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Mechanisms and Ways to Overcome Acquired Resistance of Cancer Cells to Mcl-1 Antagonists

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Abstract—Acquired drug resistance reduces the effectiveness of anticancer therapy and leads to cancer progression. Selective inhibition of anti-apoptotic proteins of the Bcl-2 family using BH3-mimetics is a promising treatment strategy for cancer patients. Recently, antagonists of the anti-apoptotic protein Mcl-1 have been actively studied in clinical trials. However, like other BH3-mimetics, they can lose their effectiveness due to the development of acquired resistance. We have found that cancer cells develop resistance to Mcl-1 inhibition through increased gene expression of other anti-apoptotic proteins, such as Bcl-2 or Bcl-xL, thereby becoming less Mcl-1-dependent. Alterations in cellular metabolism have also accompanied the development of this resistance. We have shown that combining the Mcl-1 antagonist S63845 with various anticancer compounds can overcome the resistance of malignant cells to its action.

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INTRODUCTION

During carcinogenesis, tumor cells undergo various adaptations that allow them to evade the body's defense mechanisms. One of the distinctive features of malignant clones, which enables them to actively divide and invade surrounding tissues and organs, is their avoidance of various types of programmed cell death (PCD), including apoptosis, a process that plays a vital role in protection against cancer [1-3]. The limited sensitivity of tumor cells to apoptosis underlies the progression of many oncological diseases; therefore, triggering this type of PCD is a rational strategy for anticancer therapy [4, 5].

Apoptosis is a strictly genetically controlled process. There are two primary pathways for initiating apoptosis: the extrinsic (receptor-dependent) pathway and the intrinsic (mitochondrial) pathway. The key event in the intrinsic apoptotic pathway is mitochondrial outer membrane permeabilization (MOMP), which leads to the release of cytochrome *c* and other proapoptotic factors from the intermembrane space, thereby promoting cell death [4, 5]. The proteins of the Bcl-2 (B-cell leukemia/lymphoma protein-2) family control MOMP. The functional activity of the members of this family is accomplished through the formation of various protein–protein complexes. Some proteins are responsible for MOMP activation, while other proteins inhibit this process [6-10]. According to their properties, the proapoptotic proteins of the Bcl-2 family are divided into two categories: regulatory BH3-only (Bcl-2 homology domain) and effector proteins [8, 10-12]. The latter include the multidomain proteins Bak (Bcl-2 homologous antagonist killer) and Bax (Bcl-2 associated X protein), which, upon activation, are capable of forming pores in the outer mitochondrial membrane (OMM) through the formation of homo- and hetero-oligomers. In the absence of

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apoptotic stimuli, Bax is located in the cytoplasm, and Bak is located on the OMM in monomeric form [13, 14]. The regulatory BH3-only proteins contain only one BH domain in their structure, possess various intracellular localizations (cytoplasm, OMM, cytoskeleton, etc.), and can perform two functions. Firstly, all proteins in this subgroup are capable of neutralizing the action of anti-apoptotic proteins; however, their interaction profiles and inhibitory capacities vary significantly among the different members of the Bcl-2 family. Secondly, some BH3-only proteins (Bim [Bcl-2-interacting mediator of cell death], tBid [truncated form of the Bid protein]) can also activate the effector proteins Bak and Bax with varying degrees of efficiency [15-18]. The anti-apoptotic proteins (Bcl-2, Mcl-1, Bcl-xL, etc.) localized on the OMM and in the cytoplasm promote cell survival by binding both effector and regulatory proapoptotic members of the Bcl-2 family [8, 15, 19, 20]. Thus, the proteins of the Bcl-2 family participate in forming a three-component control system of MOMP, which determines the subsequent fate of cells [8, 15, 21].

The anti-apoptotic proteins of the Bcl-2 family represent attractive targets for anticancer therapy, as an increase in their levels often underlies the resistance of tumor cells to apoptosis, leading to a decrease in the effectiveness of many chemotherapeutic agents [8, 22-24]. Over the past decades, highly selective small-molecule inhibitors of anti-apoptotic proteins of the Bcl-2 family have been actively developed. According to their mechanism of action, these inhibitors serve as “exogenous” analogs of the regulatory BH3-only proteins, which is why they are referred to as BH3-mimetics [8, 25]. To date, one drug from this group of compounds (Venetoclax, a selective antagonist of the Bcl-2 protein) has already been approved for clinical use in many countries to treat chronic lymphocytic leukemia (CLL) [8, 26].

Among all proteins of the Bcl-2 family responsible for cell survival, Mcl-1 (Myeloid cell leukemia protein-1) attracts special attention. This protein is important for maintaining embryogenesis and homeostasis in various cell types, as well as for regulating the cell cycle [9, 27]. Its gene is expressed in all tissues of the human body [20]. It is worth noting that Mcl-1 is important for mitochondrial homeostasis [28]. The molecular weight of Mcl-1 (37.2 kDa) is significantly higher than that of other anti-apoptotic proteins due to the presence of an N-terminal region containing its mitochondrial localization sequence, as well as PEST sequences (proline [P], glutamic acid [E], serine [S], and threonine [T]-rich sequences), which are regions enriched in residues of proline, glutamic acid, serine, and threonine. The presence of PEST sequences is a feature of short-lived proteins, which also distinguishes Mcl-1 [20, 29, 30]. In recent years, various negative modulators of Mcl-1 have been discovered at the transcriptional, translational, and post-translational levels [20, 29]. In particular, we have previously found that Mcl-1 in tumor cells undergoes proteasomal degradation under conditions of nutrient limitation, independent of autophagy [31]. Additionally, there are peculiarities in the Mcl-1 interactions with other members of the Bcl-2 family. For example, Mcl-1 has low affinity for the effector protein Bax and higher affinity for Bak [30, 32]. We have also demonstrated a key role of Bak in apoptosis induced by selective Mcl-1 antagonists [33]. In addition, the BH3-only protein Noxa binds to Mcl-1, facilitating its proteasomal degradation [34].

Various Mcl-1 protein antagonists (S64315/MIK665, AZD5991, AMG176, PRT1419, etc.) have been actively studied in clinical trials over the past few years [35]. However, to date, they have not been able to replicate the success of Venetoclax for several reasons. Firstly, suppression of the anti-apoptotic proteins of the Bcl-2 family can lead to serious side effects, since all of them perform multiple physiological functions. In particular, the use of Bcl-xL antagonists caused severe thrombocytopenia [8, 36], and recently it was found that Mcl-1 suppression results in impaired hematopoiesis and cardiotoxicity [35]. Secondly, Mcl-1 antagonists often possessed low efficacy in clinical trials, which may be due to the development of acquired resistance by tumor cells to Mcl-1 inhibition [35].

The development of drug resistance with subsequent loss of treatment efficacy is a serious problem arising with the use of most modern drugs, particularly antitumor agents [37]. This phenomenon has also been observed with the use of BH3-mimetics. For example, possible causes of cancer cell resistance to Venetoclax have been characterized in detail, including metabolic and proliferative adaptations of malignant clones, emergence of mutations in the genes of the target protein Bcl-2 and its proapoptotic partner Bax, compensatory effects (increased levels of other anti-apoptotic proteins), etc. [38, 39]. However, in the case of Mcl-1 antagonists, this issue has not been sufficiently studied. Therefore, this work aims to conduct a detailed study of the possible causes of the emergence of drug resistance of tumor cells to the action of selective Mcl-1 antagonists using S63845 as an example (its derivative S64315/MIK665 is being studied in the clinic [40]), as well as potential ways to overcome it.

MATERIALS AND METHODS

Cell lines and culture conditions. HeLa cervical adenocarcinoma, H23 lung adenocarcinoma, and SK-N-Be(2)c neuroblastoma cell lines (all obtained

from the Department of Toxicology at Karolinska Institutet, Stockholm, Sweden) were used in this study. Cells were grown in a CO_2 incubator (5% CO_2) at 37°C using a Dulbecco's Modified Eagle Medium (DMEM) (#C410p, PanEco, Russia) supplemented with glutamine and glucose (4.5 g/L), 10% bovine calf serum (#11965092, Gibco, USA), an antibiotic (penicillin, 100 U/mL), and an antifungal agent (streptomycin, 100 U/mL) (#15240062, Gibco). Cells were routinely checked for the absence of mycoplasma. Cells with 70-80% confluency were used in the experiments. Cells were counted using a Beckman Z2 Coulter counter (Beckman Coulter Life Sciences, USA).

Reagents. Following cell cultivation for 24-36 h, the culture medium was replaced with a fresh medium and the following chemical compounds were added: Mcl-1 antagonist S63845 (Active Biochem, China), DNA-damaging agent cisplatin (Teva Pharmaceutical, Israel), Bcl-2 and Bcl-xL antagonists – Venetoclax and A1331851, respectively (both from Selleckchem, USA), P-glycoprotein inhibitors Zosuquidar and Verapamil, GSK3 (Glycogen synthase kinase 3) inhibitor CHIR99021, ATP synthase inhibitor oligomycin, protonophore carbonyl cyanide-m-chlorophenylhydrazone (CCCP), inhibitors of electron transport chain (ETC) complexes I (rotenone) and II (thenoyltrifluoroacetone, TTFA), and the MDM2 [Mouse double minute 2 homolog] antagonist Nutlin-3a (all from Sigma, USA). All drugs were used at concentrations indicated in the respective figures. Control cells were supplemented with the solvent that corresponds to the used reagent.

Mcl-1 downregulation by RNA interference. After washing with phosphate-buffered saline (#P061, PBS, PanEco), the cells were incubated with 1 ml of transfection mixture prepared by mixing Opti-MEM transfection medium (#31985070, Gibco), 3 µl of Lipofectamine RNAiMAX transfection agent (Invitrogen, USA), and siRNA to Mcl-1 (50 µM) in an amount required to achieve working concentration (100 nM). The sense and antisense sequences of the strands of siRNA to Mcl-1 are 5′-GCATCGAACCATTAGCAGAdTdT-3′ and 5′-TCTGCTAATGGTTCGATGCdTdT-3′. Control siRNA #1 D-001810-01 (Dharmacon Reagents, UK) was used as a non-targeting siRNA. Incubation time was 24 h.

Western blot analysis. The cell pellet obtained after cell detachment by scraping was washed with cold PBS and centrifuged. Next, cells were resuspended in 30-60 µl of a RIPA lysis buffer (Bio-Rad, USA) and incubated for 20 min on ice. After centrifugation (20,000*g*, 20 min, 4°C), a portion of the supernatant was collected to measure protein concentration in the cell lysates using a Pierce BCA Protein Assay Kit (#23225, Thermo Fisher Scientific, USA), and samples for Western blot analysis were prepared from the remaining supernatant as described previously [41].

Antibodies. Primary antibodies for detection of the following proteins were used in the experiments: Bcl-2 (sc-7382) from Santa Cruz (USA); PARP [poly(ADP-ribose) polymerase] (ab74290), tubulin (ab4074), and vinculin (ab123002) (all from Abcam, UK); p89 PARP cleaved (#5625), caspase-3 (#9662), p19/17 caspase-3 cleaved (#9661), Bak (#6947), Bax (#2772), Bcl-xL (#2764), Mcl-1 (#5453), MDR1 [multidrug resistance protein 1] (#12683), GAPDH (#2118), GSK3a (#4337), and GSK3b (#9315) (all from Cell Signaling, USA). Secondary antibodies used were anti-mouse (#515-035-062) or anti-rabbit IgG (#111-035-144) horseradish peroxidase-conjugated antibodies from Jackson ImmunoResearch (USA). Dilutions of all antibodies were chosen according to the manufacturer's recommendations.

The changes in protein levels were determined using densitometric analysis. Image processing was performed with ImageJ 1.53t. Protein levels were normalized to the corresponding gel loading proteins (tubulin, vinculin, GAPDH); statistical analysis was performed using the Mann–Whitney U-test.

Flow cytometry. During sample preparation, cells were trypsinized, washed twice with cold PBS, and centrifuged (500*g*, 5 min, 4°C).

