HSC-3, and HSG cell lines and regular cell lines from oral tissues – HGF, HPLF, and HPC.[27] The cytotoxicity of HL-60 cells was assessed using the trypan blue exclusion method, while the MTT assay was used for the other lines. Doxorubicin, tested in a range of concentrations, showed higher cytotoxicity

in cancer cell lines than in healthy cell lines, and the tumor specificity (TS) index, determined based on the ratio of 50% cytotoxic concentration of cancer and healthy cell lines, was determined to be in the range of 54-255. In the apoptosis induction analysis, they compared the effect of the drug on HL-60 and HSC-2 cells at seven concentrations (0; 0.5; 1; 2; 4; 6; 8 and 16 μmol/L for HL-60 and 0; 0.1, 0.3, 10, and 30 μmol/L for HSC-2), showing activation by doxorubicin of caspases-3, -8, and -9 in both cell groups, with a clear predominance of caspase-3. In addition, gel electrophoresis performed to assess the level of genetic material damage showed DNA fragmentation in HL-60 cells and none in HSC-2 cells, despite confirmed caspase activation.[27]

Despite its widespread and well-established efficacy in cancer therapy, doxorubicin is associated with a range of adverse effects, including bone marrow suppression, nausea and vomiting, and alopecia. The cytotoxic impact of doxorubicin on healthy tissues represents a major dose-limiting factor in clinical practice. Among the most severe complications of doxorubicin therapy is cardiomyopathy, which may ultimately lead to congestive heart failure. Cardiac toxicity can be acute, with an incidence of approximately 11% in patients taking the drug, or chronic, with an incidence of approximately 1.7%.[10,28,29] Factors that increase the risk of doxorubicin-induced cardiotoxicity include dose (including single and cumulative lifetime anthracycline exposure), concomitant cardiovascular disease, previous chemotherapy, method and rate of drug administration, and patient age.[30] Although the details of the mechanisms responsible for the antibiotic's harmful effects on myocardial cells are not fully understood, the main factors mentioned include increased oxidative stress through excessive production of reactive oxygen species, mitochondrial damage, apoptosis induction, and inhibition of cardiomyocyte-specific gene expression.[10,31] The cardiotoxicity of doxorubicin has prompted the search for analogues and alternative methods of drug administration that would be less toxic.[32]

As studies show, cytostatics may also be associated with premature ovarian failure. Although alkylating agents are mainly responsible for reducing the reserve of primordial follicles in the ovaries, other chemotherapeutic agents also have a detrimental effect on organ function.[33] In their study, Ben-Aharon et al. confirmed that doxorubicin is toxic to the ovaries in female mice.[34] The antibiotic was administered intraperitoneally at a dose of 7.5 or 10 mg/kg of doxorubicin, depending on the test. They observed a significant decrease in ovarian weight in mice treated with doxorubicin, both 1 week after injection (85% of control weight) and 1 month after injection (only 52% of control weight). When examining ovulation rates, they found a drastic decrease in ovulation frequency after one week, followed by a partial increase after one month. In addition, they observed vascular changes in ovarian sections and a reduction in the number of both primary and secondary follicles.[34]

Over the years, many approaches have been developed to overcome the limitations of doxorubicin, particularly its cardiotoxicity. These include low-dose continuous infusion regimens, antibiotic administration with cardioprotective drugs, and liposomal encapsulation technology. The latter involves lipid-based drug carriers that alter drug distribution, thereby reducing its concentration in heart cells. This method was based on the assumption that liposomes cannot escape from vessels in areas with tight capillary connections, but can leave the circulation in tissues and organs lined with cells that are not tightly connected, such as cancerous tissues. Thus, encapsulating anthracyclines in liposomes results in higher concentrations in tumor tissue while limiting their effects on healthy cells, making liposomal encapsulation one of the effective methods for increasing the therapeutic index of doxorubicin and other anthracyclines.[9] Another factor reducing the therapeutic potential of doxorubicin in cancer treatment is the occurrence of multidrug resistance. The mechanism underlying resistance development is reduced intracellular drug concentration after long-term dosing, leading to upregulation of efflux systems in cancer cells.[35,36] Chen et al. confirmed the presence of multidrug resistance in the sensitive HL-60 line at progressively higher doxorubicin doses.[37] In a 2021 experiment, Al-Abbas and Shaer used the HL-60 and HL-60/ADR (chemotherapy-resistant) cell lines. They investigated the effect of doxorubicin therapy combined with coumarin, a naturally occurring compound with broad bioactivity, including antioxidant, anti-inflammatory, and anticancer properties.[36] In their study, they showed that the simultaneous use of coumarin (0, 100, 250, 500, and 1000 μg/mL) and doxorubicin (100 ng/mL) after 24 hours significantly induced apoptosis in drug-resistant HL-60/ADR cells, an effect not observed with monotherapy. The rate of cell death clearly increased with increasing coumarin concentration. Their results suggest that coumarin inhibits extracellular doxorubicin efflux, thereby increasing antibiotic efficacy against resistant human myeloid leukemia cells.[36] These and many other data in the literature suggest the existence of multiple pathways to enhance the therapeutic potential of doxorubicin in cancer treatment by circumventing its toxicity to healthy cells and cancer cells' resistance mechanisms.