(a) *Annexin V-FITC [fluorescein isothiocyanate] (annexin) and propidium iodide (PI) double staining assay*. After resuspending the cell pellet in PBS (700 µl/1,000,000 cells), approximately 200,000 cells were removed and added to 200 µl of 1x annexin-binding buffer (BD Biosciences, USA). Next, cell populations were analyzed using BD FACSCanto II flow cytometer (BD Biosciences, USA) and BD FACSDiva (BD Biosciences, USA) and FlowJo (FlowJo LLC, USA) software, as described previously [42]. The percentage of dead cells was calculated from all cells that were stained with annexin (apoptosis), PI (necrosis), or both dyes (late apoptosis); the percentage of viable cells was calculated from the cell population that was not stained with either annexin or PI [43].

(b) *SubG1 test*. Cells were fixed in a cold 70% ethanol solution in PBS at −20°C for 24 h, washed, and resuspended in PBS. Next, PI (50 µg/mL) and RNase A (100 µg/mL) were added, and the cells were incubated in the dark at room temperature for 10 min. The analysis was performed similarly to that for the annexin and PI double staining (a). The proportion of cells in the subG1 phase correlates with the intensity of apoptosis induction and reflects the percentage of cell death [43, 44]. For both flow cytometry methods, 10,000 cells were analyzed in all samples during experiments.

Real-time polymerase chain reaction (RT-PCR). Total RNA was isolated from the cell pellet using TRIzol reagent (#15596018, Thermo Fisher Scientific), and cDNA was obtained by reverse transcription

using the MMLV RT kit (Eurogen, Russia) according to the manufacturer's instructions. The qPCRmix-HS SYBR (Eurogen), specific primers for Bcl-2, Bcl-xL, and the transcription factor TATA-binding protein were added to the cDNA samples. The primer sequences are listed in Table S1, the Online Resource 1. Next, mRNA expression was assessed using the CFX96 Real-Time PCR Detection System (Bio-Rad). Transcript analysis was performed using the Pfaffl method [45]. Bcl-2 and Bcl-xL mRNA concentrations were normalized to the mRNA level of the TATA-binding protein.

Cell metabolism analysis. Cells were seeded in 96-well plates in a standard DMEM culture medium. After reaching 70-80% confluency, the cells were washed and incubated in an assay medium (DMEM without phenol red, calf serum, sodium pyruvate, and glucose) for 1-3 h at 37°C. To analyze respiration, sodium pyruvate (1 mM) and glucose (10 mM) were added to the same medium. Experiments were performed in real time using a Seahorse XF analyzer (Agilent, USA). To test respiration (the first three measurements), oligomycin (1 μM), carbonyl cyanide m-chlorophenylhydrazone (CCCP, 1 μM), and rotenone (1 μM) were added to the cells. To assess glycolysis, D-glucose (10 mM), oligomycin (1 μM), and 2-deoxyglucose (50 mM) were added to the wells. Data were normalized to the protein levels in each well.

Clonogenic activity assay. Cells (1000 per well) were seeded in triplicate in 6-well plates (Nunc, The Netherlands). After culturing (10-14 days), cells were washed twice with PBS, fixed with paraformaldehyde (4% in PBS), and stained with crystal violet (0.5% in aqueous solution). Plates were visualized using the ChemiDoc XRS+ system (Bio-Rad) and analyzed using ImageJ 1.53t.

Gene sequencing. To identify potential mutations in the *BAK1* and *MCL1* genes, Sanger sequencing and next-generation sequencing (NGS) were employed, respectively.

(a) *Sanger sequencing*. For *BAK1* sequencing, genomic DNA was isolated from the original and Mcl-1 inhibition-resistant HeLa, H23, and SK-N-Be(2)c cell lines using the QIAamp DNA Mini Kit (#56304, Qiagen, Germany). Fragments of exons 2-6 of *BAK1* were amplified by RT-PCR and sequenced using the Sanger method. The primer sequences are listed in Table S1, the Online Resource 1.

(b) NGS of *MCL1* was performed as described previously [46].

Cell viability assessment. For the experiments, cells (5,000) were seeded in 96-well plates. Cell cultivation and induction were performed using a standard DMEM culture medium with appropriate reagents. Cell viability was analyzed 24 h after induction using the MTS assay or Alamar Blue assay.

(a) *MTS assay*. After induction, cells were treated with 20 μl of MTS reagent (CellTiter 96 AQueous One Solution Cell Proliferation Assay, #G3580, Promega, USA), incubated for 2.5-3 h at 37°C, and analyzed spectrophotometrically using Varioskan Flash instrument (Thermo Fisher Scientific) at 480 nm.

(b) *Alamar Blue assay*. After induction, the culture medium was replaced with a fresh medium containing 10% Alamar Blue reagent (#DAL1100, Thermo Fisher Scientific). Samples were incubated for 3-4 h at 37°C, and fluorescence was measured using Varioskan Flash instrument (Thermo Fisher Scientific) at wavelengths of 560 nm (excitation) and 590 nm (emission).

Statistical analysis was performed using the Mann–Whitney U-test. Data processing and statistical analysis were performed using Microsoft Excel and GraphPad Prism 6 (GraphPad Software, USA). Data in histograms are presented as mean ± standard deviation (n = 4); * $p < 0.05$.

RESULTS

Generation of cancer cells with acquired resistance to the BH3 mimetic S63845. To study potential mechanisms underlying the development of acquired resistance to selective Mcl-1 antagonists in cancer cells, three cell lines of different origins (HeLa cervical adenocarcinoma, H23 lung adenocarcinoma, and SK-N-Be(2)c neuroblastoma) were selected based on their inherent sensitivity to Mcl-1 inhibition [33]. It is worth noting that all cancer cells used in this study are characterized by impaired functional activity of the transcription factor p53. The H23 [47] and SK-N-Be(2)c [48] cell lines contain mutant p53 protein. In contrast, in HeLa cells, the functional activity of the wild-type p53 protein is suppressed due to the presence of the E6 protein of human papillomavirus (HPV) type 18, which leads to rapid degradation of p53 [49]. The presence of wild-type p53 protein could maximize the effectiveness of BH3-mimetics [50]; however, its absence or altered functional activity does not prevent the initiation of cancer cell death upon exposure to these drugs due to their mechanism of action. Moreover, Venetoclax, which is approved for clinical use, can also be used as a therapeutic option for patients with CLL and 17p deletion or TP53 mutation [51].

A "pulse" method was used to generate resistant cells reproducing effects of chemotherapeutic courses in patients that determine its clinical significance [52]. This method includes culturing of the original cell lines with gradually increasing concentrations of the drug, alternating with drug-free cell culture. Nanomolar concentrations of S63845 (from 125 to 500 nM)

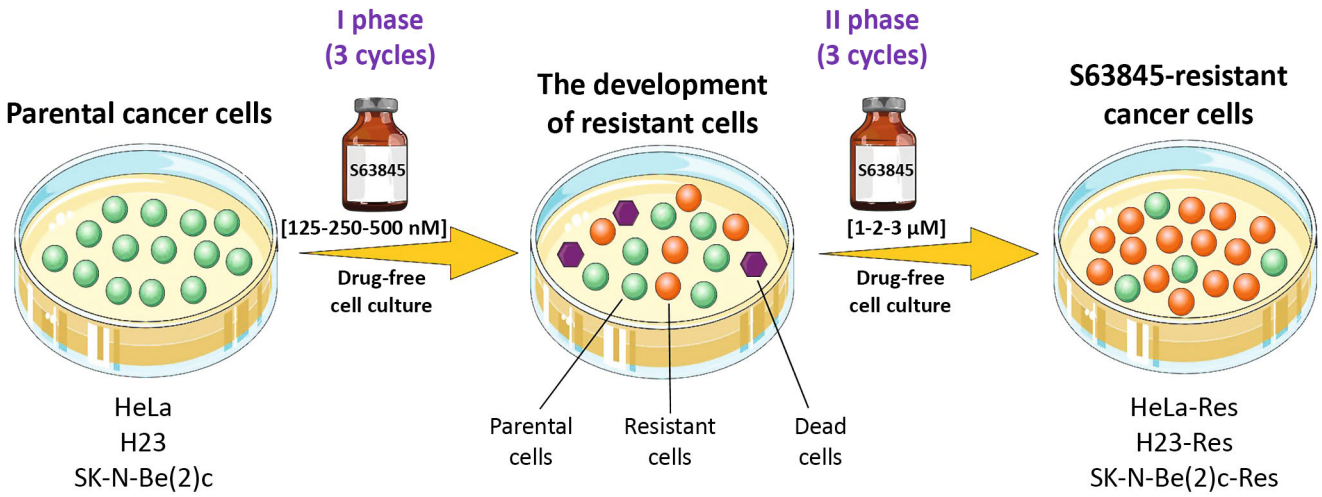

Fig. 1. Generation of HeLa, H23, and SK-N-Be(2)c cancer cells with acquired resistance to the Mcl-1 antagonist S63845. HeLa-Res, H23-Res, and SK-N-Be(2)c-Res are cancer cells resistant to S63845 (hereinafter). The figure was created using Servier Medical Art (https://smart.servier.com/) under a CC BY 4.0 license (https://creativecommons.org/licenses/by/4.0/).

were used in the first stage, and micromolar (up to 1 μM for H23 and up to 3 μM for HeLa and SK-N-Be(2)c) concentrations of this BH3-mimetic were used in the second stage. Both stages consisted of three cycles. Each cycle sequentially included incubation of cells with S63845 for 24 h, culturing cells without S63845 addition for 96 h, and the reseeding of surviving cells (Fig. 1).

Resistance to Mcl-1 inhibition in the selected models was tested using two independent approaches: Western blot (WB) analysis and flow cytometry. Both parental and developed cells were treated with S63845 at concentrations ranging from 250 nM to 3 μM. According to the results of WB analysis, in all cases, the developed cells were characterized by lower sensitivity to the BH3-mimetic as compared to parental cells (Fig. 2a; Fig. S1a; Fig. S2a in the Online Resource 1). Cell death was assessed during WB analysis based on the degree of cleavage of two key apoptosis markers: the effector caspase-3 and its substrate, the repair protein PARP [43, 53]. The greatest differences in the response to S63845 were observed in cells treated with micromolar concentrations (1 and 3 μM) in all lines. In resistant cells, there was a marked decrease in the cleavage of full-length forms of caspase-3 and PARP and accumulation of their fragments – p19/17 caspase-3 and p89 PARP (Fig. 2a; Fig. S1a; Fig. S2a in the Online Resource 1). Flow cytometry results confirmed the presence of acquired resistance to S63845 in the derived HeLa (Fig. S1b in the Online Resource 1), H23 (Fig. S2b in the Online Resource 1), and SK-N-Be(2)c cells (Fig. 2b). In particular, upon incubation with S63845 (3 μM), the number of subG1 fractions was more than 2-fold lower in resistant SK-N-Be(2)c cells compared to the wild-type cells (Fig. 2b).

It is worth noting that the increased concentration of the Mcl-1 antagonist S63845 from 1 to 3 μM did not influence the level of H23 cell death. Moreover, the reduced sensitivity to S63845 in resistant H23 cells persisted up to a concentration of 1 μM BH3-mimetic. In contrast, the use of 3 μM S63845 led to overcoming this type of resistance, which distinguishes this cell model from HeLa and SK-N-Be(2)c cell lines (Fig. S2, a, b in the Online Resource 1).

Assessment of the contribution of the anti-apoptotic proteins Bcl-xL and Bcl-2 to the development of acquired tumor cell resistance to the BH3-mimetic S63845. We have previously shown that not only the initial insensitivity but also the acquired resistance of tumor cells to Mcl-1 inhibition can be mediated by high levels of the anti-apoptotic protein Bcl-xL [33]. Here, densitometric analysis of the WB data revealed that not only could Bcl-xL levels be significantly increased in resistant cells compared to parental cells, as was observed in the HeLa-Res cell line (Fig. S1c in the Online Resource 1), but also that the level of another anti-apoptotic protein, Bcl-2, could be increased, as was observed in SK-N-Be(2)c-Res cells (Fig. 2c). Moreover, the development of resistance to Mcl-1 inhibition may not be accompanied by the changes in the levels of other anti-apoptotic proteins, as was observed in H23-Res cells (Fig. S2c in Online Resource 1). Furthermore, in all three cell models, the Mcl-1 protein level in resistant cells remained unchanged compared to parental tumor lines (Fig. S3 in the Online Resource 1). It was hypothesized that, as tumor cell lines develop resistance to Mcl-1 inhibition, cells with increased expression of Bcl-xL and Bcl-2 protein genes are accumulated compared to the original cells. The data of RT-PCR analysis of mRNA levels for these anti-apoptotic proteins confirmed this

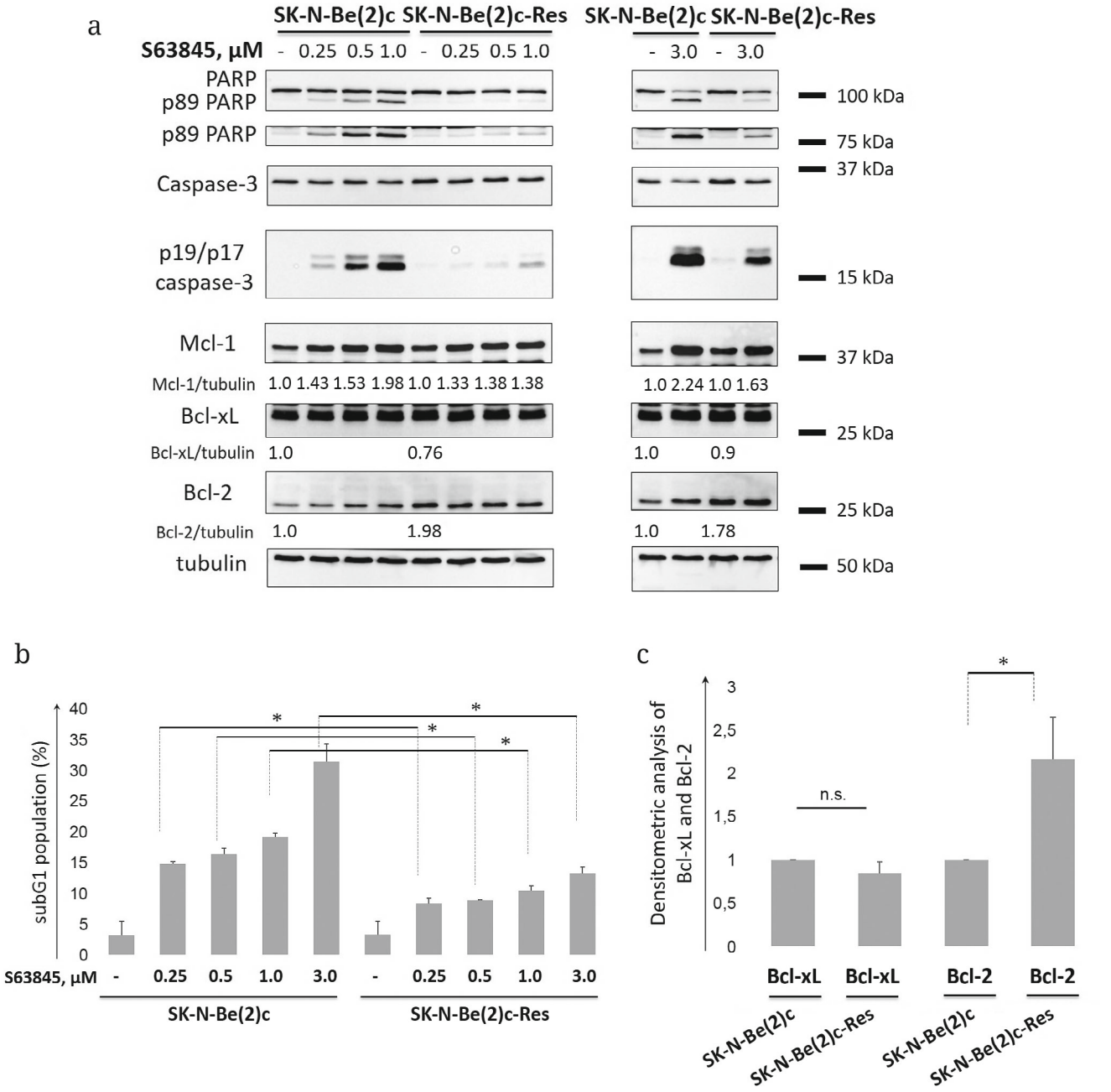

Fig. 2. Analysis of the efficacy of the BH3-mimetic S63845 (0.25-3 μM, 24 h) in SK-N-Be(2)c and SK-N-Be(2)c-Res cells. Results of WB (a), subG1 (b), and densitometric analysis of Bcl-xL and Bcl-2 in SK-N-Be(2)c and SK-N-Be(2)c-Res cells (c). Data are presented as mean ± standard deviation, $n = 4$; * $p < 0.05$, n.s. – not significant (U-test).

hypothesis: the increased Bcl-xL and Bcl-2 mRNA levels compared to the original cell lines were observed in HeLa-Res and SK-N-Be(2)c-Res cells, respectively (Fig. 3, a, b).

The observed changes in the Bcl-2 protein profile in resistant cells may indicate a reduced role of Mcl-1 in maintaining their viability compared to other anti-apoptotic proteins. This was confirmed by assessing cell death triggered by Mcl-1 suppression using RNA interference. The WB and flow cytometry analysis data revealed that two cell models with acquired resistance to S63845, where increased levels of Bcl-xL (HeLa) or Bcl-2 (SK-N-Be(2)c) were observed, and were characterized by lower sensitivity to the genetic Mcl-1 suppression (Fig. 4, a, b, d, e). At the same time, compared with the parental H23 line, in H23-Res cells, where the profile of Bcl-2 family proteins remained unchanged, the proportion of dead cells upon Mcl-1 silencing did not change (Fig. 4, c, f).

The results obtained clearly demonstrate a compensatory phenomenon observed among different members of the Bcl-2 family: inhibition of one

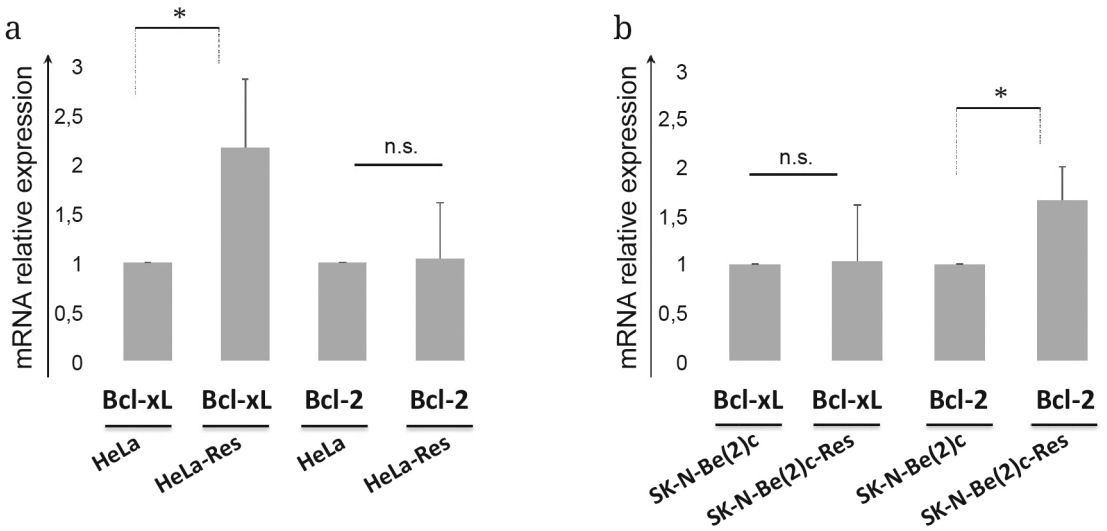

Fig. 3. Analysis of Bcl-xL and Bcl-2 mRNA levels in HeLa and HeLa-Res (a) and SK-N-Be(2)c and SK-N-Be(2)c-Res (b) cells. Data are presented as mean ± standard deviation, $n = 4$; * $p < 0.05$, n.s. – not significant (U-test).

anti-apoptotic protein could lead to an increase in the expression of genes encoding other cell survival proteins, resulting in the emergence of acquired resistance [8, 35]. However, the example of H23 cells clearly illustrates the multifactorial nature of drug resistance development: an increase in gene expression encoding Bcl-2 or Bcl-xL proteins is a probable, but not the only reason, underlying resistance to the action of selective Mcl-1 antagonists.

Assessing the contribution of P-glycoprotein to the development of acquired cancer cell resistance to the BH3-mimetic S63845. The development of drug resistance could be accompanied by increased expression of genes encoding multidrug resistance proteins, such as P-glycoprotein (MDR1). Proteins of this group are located on the plasma membrane and mediate the ATP-dependent reverse transport of many substances from cells [54, 55]. This phenomenon has also been demonstrated for Mcl-1 antagonists, particularly for S63845, which was used in this study [56].

To prevent the development of tumor cell resistance to the BH3-mimetic S63845 due to its export during the development of resistance (Fig. 1), the MDR1 inhibitor Verapamil (50 μM) was added to the cells, combined with the Mcl-1 antagonist. In addition, comparative WB analysis of cell death was performed in the original and the Mcl-1 inhibition-resistant cells using a combination of S63845 and another MDR1 inhibitor, Zosuquidar (Fig. S4 in the Online Resource 1). MDR1 was undetectable in both HeLa/H23 and HeLa-Res/H23-Res cells. Furthermore, Zosuquidar alone did not induce cell death in either wild-type or resistant HeLa and H23 cells, nor did it enhance S63845-mediated cell death in these cell models (Fig. S4, a, b in the Online Resource 1).

In SK-N-Be(2)c neuroblastoma cells, MDR1 was present in both parental and S63845-resistant cells; however, its content in the latter cells did not increase, as determined by densitometric analysis. As in other cell types, Zosuquidar did not induce death as a single agent, but it equally effectively enhanced S63845-dependent apoptosis (~2-fold decrease in cell viability according to the MTS assay) in both SK-N-Be(2)c and SK-N-Be(2)c-Res cells without overcoming resistance of neuroblastoma cells to the Mcl-1 antagonist (Fig. S4, c-e in the Online Resource 1). Thus, an increase in MDR1 does not underlie acquired resistance of tumor cells to the BH3-mimetic S63845.

A search for possible mutations in *BAK1* and *MCL1* as causes of acquired cancer cell resistance to the BH3-mimetic S63845. Investigation of the Bcl-2 antagonist Venetoclax revealed, among other factors, that mutations in the target protein gene or the Bax effector protein gene could lead to the development of tumor cell resistance to this BH3-mimetic [38, 39]. Taking this data into account, we searched for possible mutations in the Bak effector protein gene, since Mcl-1 has a higher affinity for Bak, while Bcl-2 preferentially interacts with Bax [15]. Using Sanger sequencing, it was found that in two of the three S63845-resistant cell populations (HeLa-Res and H23-Res), there is a single mutation, BAK1(NM_001188.4):c.309G>A p.(Thr103=), compared to the original lines (HeLa and H23, respectively). This mutation is located in exon 4 of *BAK1* and is a synonymous single-nucleotide substitution that does not affect the protein structure. No changes in *BAK1* were observed in resistant SK-N-Be(2)c neuroblastoma cells.