Conclusion

To summarize the gathered information, doxorubicin clearly affects HL-60 cells by disrupting cell cycle progression and activating apoptotic pathways, leading to a marked reduction in their proliferative capacity. Although its anticancer activity is well established, the

therapeutic impact of the drug continues to be limited by toxicity toward healthy tissues and the emergence of resistance. Ongoing research exploring molecular mechanisms, protective strategies, and combination approaches offers promising directions for improving the safety and effectiveness of doxorubicin-based therapies in hematologic malignancies.

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Conceptualization, D.R. and E.K.; Methodology, D.R.; Writing – Original Draft Preparation, D.R. and E.K; Writing – Review & Editing, E.K.; Supervision, E.K.

Conflicts of interest
The authors declare no conflict of interest.

Data availability
No datasets were generated or analyzed during the current study.

Ethics approval
Not applicable

References
1. Cieślik A. Epidemiology of leukaemias in Poland. *Stud Med*. 2011;21(1):53-60.
2. Wafa A, Moassass F, Liehr T, Al-Ablog A, Al-Achkar W. Acute promyelocytic leukemia with the translocation t(15;17)(q22;q21) associated with t(1;2)(q42~43;q11.2~12): a case report. *J Med Case Rep.* 2016;10:203. doi:10.1186/s13256-016-0982-8.
3. Wang Z, Chen Z. Acute promyelocytic leukemia: From highly fatal to highly curable. *Blood.* 2008;111(5):2505-2515. doi:10.1182/blood-2007-07-102798
4. Podhorecka M, Macheta A. Acute promyelocytic leukemia - modern approach to disease pathogenesis and differentiation treatment. *Postepy Hig Med Dosw (online).* 2013;67:1083-1089. doi:10.5604/17322693.1077467
5. Seferyńska I, Warzocha K. A registry report from the Institute of Hematology and Transfusion Medicine on adult morbidity for acute leukemias between 2004–2010 in Poland made on behalf of the Polish Adult Leukemia Group (PALG). *Hematologia.* 2014;5(2):162-172. doi: 10.5114/hcp.2014.44988
6. Sanz MA, Martín G, González M, et al. Risk-adapted treatment of acute promyelocytic leukemia with all-trans-retinoic acid and anthracycline monochemotherapy: A multicenter study by the PETHEMA group. *Blood.* 2004;103(4):1237-1243. doi:10.1182/blood-2003-07-2462
7. Saeed S, Logie C, Stunnenberg HG, Martens JH. Genome-wide functions of PML-RARα in acute promyelocytic leukaemia. *Br J Cancer*. 2011;104(4):554-8. doi: 10.1038/sj.bjc.6606095.
8. Wierzbowska A. Ostra białaczka szpikowa. *Onkol Prakt Klin – Edukacja*. 2020;6(A):119-144. doi:10.5603/owpk_edu.70553
9. Rivankar S. An overview of doxorubicin formulations in cancer therapy. *J Cancer Res Ther.* 2014;10(4):853-858. doi:10.4103/0973-1482.139267
10. Johnson-Arbor K, Dubey R. Doxorubicin. In: *StatPearls.* Treasure Island (FL): StatPearls Publishing; 2023.
11. Denard B, Lee C, Ye J. Doxorubicin blocks proliferation of cancer cells through proteolytic activation of CREB3L1. *eLife.* 2012;1:e00090. doi:10.7554/eLife.00090
12. Patel AG, Kaufmann SH. Cancer: How does doxorubicin work? *eLife.* 2012;1:e00387. doi:10.7554/eLife.00387
13. Hou W, Liu Y, Liang H, Wu S. Induction of differentiation of the acute myeloid leukemia cell line HL-60 by SN3-L6 and all-trans retinoic acid. Cell Death Discov. 2020;6:84. doi:10.1038/s41420-020-00354-3
14. Basu J, Madhulika S, Murmu KC, Mohanty S, Samal P, Das A, Mahapatra S, Saha S, Sinha I, Prasad P. Molecular and epigenetic alterations in normal and malignant myelopoiesis in human leukemia 60 (HL60) promyelocytic cell line model. *Front Cell Dev Biol*. 2023;11:1060537. doi:10.3389/fcell.2023.1060537.
15. Sewastianik T, Prochorec-Sobieszek M, Juszczyński P. Deregulation of MYC transcription factor in lymphoid tumors — molecular, pathogenetic, clinical and therapeutic implications. *Hematologia.* 2012;3(4):313-326.
16. Reece JB, Urry LA, Cain ML, et al. Campbell Biology. 10th ed. Poznań: Dom Wydawniczy REBIS; 2016:234-249.
17. Visconti R, Della Monica R, Grieco D. Cell cycle checkpoint in cancer: a therapeutically targetable double-edged sword. *J Exp Clin Cancer Res*. 2016;35:153. doi:10.1186/s13046-016-0433-9
18. Israels ED, Israels LG. The cell cycle. *Oncologist*. 2000;5(6):510-513. doi:10.1634/theoncologist.5-6-510
19. Williams GH, Stoeber K. The cell cycle and cancer. *J Pathol.* 2011;226(2):352-364. doi:10.1002/path.3022
20. Gąsior Ł, Daszkiewicz R, Michniak K. Role of gamma H2A.X in reproductive cells. *Postepy Biol Komór Cell*. 2016;43(1):53-72.
21. Gilewski TA, Dang C, Surbone A, et al. Cytokinetics. In: Bast RC Jr, Kufe DW, Pollock RE, et al., editors. Holland-Frei Cancer Medicine. 5th edition. Hamilton (ON): BC Decker; 2000. pp 511-538.
22. Gasińska A, Biesaga B. The two aspects of tumour hypoxia. *Nowotwory J Oncol.* 2010;60(4):332. doi:10.5603/njo.52151
23. Afford S, Randhawa S. Apoptosis. *Mol Pathol*. 2000;53(2):55-63. doi:10.1136/mp.53.2.55