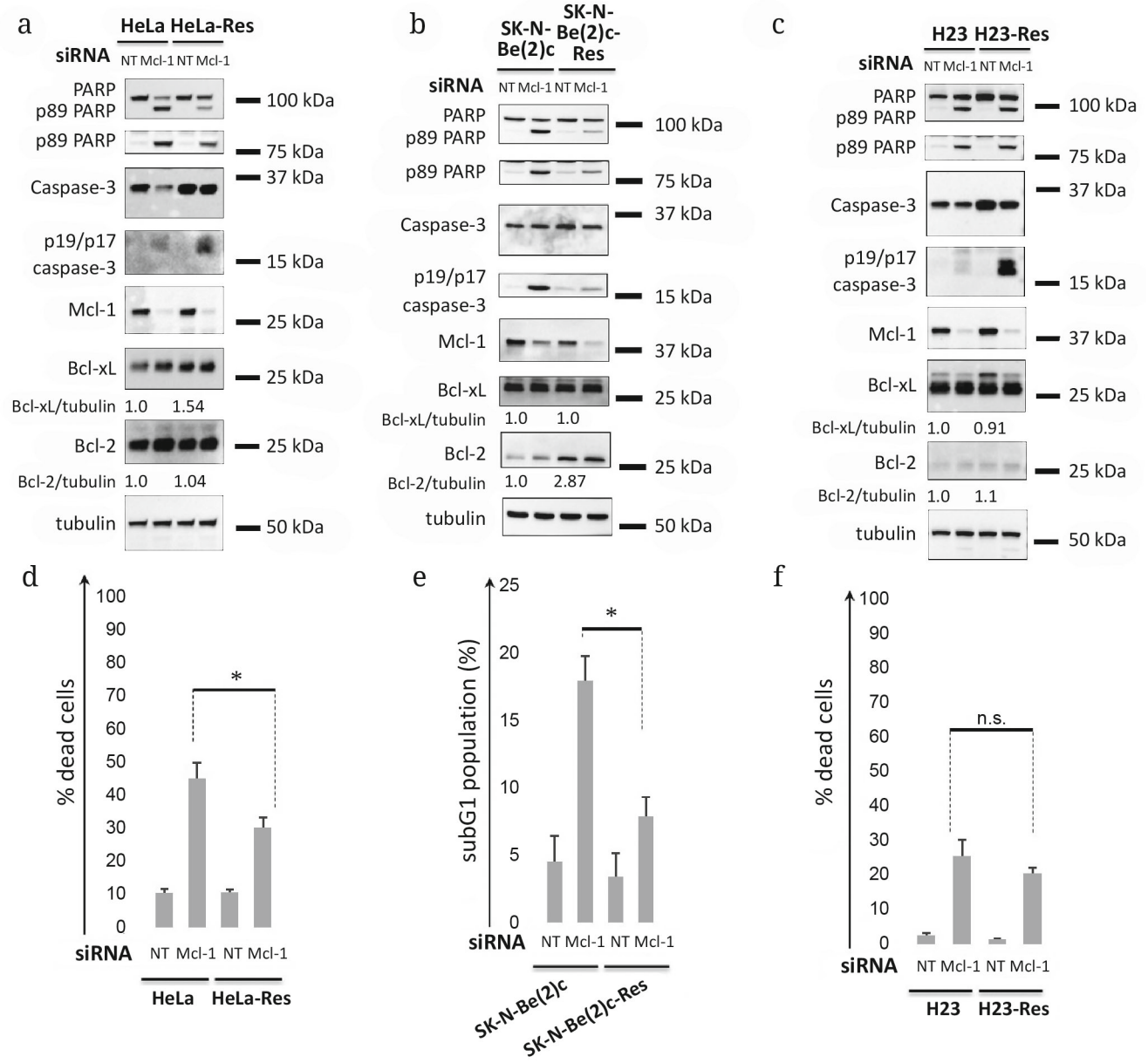

Fig. 4. Analysis of cell death induced by genetic silencing of Mcl-1 using RNA interference. Results of WB (a-c), flow cytometry with annexin and PI double staining (d, f), and subG1 (e) in HeLa/HeLa-Res (a, d), SK-N-Be(2)c/SK-N-Be(2)c-Res (b, e), and H23/H23-Res (c, f) cells. Transfection period: 24 h. NT (non-target) – non-target siRNA. Data are presented as mean ± standard deviation, $n = 4$; * $p < 0.05$, n.s. – not significant (U-test).

HeLa/HeLa-Res and H23/H23-Res cell lines were subsequently examined for mutations in *MCL1* using NGS; however, no changes in *MCL1* were detected. Additionally, not only an increase in the anti-apoptotic proteins but also a decrease in the proapoptotic proteins (primarily the Bak and Bax effector proteins) of the Bcl-2 family could mediate acquired chemotherapy resistance, as has been demonstrated, for example, after treatment with the monoclonal antibody drug Rituximab [57]. In our study, WB analysis of all three models did not reveal any significant changes in Bak and Bax protein levels in resistant cells compared to parental cells (Fig. S5 in the Online Resource 1).

Comparative assessment of proliferative activity and metabolism in parental and resistant to the BH3-mimetic S63845 cells. Malignant clones are capable of altering their proliferative activity and developing various metabolic adaptations during the development of resistance to chemotherapy. We initially analyzed potential changes in proliferation rate in cell populations resistant to Mcl-1 suppression using a clonogenic assay. In all three models, there was no statistically significant change in the proliferation

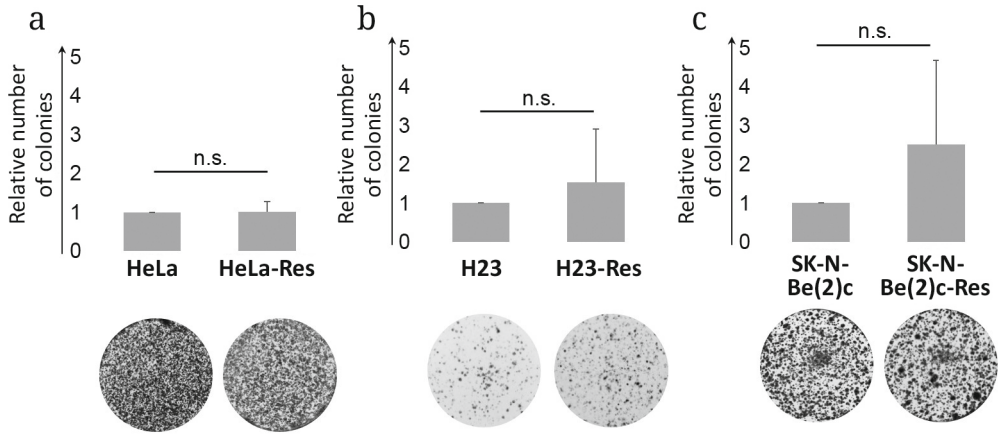

Fig. 5. Assessment of proliferative activity. Results of clonogenic assay for parental and S63845-resistant HeLa (a), H23 (b), and SK-N-Be(2)c (c) cells. Data are presented as mean ± standard deviation, $n = 4$; * $p < 0.05$, n.s. – not significant (U-test).

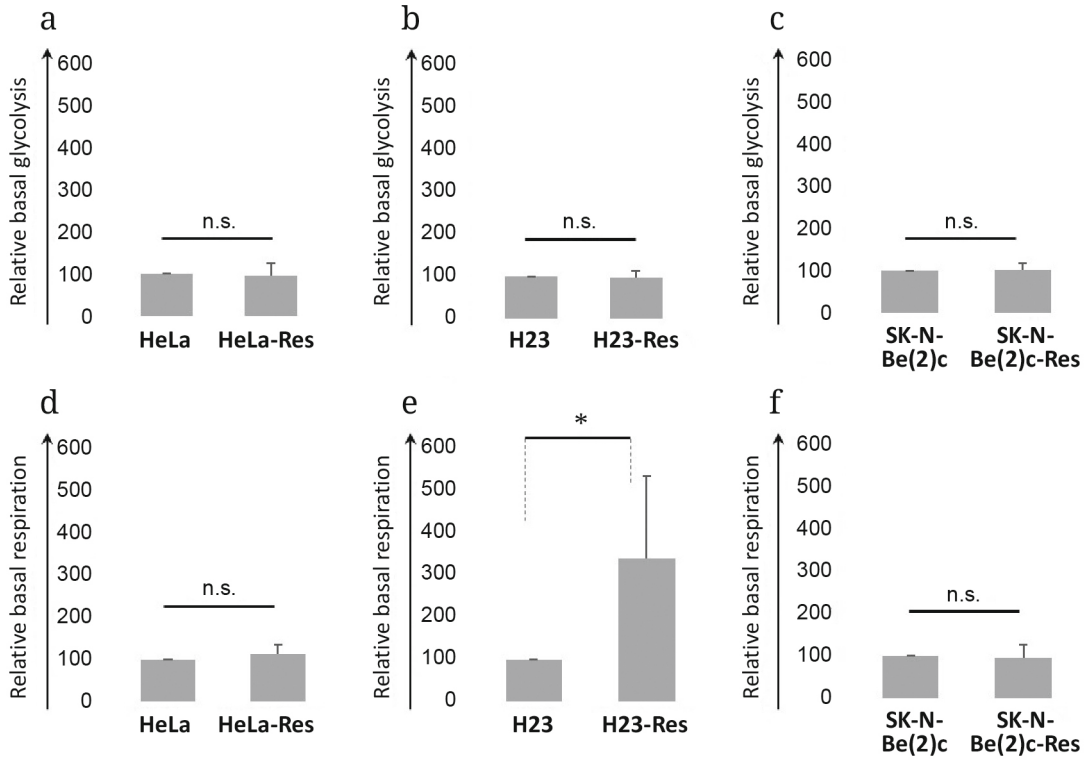

Fig. 6. Basal glycolysis (a-c) and respiration (d-f) in parental and S63845-resistant HeLa (a, d), H23 (b, e), and SK-N-Be(2)c (c, f) cells. Data are presented as mean ± standard deviation, $n = 4$ (e); $n = 3$ (a-d, e); * $p < 0.05$, n.s. – not significant (U-test).

rate of resistant cells compared to the wild-type cells (Fig. 5, a-c).

Next, parental and S63845-resistant cells from all three lines were examined for possible metabolic changes using the Seahorse technology (Fig. 6).

In all three experimental models, basal glycolytic activity was unchanged in resistant clones compared to the original cell lines (Fig. 6, a-c). Basal respiration also remained unchanged in the HeLa/HeLa-Res and SK-N-Be(2)c/SK-N-Be(2)c-Res pairs (Fig. 6, d, f). However, a significant increase in cellular respiration was observed in resistant H23 cells compared to the parental H23 line (Fig. 6e), which likely contributes to the development of acquired resistance to Mcl-1 inhibition.

Analysis of the efficiency of cisplatin and other BH3-mimetics in overcoming cancer cell resistance to Mcl-1 inhibition. To overcome acquired cancer cell resistance to the BH3-mimetic S63845, it is worthwhile to combine this compound with other chemotherapeutic agents. We previously demonstrated that combining the DNA-damaging agent cisplatin or selective

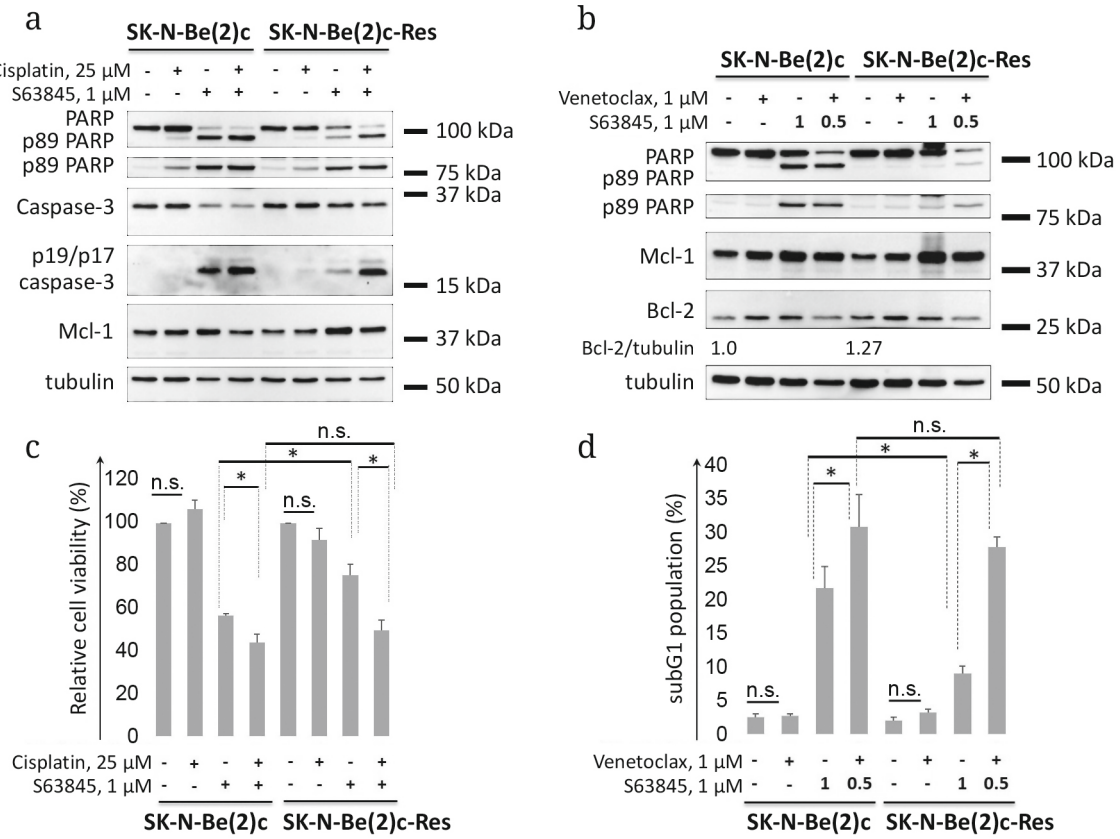

Fig. 7. Evaluation of the efficacy of cisplatin (a, c) and Venetoclax (b, d) in overcoming resistance of SK-N-Be(2)c cells to S63845. WB (a, b), MTS assay (c), and subG1 (d) results. Incubation period: 24 h. Data are presented as mean ± standard deviation, $n = 4$; * $p < 0.05$, n.s. – not significant (U-test).

antagonists of the anti-apoptotic proteins Bcl-2/Bcl-xL with S63845 can effectively overcome cancer cell resistance to the latter in HeLa and H23 cells [33]. In our work, similar results were obtained in SK-N-Be(2)c cells (Fig. 7; Fig. S6 in the Online Resource 1).

Thus, cisplatin (Fig. 7, a, c), Venetoclax (Fig. 7, b, d), and the BH3-mimetic to Bcl-xL A1331851 (Fig. S6, a-c in the Online Resource 1), when used in combination with S63845, enhanced the cytotoxicity of the latter and led to the comparable induction of cell death in both parental and Mcl-1 inhibition-resistant neuroblastoma cells, overcoming this type of resistance. Furthermore, as we have previously shown in other models [33], co-inhibition of Mcl-1 and Bcl-xL using the corresponding BH3-mimetics at low concentrations (100 nM) led to the pronounced activation of apoptosis in SK-N-Be(2)c and SK-N-Be(2)c-Res cells after just 6 h (Fig. S6a in the Online Resource 1).

Analysis of the efficiency of the GSK3 inhibitor to overcome cancer cell resistance to Mcl-1 inhibition. GSK3 is a serine–threonine protein kinase involved in regulating multiple intracellular signaling pathways. For example, in addition to its primary function of controlling glycogen metabolism, GSK3 plays a key role in carcinogenesis by activating the NF-κB signaling pathway, one of the key pathways that promotes cell survival. Activation of the NF-κB-dependent cascade could lead to inhibition of apoptosis, promote survival of malignant clones, and underlie resistance to chemotherapy. Therefore, GSK3 is a promising target for anticancer therapy [58-60]. Earlier studies have demonstrated the ability of GSK3 to regulate Mcl-1 [61, 62]. Therefore, a combination of inhibitors of both proteins could be effective in the induction of cancer cell death. Here, we assessed the feasibility of using CHIR99021, an inhibitor of the two major GSK3 isoforms GSK3α and GSK3β, to overcome cancer cell resistance to the BH3-mimetic S63845 (Fig. 8, a, b).

WB and flow cytometry analyses revealed that CHIR99021, used as a single agent, did not induce cell death in either parental or S63845-resistant HeLa cells. However, it did enhance S63845-mediated cell death in wild-type and, especially, in resistant HeLa clones, thereby overcoming resistance of this line to Mcl-1 inhibition (Fig. 8). Similar results were observed in H23/H23-Res (Fig. S7, a, c in the Online Resource 1) and SK-N-Be(2)c/SK-N-Be(2)c-Res (Fig. S7, b, d in the Online Resource 1) cells, as determined by WB and the MTS assay.

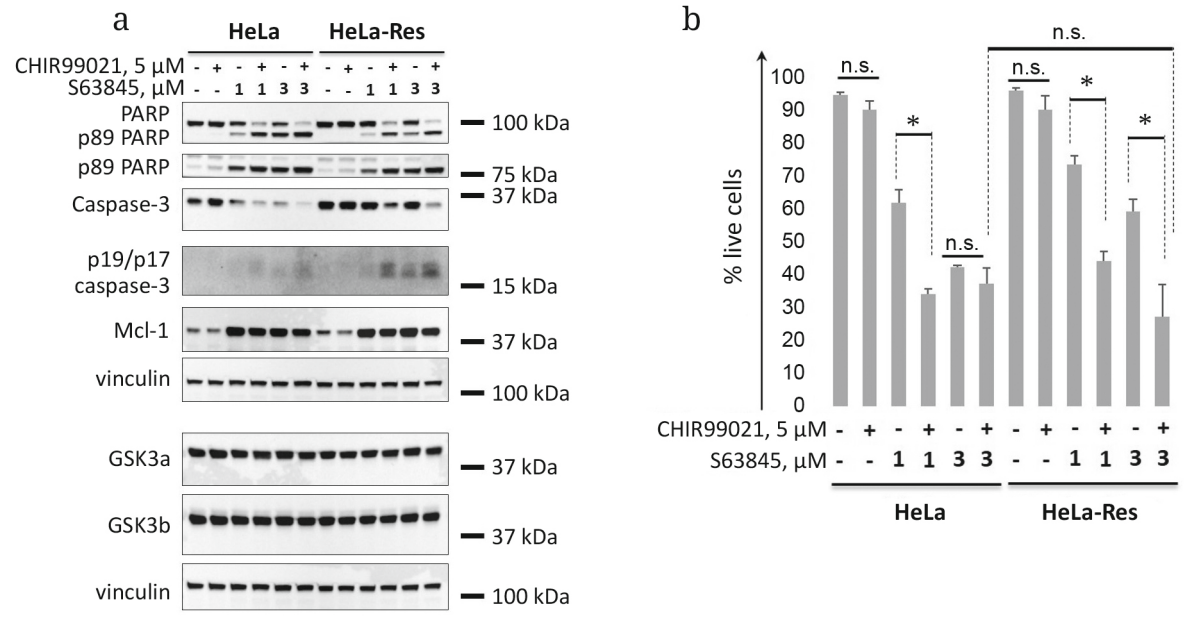

Fig. 8. Evaluation of the efficacy of the GSK3 inhibitor CHIR99021 in overcoming resistance of HeLa cells to S63845. Results of WB (a) and flow cytometry with annexin and PI double staining (b). Incubation period: 24 h. Data are presented as mean ± standard deviation, $n = 4$; * $p < 0.05$, n.s. – not significant (U-test).

Analysis of the efficiency of MDM2 antagonist and inhibitors of cellular respiration in overcoming resistance of H23 cells to Mcl-1 inhibition. The transcription factor p53 is a critical tumor suppressor; however, its functional activity is typically suppressed in tumor cells due to either MDM2, a negative p53 regulator, or mutations in the structure of the p53 gene. In the first case, MDM2 antagonists, which have been actively studied in clinical trials over the past few years, could be utilized. In the second case, reactivators of mutant p53 could be used [63-65]. However, it has been found that MDM2 antagonists can also exhibit antitumor activity in cells regardless of the presence of p53 and its status [66-68]. Here, using the H23 cells, we found that the MDM2 antagonist Nutlin-3a, progenitor of this group of compounds [69], alone did not cause death of the original and S63845-resistant cells. However, it enhanced the cytotoxicity of S63845 in resistant clones, thereby overcoming their resistance to Mcl-1 inhibition (Fig. S8 in the Online Resource 1).

Next, we analyzed the feasibility of targeting cellular respiration to overcome the resistance of H23 cells to Mcl-1 inhibition. For this purpose, the ATP synthase inhibitor oligomycin, the protonophore CCCP, and inhibitors of ETC complexes I (rotenone) and II (TTFA) were used (Fig. 9, Fig. S9 in the Online Resource 1).

According to the results of WB analysis and the Alamar Blue assay, we found that only rotenone alone reduced the viability of the original H23 cells (Fig. 9, b, d). At the same time, rotenone, TTFA, and oligomycin statistically significantly reduced viability of resistant H23 cells (by 10-25%) according to the Alamar Blue assay; however, according to the WB results, they did not cause cleavage of the apoptotic markers (caspase-3 and/or PARP protein) (Fig. 9, Fig. S9 in the Online Resource 1). Since the Alamar Blue assay, like the MTT/MTS tests, evaluates not only the direct viability of cells, but also their metabolic and proliferative activities [70], it can be assumed that the above-mentioned agents in H23-Res cells suppressed their metabolism, but did not cause initiation of their death at the indicated concentrations. As noted above, the level of respiration in resistant H23 cells is higher than in parental H23 cells, and, therefore, suppression of respiration affected their ability to proliferate. In combination with the Mcl-1 antagonist S63845, all compounds, except CCCP, slightly enhanced cytotoxicity in parental H23 cells and more significantly increased S63845-induced death in H23-Res cells (Fig. 9, Fig. S9 in the Online Resource 1), thereby overcoming resistance to Mcl-1 inhibition and confirming the contribution of metabolic alterations to this type of resistance.

DISCUSSION

The development of acquired drug resistance to chemotherapeutic agents is one of the most important problems in oncology, as decreased sensitivity of malignant clones to therapy leads to cancer progression and subsequent death of patients. As previously noted,

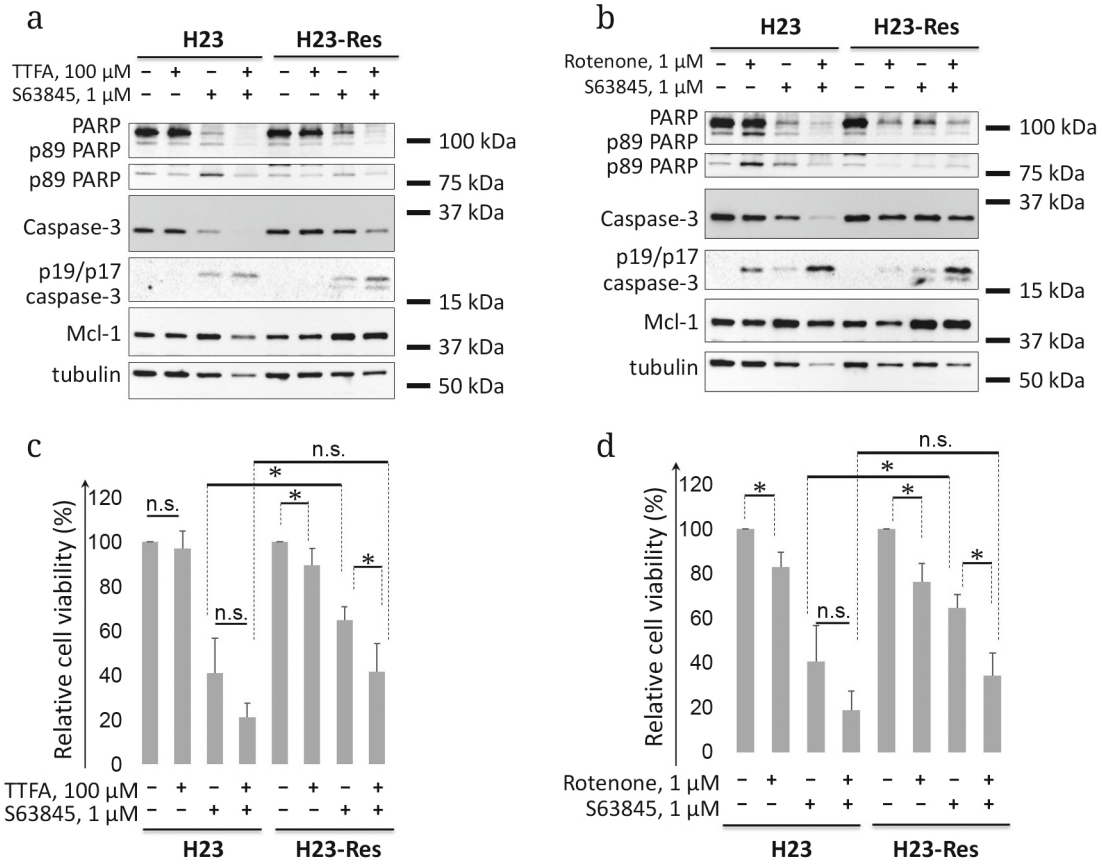

Fig. 9. Evaluation of the efficacy of TTFA (a, c) and rotenone (b, d) in overcoming resistance of H23 cells to S63845. Results of WB (a, b) and Alamar Blue assay (c,d). Incubation period: 24 h. Data are presented as mean ± standard deviation, $n = 4$; * $p < 0.05$, n.s. – not significant (U-test).