24. Kopiński P, Chorostowska-Wynimko J, Dyczek A, Giżycka A. Apoptosis of alveolar lymphocytes. Part 1: pathways of lymphocyte apoptosis. *Adv Respir Med*. 2014;82(2):170-182. doi:10.5603/PiAP.2014.0023
25. Żuryń A, Grzanka A, Stępień A, Grzanka D, Dębski R, Smoliński D. Expression of cyclin A in human leukemia cell line HL-60 following treatment with doxorubicin and etoposide: the potential involvement of cyclin A in apoptosis. *Oncol Rep*. 2007;17:1013-1019. doi:10.3892/or.17.5.101
26. Stenzel A, Żuryń A, Grzanka AA, Grzanka A. Cyclins as a markers of cancer diseases. *Nowotwory J Oncol*. 2012;62(2):115-122. doi:10.5603/njo.19142.
27. Suzuki F, Hashimoto K, Kikuchi H, et al. Induction of tumor-specific cytotoxicity and apoptosis by doxorubicin. *Anticancer Res.* 2005;25:887-894.
28. Chatterjee K, Zhang J, Honbo N, Karliner JS. Doxorubicin cardiomyopathy. *Cardiology.* 2010;115(2):155-162. doi:10.1159/000265166
29. Sheibani M, Azizi Y, Shayan M, et al. Doxorubicin-induced cardiotoxicity: An overview on pre-clinical therapeutic approaches. *Cardiovasc Toxicol*. 2022;22:292-310. doi:10.1007/s12012-022-09721-1
30. Pai VB, Nahata MC. Cardiotoxicity of chemotherapeutic agents: incidence, treatment and prevention. *Drug Saf*. 2000;22(4):263-302. doi:10.2165/00002018-200022040-00002
31. Christidi E, Brunham LR. Regulated cell death pathways in doxorubicin-induced cardiotoxicity. *Cell Death Dis.* 2021;12:339. doi:10.1038/s41419-021-03614-x
32. Kong CY, Guo Z, Song P, Zhang X, Yuan YP, Teng T, Yan L, Tang QZ. Underlying the mechanisms of doxorubicin-induced acute cardiotoxicity: oxidative stress and cell death. *Int J Biol Sci*. 2022;18(2):760-770. doi:10.7150/ijbs.65258
33. Oktem O, Oktay K. Quantitative assessment of the impact of chemotherapy on ovarian follicle reserve and stromal function. *Cancer.* 2007;110:2222-2229. doi:10.1002/cncr.23071
34. Ben-Aharon I, Bar-Joseph H, Tzarfaty G, et al. Doxorubicin-induced ovarian toxicity. *Reprod Biol Endocrinol.* 2010;8:20. doi:10.1186/1477-7827-8-20
35. Kostrzewa-Nowak, D., Paine, M., Wolf, C. *et al.*The role of bioreductive activation of doxorubicin in cytotoxic activity against leukaemia HL60-sensitive cell line and its multidrug-resistant sublines. *Br J Cancer*, 2005;93:89-97. doi:10.1038/sj.bjc.6602639
36. Al-Abbasa NS, Shaer NA. Combination of coumarin and doxorubicin induces drug-resistant acute myeloid leukemia cell death. *Heliyon*. 2021;7:e06255. doi:10.1016/j.heliyon.2021.e06255
37. Chen J, Wei H, Cheng J, et al. Characteristics of doxorubicin-selected multidrug-resistant human leukemia HL-60 cells with tolerance to arsenic trioxide and contribution of leukemia stem cells. *Oncol Lett*. 2018;15(2):1255-1262. doi:10.3892/ol.2017.7353