inhibition of anti-apoptotic proteins of the Bcl-2 family represents a promising approach for treating cancer patients. Approximately 20 years ago, the first non-selective BH3-mimetic, ABT-737, was developed, followed by the development of selective antagonists of Bcl-2, Bcl-xL, and Mcl-1. However, limited efficacy and observed side effects have not allowed these BH3-mimetics, except for Venetoclax, to be approved for clinical use to date [9]. It should be noted that various approaches to inhibit anti-apoptotic proteins of the Bcl-2 family, including Mcl-1, are in progress [10]. For example, in 2025, a study was launched to evaluate the new compound S227928 in patients with myelodysplastic syndrome, acute myeloid leukemia (AML), and chronic myelomonocytic leukemia, both as an individual agent and in combination with Venetoclax (NCT06563804). S227928 is the Mcl-1 antagonist S64315/MIK665 conjugated to a monoclonal antibody that targets the membrane protein CD74, the gene of which is actively expressed in AML cells [40]. Establishing potential reasons for resistance to Mcl-1 inhibition and identifying approaches to overcome it may contribute to the successful completion of clinical trials for this group of compounds in the future. To generate cell populations resistant to Mcl-1 inhibition, we selected three cell lines of different origins (HeLa, H23, and SK-N-Be(2)c), which are highly sensitive to the suppression of this protein [33]. There are two main strategies for obtaining resistant clones. The first involves culturing tumor cells with high concentrations of chemotherapeutic agents for several months. In this case, the clones most adapted to therapy survive; however, this scheme could lead to artifactual results, since such conditions are not present in the patient's body. Another strategy involves alternating short periods of incubation of the original cells with gradually increasing concentrations of anticancer agents and drug-free periods. This "pulse" approach was chosen in our study, as it mimics the course of treatment for cancer patients [52, 70, 71].

Subsequent analysis revealed that in all three cell lines, the observed resistance to the Mcl-1 antagonist S63845 is not associated with MDR1-linked drug efflux systems. In two of the three cell models of this type of resistance, clones with increased expression of the genes encoding other anti-apoptotic proteins, Bcl-2 in SK-N-Be(2)c cells or Bcl-xL in HeLa

cells, were generated. Moreover, these resistant cells became less sensitive to death triggered by the genetic silencing of Mcl-1. Thus, survival of resistant SK-N-Be(2)c and HeLa cells depends less on Mcl-1 and more on other Bcl-2 family anti-apoptotic proteins, clearly demonstrating the aforementioned compensatory phenomenon of these proteins. Earlier studies have revealed that the loss of cancer cell sensitivity to Venetoclax is accompanied by increased levels of the Bcl-xL and Mcl-1 proteins [8, 20, 35].

While studying the mechanisms of malignant clones' resistance to Venetoclax, it was suggested that the Bcl-2 protein could mutate and alter its structure, thus preventing binding of Venetoclax to its target and neutralizing the anti-apoptotic activity of Bcl-2 [8, 72]. Novel Bcl-2 antagonists, triggering cell death in Venetoclax-resistant cells, have already been developed [73]. Furthermore, mutations could occur in the Bax protein gene, which primarily interacts with Bcl-2, disrupting functional activity of the effector protein and blocking initiation of apoptosis [38]. In our study, no mutations were detected that affected the structure and function of either Mcl-1 or its proapoptotic partner Bak. Currently, little data on mutations in the Mcl-1 structure have been found that could interfere with its inhibition by selective antagonists. Mutations in the *MCL1* and *BAK* genes, which are rare in tumors [35, 74], appear to be an unlikely cause of this type of resistance; however, this issue requires further clarification [35].

The presence of drug resistance is often characterized not only by the decrease in cell death activation but also by metabolic adaptations and changes in proliferation rates, and both modifications could change in opposite directions. Indeed, previously we found that resistance to cisplatin is accompanied by a decrease in proliferation and metabolism of mutant clones [70], while the opposite pattern was observed in the case of resistance to the MDM2 antagonist RG7388 [46]. In other models of cisplatin resistance, resistant clones could switch either to the predominantly anaerobic type of metabolism, increasing glycolysis and slowing oxidative phosphorylation, or to an aerobic type with opposite metabolic changes [75]. Here, proliferative activity of wild-type and S63845-resistant cells has not changed, and no changes in glycolysis occurred. Only in resistant H23 cells was an increase in cellular respiration detected. Since the profile of anti-apoptotic proteins of the Bcl-2 family remained unchanged, the detected metabolic adaptation could determine resistance to Mcl-1 inhibition. Its importance for the survival of H23 cells was confirmed by us using various inhibitors of cellular respiration, which enhanced the cytotoxicity of S63845 and led to overcoming the resistance to its action.

Finally, combining various chemotherapeutic agents is a rational approach in clinical practice, allowing both to prevent the development of acquired resistance by using reduced concentrations of each drug, thereby reducing the toxic effect on healthy cells, and to overcome the formed resistance by enhancing the cytotoxic effect compared to monotherapy. A promising direction is the combined use of various BH3-mimetics, which could reduce the above-mentioned compensatory phenomenon. In addition, combinations of BH3-mimetics and DNA-damaging agents, or MDM2 antagonists, have the potential for high therapeutic effect [35]. The feasibility of combining S63845 with Venetoclax, a Bcl-xL antagonist, cisplatin, and MDM2 antagonists was demonstrated by us in various experimental models, both in this study and in several previous studies [33, 46]. For example, it has recently been shown that the MDM2 antagonist RG7388 could induce both apoptosis and pyroptosis, mediated by, among other factors, reactive oxygen species (ROS) in H23 cells expressing mutant p53 protein [76]. Since we have demonstrated that H23-Res cells exhibit increased cellular respiration, potentially resulting in higher ROS levels, it is likely that the mechanism described above for RG7388 explains the effect of overcoming H23 cell resistance to S63845 when combined with the MDM2 antagonist Nutlin-3a. In addition, we demonstrated for the first time the effectiveness of the combination of selective GSK3 and Mcl-1 antagonists in overcoming cancer cell resistance to the action of the latter.

CONCLUSION

Three cell types, HeLa, H23, and SK-N-Be(2)c, were used to study possible reasons for acquired resistance of cancer cells to the BH3-mimetic S63845, the Mcl-1 antagonist. Various potential mechanisms for avoiding cell death during Mcl-1 inhibition were identified in resistant populations: increased expression of the Bcl-xL or Bcl-2 genes was found in HeLa and SK-N-Be(2)c cells, respectively, and no changes in the levels of anti-apoptotic Bcl-2 family proteins were observed in H23 cells. However, an increased cellular respiration was detected in H23 cells, clearly demonstrating multifactorial mechanisms underlying acquired drug resistance. No mutations that could alter the structure or function of Mcl-1 or its proapoptotic partner, Bak, were found in all selected cell lines. Cell proliferative activity was unchanged in S63845-resistant cell lines, and metabolic adaptations were detected only in H23 cells. Use of the MDM2 antagonist Nutlin-3a or inhibitors of cellular respiration (oligomycin, rotenone, and TTFA) enhanced cytotoxicity of S63845 in resistant H23 cells, which led to overcoming resistance to its action.

Finally, in addition to combining S63845 with cisplatin or BH3 mimetics, the feasibility of combining the Mcl-1 antagonist with the GSK3 inhibitor CHIR99021 was demonstrated in all models.

Abbreviations

Bak	Bcl-2 homologous antagonist killer
Bax	Bcl-2-associated X protein
Bcl-2	B-cell leukemia/lymphoma protein-2
BH-domain	Bcl-2 homology domain
CCCP	carbonyl cyanide-m-chlorophenylhydrazone
FITC	fluorescein isothiocyanate
GSK3	glycogen synthase kinase 3
MDM2	mouse double minute 2 homolog
Mcl-1	myeloid cell leukemia protein-1
MDR1	multidrug resistance protein 1
MOMP	mitochondrial outer membrane permeabilization
NGS	next-generation sequencing
p19/17	catalytically active fragments of effector caspase-3
caspase-3	
PARP	poly(ADP-ribose) polymerase
p89 PARP	cleaved fragment of the PARP protein
PCR	polymerase chain reaction
TTFA	thenoyltrifluoroacetone

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1134/S0006297925602710.

Contributions

N. V. Pervushin, B. Zhivotovsky, and G. S. Kopeina conceived and supervised the study; N. V. Pervushin, B. Y. Valdez Fernandez, V. V. Senichkin, M. A. Yaprynt-seva, and V. S. Pavlov performed the experiments; N. V. Pervushin, V. V. Senichkin, B. Zhivotovsky, and G. S. Kopeina discussed the study results; N. V. Pervushin wrote the text and prepared the figures; B. Zhivotovsky and G. S. Kopeina edited the text.

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Ethics approval and consent to participate

This work does not contain any studies involving human and animal subjects.

Conflict of interest

The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. Hanahan, D., and Weinberg, R. A. (2011) Hallmarks of cancer: the next generation, *Cell*, **144**, 646-674, https://doi.org/10.1016/j.cell.2011.02.013.
2. Hanahan, D. (2022) Hallmarks of cancer: new dimensions, *Cancer Discov.*, **12**, 31-46, https://doi.org/10.1158/2159-8290.CD-21-1059.
3. Mustafa, M., Ahmad, R., Tantry, I. Q., Ahmad, W., Siddiqui, S., Alam, M., Abbas, K., Moinuddin, Hassan, M. I., Habib, S., and Islam, S. (2024) Apoptosis: a comprehensive overview of signaling pathways, morphological changes, and physiological significance and therapeutic implications, *Cells*, **13**, 1838, https://doi.org/10.3390/cells13221838.
4. Elmore, S. (2007) Apoptosis: a review of programmed cell death, *Toxicol. Pathol.*, **35**, 495-516, https://doi.org/10.1080/01926230701320337.
5. Moyer, A., Tanaka, K., and Cheng, E. H. (2025) Apoptosis in cancer biology and therapy, *Annu. Rev. Pathol.*, **20**, 303-328, https://doi.org/10.1146/annurev-pathmechdis-051222-115023.
6. Czabotar, P. E., Lessene, G., Strasser, A., and Adams, J. M. (2014) Control of apoptosis by the Bcl-2 protein family: implications for physiology and therapy, *Nat. Rev. Mol. Cell Biol.*, **15**, 49-63, https://doi.org/10.1038/nrm3722.
7. Czabotar, P. E., and Garcia-Saez, A. J. (2023) Mechanisms of Bcl-2 family proteins in mitochondrial apoptosis, *Nat. Rev. Mol. Cell Biol.*, **24**, 732-748, https://doi.org/10.1038/s41580-023-00629-4.
8. Senichkin, V. V., Pervushin, N. V., Zuev, A. P., Zhivotovsky, B., and Kopeina, G. S. (2020) Targeting Bcl-2 family proteins: what, where, when? *Biochemistry (Moscow)*, **85**, 1210-1226, https://doi.org/10.1134/S0006297920100090.
9. Croce, C. M., Vaux, D., Strasser, A., Opferman, J. T., Czabotar, P. E., and Fesik, S. W. (2025) The Bcl-2 protein family: from discovery to drug development, *Cell Death Differ.*, **32**, 1369-1381, https://doi.org/10.1038/s41418-025-01481-z.
10. Vogler, M., Braun, Y., Smith, V. M., Westhoff, M.-A., Pereira, R. S., Pieper, N. M., Anders, M., Callens, M., Vervliet, T., Abbas, M., Macip, S., Schmid, R., Bultynck, G., and Dyer, M. J. (2025) The BCL2 family: from apoptosis mechanisms to new advances in targeted therapy, *Signal Transduct. Targeted Ther.*, **10**, 91, https://doi.org/10.1038/s41392-025-02176-0.
11. Adams, J. M., and Cory, S. (1998) The Bcl-2 protein family: arbiters of cell survival, *Science*, **281**, 1322-1326, https://doi.org/10.1126/science.281.5381.1322.
12. Burlacu, A. (2003) Regulation of apoptosis by Bcl-2 family proteins, *J. Cell. Mol. Med.*, **7**, 249-257, https://doi.org/10.1111/j.1582-4934.2003.tb00225.x.
13. Westphal, D., Kluck, R. M., and Dewson, G. (2014) Building blocks of the apoptotic pore: how Bax and

Bak are activated and oligomerize during apoptosis, *Cell Death Differ.*, **21**, 196-205, https://doi.org/10.1038/cdd.2013.139.
14. Peña-Blanco, A., and García-Sáez, A. J. (2018) Bax, Bak and beyond – mitochondrial performance in apoptosis, *FEBS J.*, **285**, 416-431, https://doi.org/10.1111/febs.14186.
15. Kale, J., Osterlund, E. J., and Andrews, D. W. (2018) Bcl-2 family proteins: changing partners in the dance towards death, *Cell Death Differ.*, **25**, 65-80, https://doi.org/10.1038/cdd.2017.186.
16. Glab, J. A., Mbogo, G. W., and Puthalakath, H. (2017) BH3-only proteins in health and disease, *Int. Rev. Cell Mol. Biol.*, **328**, 163-196, https://doi.org/10.1016/bs.ircmb.2016.08.005.
17. Doerflinger, M., Glab, J. A., and Puthalakath, H. (2015) BH3-only proteins: a 20-year stock-take, *FEBS J.*, **282**, 1006-1016, https://doi.org/10.1111/febs.13190.
18. Pervushin, N. V., Nilov, D. K., Zhivotovsky, B., and Kopeina, G. S. (2025) Bcl-2 modifying factor (Bmf): "a mysterious stranger" in the Bcl-2 family proteins, *Cell Death Differ.*, https://doi.org/10.1038/s41418-025-01562-z.
19. Pervushin, N. V., Kopeina, G. S., and Zhivotovsky, B. (2023) Bcl-B: an "unknown" protein of the Bcl-2 family, *Biol. Direct*, **18**, 69, https://doi.org/10.1186/s13062-023-00431-4.
20. Pervushin, N. V., Senichkin, V. V., Zhivotovsky, B., and Kopeina, G. S. (2020) Mcl-1 as a "barrier" in cancer treatment: can we target it now? *Int. Rev. Cell Mol. Biol.*, **351**, 23-55, https://doi.org/10.1016/bs.ircmb.2020.01.002.
21. Kaloni, D., Diepstraten, S. T., Strasser, A., and Kelly, G. L. (2023) Bcl-2 protein family: attractive targets for cancer therapy, *Apoptosis*, **28**, 20-38, https://doi.org/10.1007/s10495-022-01780-7.
22. Garner, T. P., Lopez, A., Reyna, D. E., Spitz, A. Z., and Gavathiotis, E. (2017) Progress in targeting the Bcl-2 family of proteins, *Curr. Opin. Chem. Biol.*, **39**, 133-142, https://doi.org/10.1016/j.cbpa.2017.06.014.
23. Cavallo, M. R., Yo, J. C., Gallant, K. C., Cunanan, C. J., Amirfallah, A., Daniali, M., Sanders, A. B., Aplin, A. E., Pribitkin, E. A., and Hartsough, E. J. (2024) Mcl-1 mediates intrinsic resistance to RAF inhibitors in mutant BRAF papillary thyroid carcinoma, *Cell Death Discov.*, **10**, 175, https://doi.org/10.1038/s41420-024-01945-0.
24. Dolnikova, A., Kazantsev, D., Klanova, M., Pokorna, E., Sovilj, D., Kelemen, C. D., Tuskova, L., Hoferkova, E., Mraz, M., Helman, K., Curik, N., Machova Polakova, K., Andera, L., Trneny, M., and Klener, P. (2024) Blockage of Bcl-xL overcomes venetoclax resistance across BCL^{2+} lymphoid malignancies irrespective of BIM status, *Blood Adv.*, **8**, 3532-3543, https://doi.org/10.1182/bloodadvances.2024012906.
25. Townsend, P. A., Kozhevnikova, M. V., Cexus, O. N. F., Zamyatnin, A. A., and Soond, S. M. (2021) BH3-mimetics: recent developments in cancer therapy, *J. Exp. Clin. Cancer Res.*, **40**, 355, https://doi.org/10.1186/s13046-021-02157-5.
26. Seymour, J. (2019) Venetoclax, the first Bcl-2 inhibitor for use in patients with chronic lymphocytic leukemia, *Clin. Adv. Hematol. Oncol.*, **17**, 440-443.
27. Brinkmann, K., McArthur, K., Malelang, S., Gibson, L., Tee, A., Elahee Doomun, S. N., Rowe, C. L., Arandjelovic, P., Marchingo, J. M., D'Silva, D., Bachem, A., Monard, S., Whelan, L. G., Dewson, G., Putoczki, T. L., Bouillet, P., Fu, N. Y., Brown, K. K., Kueh, A. J., Wimmer, V. C., Herold, M. J., Thomas, T., Voss, A. K., and Strasser, A. (2025) Relative importance of the anti-apoptotic versus apoptosis-unrelated functions of Mcl-1 *in vivo*, *Science*, **389**, 1003-1011, https://doi.org/10.1126/science.adw1836.
28. Perciavalle, R. M., Stewart, D. P., Koss, B., Lynch, J., Milasta, S., Bathina, M., Temirov, J., Cleland, M. M., Pelletier, S., Schuetz, J. D., Youle, R. J., Green, D. R., and Opferman, J. T. (2012) Anti-apoptotic Mcl-1 localizes to the mitochondrial matrix and couples mitochondrial fusion to respiration, *Nat. Cell Biol.*, **14**, 575-583, https://doi.org/10.1038/ncb2488.
29. Senichkin, V. V., Streletskaia, A. Y., Gorbunova, A. S., Zhivotovsky, B., and Kopeina, G. S. (2020) Saga of Mcl-1: regulation from transcription to degradation, *Cell Death Differ.*, **27**, 405-419, https://doi.org/10.1038/s41418-019-0486-3.
30. Senichkin, V. V., Streletskaia, A. Y., Zhivotovsky, B., and Kopeina, G. S. (2019) Molecular comprehension of Mcl-1: from gene structure to cancer therapy, *Trends Cell Biol.*, **29**, 549-562, https://doi.org/10.1016/j.tcb.2019.03.004.
31. Pervushin, N. V., Senichkin, V. V., Kapusta, A. A., Gorbunova, A. S., Kaminskyy, V. O., Zhivotovsky, B., and Kopeina, G. S. (2020) Nutrient deprivation promotes Mcl-1 degradation in an autophagy-independent manner, *Biochemistry (Moscow)*, **85**, 1235-1244, https://doi.org/10.1134/S0006297920100119.
32. Srivastava, S., Sekar, G., Ojoawo, A., Aggarwal, A., Ferreira, E., Uchikawa, E., Yang, M., Grace, C. R., Dey, R., Lin, Y.-L., Guibao, C. D., Jayaraman, S., Mukherjee, S., Kossiakoff, A. A., Dong, B., Myasnikov, A., and Moldoveanu, T. (2025) Structural basis of BAK sequestration by Mcl-1 in apoptosis, *Mol. Cell*, **85**, 1606-1623.e10, https://doi.org/10.1016/j.molcel.2025.03.013.
33. Senichkin, V. V., Pervushin, N. V., Zamaraev, A. V., Sazonova, E. V., Zuev, A. P., Streletskaia, A. Y., Prikazchikova, T. A., Zatsepin, T. S., Kovaleva, O. V., Tchevkina, E. M., Zhivotovsky, B., and Kopeina, G. S. (2021) Bak and Bcl-xL participate in regulating sensitivity of solid tumor derived cell lines to Mcl-1 inhibitors, *Cancers*, **14**, 181, https://doi.org/10.3390/cancers14010181.
34. Gomez-Bougie, P., Ménoret, E., Juin, P., Dousset, C., Pellat-Deceunynck, C., and Amiot, M. (2011) Noxa controls Mule-dependent Mcl-1 ubiquitination through

the regulation of the Mcl-1/USP9X interaction, *Biochem. Biophys. Res. Commun.*, **413**, 460-464, https://doi.org/10.1016/j.bbrc.2011.08.118.

35. Tantawy, S. I., Timofeeva, N., Sarkar, A., and Gandhi, V. (2023) Targeting Mcl-1 protein to treat cancer: opportunities and challenges, *Front. Oncol.*, **13**, 1226289, https://doi.org/10.3389/fonc.2023.1226289.
36. Mason, K. D., Carpinelli, M. R., Fletcher, J. I., Collinge, J. E., Hilton, A. A., Ellis, S., Kelly, P. N., Ekert, P. G., Metcalf, D., Roberts, A. W., Huang, D. C. S., and Kile, B. T. (2007) Programmed anuclear cell death delimits platelet life span, *Cell*, **128**, 1173-1186, https://doi.org/10.1016/j.cell.2007.01.037.
37. Vasan, N., Baselga, J., and Hyman, D. M. (2019) A view on drug resistance in cancer, *Nature*, **575**, 299-309, https://doi.org/10.1038/s41586-019-1730-1.
38. Moujalled, D. M., Brown, F. C., Chua, C. C., Dengler, M. A., Pomilio, G., Anstee, N. S., Litalien, V., Thompson, E., Morley, T., MacRaild, S., Tiong, I. S., Morris, R., Dun, K., Zordan, A., Shah, J., Banquet, S., Halilovic, E., Morris, E., Herold, M. J., Lessene, G., Adams, J. M., Huang, D. C. S., Roberts, A. W., Blombery, P., and Wei, A. H. (2023) Acquired mutations in BAX confer resistance to BH3-mimetic therapy in acute myeloid leukemia, *Blood*, **141**, 634-644, https://doi.org/10.1182/blood.2022016090.
39. Zielonka, K., and Jamroziak, K. (2024) Mechanisms of resistance to venetoclax in hematologic malignancies, *Adv. Clin. Exp. Med.*, **33**, 1421-1433, https://doi.org/10.17219/acem/181145.
40. Maragno, A.-L., Seiss, K., Newcombe, R., Mistry, P., Schnell, C. R., Von Arx, F., Koenig, J., Malamas, A. S., Le Toumelin-Braizat, G., Bresson, L., Rocchetti, F., Demarles, D., Kurth, E., Renteria, L., Hainzl, D., Engelhardt, V., Liot, C., Madelain, V., Kostova, V., Starck, J.-B., Valour, D., Franzetti, G.-A., Colland, F., Vostiarova, H., Tschantz, W. R., Bachmann, N., Palacio-Ramirez, S., Burger, M. T., Campbell, J. M., Chen, Z., Koenig, R., McNeill, E., Mo, R., Nakajima, K., Palermo, M. G., Shen, Y., Yu, B., Zambrowski, M., Zhang, A., Zecri, F., Broniscer, A., Geneste, O., Horton, K., and D'Alessio, J. A. (2024) S227928: A novel anti-CD74 ADC with Mcl-1 inhibitor payload for the treatment of acute myeloid leukemia (AML) and other hematologic malignancies, *Blood*, **144**, 1381, https://doi.org/10.1182/blood-2024-210048.
41. Gongalsky, M. B., Tsurikova, U. A., Kudryavtsev, A. A., Pervushin, N. V., Sviridov, A. P., Kumeria, T., Egoshina, V. D., Tyurin-Kuzmin, P. A., Naydov, I. A., Gonchar, K. A., Kopeina, G. S., Andreev, V. G., Zhivotovsky, B., and Osminkina, L. A. (2025) Amphiphilic photoluminescent porous silicon nanoparticles as effective agents for ultrasound-amplified cancer therapy, *ACS Appl. Mater. Interf.*, **17**, 374-385, https://doi.org/10.1021/acsami.4c15725.
42. Gongalsky, M. B., Pervushin, N. V., Maksutova, D. E., Tsurikova, U. A., Putintsev, P. P., Gyuppenen, O. D., Evstratova, Y. V., Shalygina, O. A., Kopeina, G. S., Kudryavtsev, A. A., Zhivotovsky, B., and Osminkina, L. A. (2021) Optical monitoring of the biodegradation of porous and solid silicon nanoparticles, *Nanomaterials (Basel, Switzerland)*, **11**, 2167, https://doi.org/10.3390/nano11092167.
43. Crowley, L. C., Marfell, B. J., Scott, A. P., Boughaba, J. A., Chojnowski, G., Christensen, M. E., and Waterhouse, N. J. (2016) Dead cert: measuring cell death, *Cold Spring Harb. Protocols*, **2016**, pdb.top070318, https://doi.org/10.1101/pdb.top070318.
44. Plesca, D., Mazumder, S., and Almasan, A. (2008) DNA damage response and apoptosis, *Methods Enzymol.*, **446**, 107-122, https://doi.org/10.1016/S0076-6879(08)01606-6.
45. Pfaffl, M. W. (2001) A new mathematical model for relative quantification in real-time RT-PCR, *Nucleic Acids Res.*, **29**, e45, https://doi.org/10.1093/nar/29.9.e45.
46. Pervushin, N. V., Nilov, D. K., Pushkarev, S. V., Shipunova, V. O., Badlaeva, A. S., Yapryntseva, M. A., Kopytova, D. V., Zhivotovsky, B., and Kopeina, G. S. (2024) BH3-mimetics or DNA-damaging agents in combination with RG7388 overcome p53 mutation-induced resistance to MDM2 inhibition, *Apoptosis*, **29**, 2197-2213, https://doi.org/10.1007/s10495-024-02014-8.
47. Leroy, B., Girard, L., Hollestelle, A., Minna, J. D., Gazdar, A. F., and Soussi, T. (2014) Analysis of TP53 mutation status in human cancer cell lines: a reassessment, *Hum. Mutat.*, **35**, 756-765, https://doi.org/10.1002/humu.22556.
48. Al-Ghabkari, A., and Narendran, A. (2019) *In vitro* characterization of a potent p53-MDM2 Inhibitor, RG7112 in neuroblastoma cancer cell lines, *Cancer Biother. Radiopharmaceut.*, **34**, 252-257, https://doi.org/10.1089/cbr.2018.2732.
49. Ajay, A. K., Meena, A. S., and Bhat, M. K. (2012) Human papillomavirus 18 E6 inhibits phosphorylation of p53 expressed in HeLa cells, *Cell Biosci.*, **2**, 2, https://doi.org/10.1186/2045-3701-2-2.
50. Diepstraten, S. T., Yuan, Y., La Marca, J. E., Young, S., Chang, C., Whelan, L., Ross, A. M., Fischer, K. C., Pomilio, G., Morris, R., Georgiou, A., Litalien, V., Brown, F. C., Roberts, A. W., Strasser, A., Wei, A. H., and Kelly, G. L. (2024) Putting the STING back into BH3-mimetic drugs for TP53-mutant blood cancers, *Cancer Cell*, **42**, 850-868.e9, https://doi.org/10.1016/j.ccell.2024.04.004.
51. Gentile, M., Petrungaro, A., Uccello, G., Vigna, E., Recchia, A. G., Caruso, N., Bossio, S., De Stefano, L., Palummo, A., Storino, F., Martino, M., and Morabito, F. (2017) Venetoclax for the treatment of chronic lymphocytic leukemia, *Expert Opin. Invest. Drugs*, **26**, 1307-1316, https://doi.org/10.1080/13543784.2017.1386173.

52. McDermott, M., Eustace, A. J., Busschots, S., Breen, L., Crown, J., Clynes, M., O'Donovan, N., and Stordal, B. (2014) *In vitro* development of chemotherapy and targeted therapy drug-resistant cancer cell lines: a practical guide with case studies, *Front. Oncol.*, **4**, 40, https://doi.org/10.3389/fonc.2014.00040.
53. Lazebnik, Y. A., Kaufmann, S. H., Desnoyers, S., Poirier, G. G., and Earnshaw, W. C. (1994) Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE, *Nature*, **371**, 346-347, https://doi.org/10.1038/371346a0.
54. Gottesman, M. M., Pastan, I., and Ambudkar, S. V. (1996) P-glycoprotein and multidrug resistance, *Curr. Opin. Genetics Dev.*, **6**, 610-617, https://doi.org/10.1016/s0959-437x(96)80091-8.
55. Dong, J., Yuan, L., Hu, C., Cheng, X., and Qin, J.-J. (2023) Strategies to overcome cancer multidrug resistance (MDR) through targeting P-glycoprotein (ABCB1): an updated review, *Pharmacol. Ther.*, **249**, 108488, https://doi.org/10.1016/j.pharmthera.2023.108488.
56. Bolomsky, A., Miettinen, J. J., Malyutina, A., Besse, A., Huber, J., Fellinger, S., Breid, H., Parsons, A., Klavins, K., Hannich, J. T., Kubicek, S., Caers, J., Hübl, W., Schreder, M., Zojer, N., Driessen, C., Tang, J., Besse, L., Heckman, C. A., and Ludwig, H. (2021) Heterogeneous modulation of Bcl-2 family members and drug efflux mediate Mcl-1 inhibitor resistance in multiple myeloma, *Blood Adv.*, **5**, 4125-4139, https://doi.org/10.1182/bloodadvances.2020003826.
57. Olejniczak, S. H., Hernandez-Ilizaliturri, F. J., Clements, J. L., and Czuczman, M. S. (2008) Acquired resistance to rituximab is associated with chemotherapy resistance resulting from decreased Bax and Bak expression, *Clin. Cancer Res.*, **14**, 1550-1560, https://doi.org/10.1158/1078-0432.CCR-07-1255.
58. Dolcet, X., Llobet, D., Pallares, J., and Matias-Guiu, X. (2005) NF-kB in development and progression of human cancer, *Virchows Arch.*, **446**, 475-482, https://doi.org/10.1007/s00428-005-1264-9.
59. Lin, J., Song, T., Li, C., and Mao, W. (2020) GSK-3β in DNA repair, apoptosis, and resistance of chemotherapy, radiotherapy of cancer, *Biochim. Biophys. Acta*, **1867**, 118659, https://doi.org/10.1016/j.bbamcr.2020.118659.
60. Duda, P., Akula, S. M., Abrams, S. L., Steelman, L. S., Martelli, A. M., Cocco, L., Ratti, S., Candido, S., Libra, M., Montalto, G., Cervello, M., Gizak, A., Rakus, D., and McCubrey, J. A. (2020) Targeting GSK3 and associated signaling pathways involved in cancer, *Cells*, **9**, 1110, https://doi.org/10.3390/cells9051110.
61. Wang, R., Xia, L., Gabrilove, J., Waxman, S., and Jing, Y. (2013) Downregulation of Mcl-1 through GSK-3β activation contributes to arsenic trioxide-induced apoptosis in acute myeloid leukemia cells, *Leukemia*, **27**, 315-324, https://doi.org/10.1038/leu.2012.180.
62. Kang, X.-H., Zhang, J.-H., Zhang, Q.-Q., Cui, Y.-H., Wang, Y., Kou, W.-Z., Miao, Z.-H., Lu, P., Wang, L.-F., Xu, Z.-Y., and Cao, F. (2017) Degradation of Mcl-1 through GSK-3β activation regulates apoptosis induced by bufalin in non-small cell lung cancer H1975 cells, *Cell. Physiol. Biochem.*, **41**, 2067-2076, https://doi.org/10.1159/000475438.
63. Fallatah, M. M. J., Law, F. V., Chow, W. A., and Kaiser, P. (2023) Small-molecule correctors and stabilizers to target p53, *Trends Pharmacol. Sci.*, **44**, 274-289, https://doi.org/10.1016/j.tips.2023.02.007.
64. Hassin, O., and Oren, M. (2023) Drugging p53 in cancer: one protein, many targets, *Nature Reviews. Drug Discovery*, **22**, 127-144, https://doi.org/10.1038/s41573-022-00571-8.
65. Levine, A. J. (2020) p53: 800 million years of evolution and 40 years of discovery, *Nat. Rev. Cancer*, **20**, 471-480, https://doi.org/10.1038/s41568-020-0262-1.
66. Shuvalov, O., Kizenko, A., Shakirova, A., Fedorova, O., Petukhov, A., Aksenov, N., Vasileva, E., Daks, A., and Barlev, N. (2018) Nutlin sensitizes lung carcinoma cells to interferon-alpha treatment in MDM2-dependent but p53-independent manner, *Biochem. Biophys. Res. Commun.*, **495**, 1233-1239, https://doi.org/10.1016/j.bbrc.2017.11.118.
67. Lau, L. M. S., Nugent, J. K., Zhao, X., and Irwin, M. S. (2008) HDM2 antagonist Nutlin-3 disrupts p73-HDM2 binding and enhances p73 function, *Oncogene*, **27**, 997-1003, https://doi.org/10.1038/sj.onc.1210707.
68. Ambrosini, G., Sambol, E. B., Carvajal, D., Vassilev, L. T., Singer, S., and Schwartz, G. K. (2007) Mouse double minute antagonist Nutlin-3a enhances chemotherapy-induced apoptosis in cancer cells with mutant p53 by activating E2F1, *Oncogene*, **26**, 3473-3481, https://doi.org/10.1038/sj.onc.1210136.
69. Vassilev, L. T., Vu, B. T., Graves, B., Carvajal, D., Podlaski, F., Filipovic, Z., Kong, N., Kammlott, U., Lukacs, C., Klein, C., Fotouhi, N., and Liu, E. A. (2004) In vivo activation of the p53 pathway by small-molecule antagonists of MDM2, *Science*, **303**, 844-848, https://doi.org/10.1126/science.1092472.
70. Sazonova, E. V., Yapryntseva, M. A., Pervushin, N. V., Tsvetcov, R. I., Zhivotovsky, B., and Kopeina, G. S. (2024) Cancer drug resistance: targeting proliferation or programmed cell death, *Cells*, **13**, 388, https://doi.org/10.3390/cells13050388.
71. Amaral, M. V. S., de Sousa Portilho, A. J., da Silva, E. L., de Oliveira Sales, L., da Silva Maués, J. H., de Moraes, M. E. A., and Moreira-Nunes, C. A. (2019) Establishment of drug-resistant cell lines as a model in experimental oncology: a review, *Anticancer Res.*, **39**, 6443-6455, https://doi.org/10.21873/anticanres.13858.
72. Ong, F., Kim, K., and Konopleva, M. Y. (2022) Venetoclax resistance: mechanistic insights and future strategies, *Cancer Drug Resist.*, **5**, 380-400, https://doi.org/10.20517/cdr.2021.125.

73. Liu, J., Li, S., Wang, Q., Feng, Y., Xing, H., Yang, X., Guo, Y., Guo, Y., Sun, H., Liu, X., Yang, S., Mei, Z., Zhu, Y., Cheng, Z., Chen, S., Xu, M., Zhang, W., Wan, N., Wang, J., Ma, Y., Zhang, S., Luan, X., Xu, A., Li, L., Wang, H., Yang, X., Hong, Y., Xue, H., Yuan, X., Hu, N., Song, X., Wang, Z., Liu, X., Wang, L., and Liu, Y. (2024) Sonrotoclax overcomes BCL2 G101V mutation-induced venetoclax resistance in preclinical models of hematologic malignancy, *Blood*, **143**, 1825-1836, https://doi.org/10.1182/blood.2023019706.
74. Sakamoto, I., Yamada, T., Ohwada, S., Koyama, T., Nakano, T., Okabe, T., Hamada, K., Kawate, S., Takeyoshi, I., Iino, Y., and Morishita, Y. (2004) Mutational analysis of the BAK gene in 192 advanced gastric and colorectal cancers, *Int. J. Mol. Med.*, **13**, 53-55, https://doi.org/10.3892/ijmm.13.1.53.
75. Pervushin, N. V., Yapryntseva, M. A., Panteleev, M. A., Zhivotovsky, B., and Kopeina, G. S. (2024) Cisplatin resistance and metabolism: simplification of complexity, *Cancers*, **16**, 3082, https://doi.org/10.3390/cancers16173082.
76. Tang, G., Cao, X., Chen, J., Hui, F., Xu, N., Jiang, Y., Lu, H., Xiao, H., Liang, X., Ma, M., Qian, Y., Liu, D., Wang, Z., Liu, S., Yu, G., and Sun, L. (2025) Repurposing MDM2 inhibitor RG7388 for TP53-mutant NSCLC: a p53-independent pyroptotic mechanism via ROS/p-p38/NOXA/caspase-3/GSDME axis, *Cell Death Dis.*, **16**, 452, https://doi.org/10.1038/s41419-025-07770-2.