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Venetoclax Drug Increases the Apoptosis of T and B Acute Lymphoblastic Leukemia Cells by Reducing the Expression of *BCL-2*

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ABSTRACT

Venetoclax, a specific inhibitor of the BCL2 protein, is administered for the treatment of acute lymphoblastic leukemia. However, despite being utilized in conjunction with chemotherapy, the drug exhibits instances of resistance. The exact mechanisms responsible for this resistance remain relatively obscure. Within the context of this investigation, the study aimed to explore the involvement of anti- and pro-apoptotic proteins as one of the potential mechanisms underlying this resistance phenomenon. Blast cells were extracted from patients diagnosed with B&T acute lymphoid leukemia. Subsequently, these cells were subjected to a cultivation process. Following the cultivation, treatment with the Venetoclax drug was administered to both groups of B&T cells. Additionally, one group from each cell type was designated as a control. The relative expression levels of genes *BCL-2*, *MCL-1*, and *BIM* were assessed in comparison to the control group. Annexin V–fluorescein isothiocyanate and propidium iodide staining was done to check cell apoptosis. The results showed a significant increase in the expression of BIM gene and a significant decrease in *BCL-2* gene compared to the control group, but the change in the expression of *MCL-1* gene was not significant. Also, an increase in apoptosis was observed in the treatment groups compared to the control. Although it was shown that changes in the expression of pro- and anti-apoptotic genes can lead to an increase in cell apoptosis and a decrease in the number of blast cells, more studies are needed to investigate the simultaneous effect of Venetoclax drug with other drugs and also in the form of a clinical trial.

Keywords: Venetoclax, acute lymphoblastic leukemia, apoptosis, *BCL-2*

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Introduction

Acute leukemia is a varied group of cancerous growths in the body. They occur when specific cells in the bone marrow rapidly multiply abnormally. These cells can come from either lymphoid or myeloid cells that have turned into cancer (1). Roughly 6000 unused cases of acute lymphoblastic leukemia (ALL) are analyzed in the United States annually. ALL is the most common type of cancer in children, with about 25% of all cancer cases diagnosed as ALL. Around 60% of ALL cases occur in children and young people under 20 year old. In a year, there are 36. 2 cases per 1 million people, with the highest number of cases occurring between the ages of 2 and 5, with more than 90 cases per 1 million people (2, 3). This type of cancer is divided into two groups: B-cell precursors and T-cell precursors. B-cell precursors comprise about 85% of the cases, while the remaining 15% have a T-cell phenotype (4).

T-cell acute lymphoblastic leukemia (T-ALL) could be a harmful clonal development of immature T cells that accounts for 10% to 15% of childhood and 25% of grown-up ALL cases. With more extensive utilization of serious chemotherapy, the estimate for youth T-ALL has moved forward strikingly: about 80% of patients can be cured right now (5, 6). The overexpression of BCL-2 has been extensively documented in acute lymphoblastic leukemia (ALL) via specific molecular and cytogenetic characteristics, indicating that these characteristics may exhibit heightened sensitivity to BCL-2 inhibition (7).

Apoptosis represents an intricately regulated mechanism of cellular demise. Diverging from necrosis, an abrupt mode of cellular extinction, apoptosis embodies a deliberate and reasoned choice to relinquish particular cells, thereby augmenting the overall advantages of the organism. This is an ordinary physiological course of action systematically executed within multicellular organisms (8). Apoptosis is imperative in overseeing the appropriate progression and preservation of tissue equilibrium in multicellular organisms by eliminating impaired or outdated cells. Nevertheless, the dysregulation of apoptosis may give rise to diverse human pathologies, such as cancer (9).

The processes governing apoptosis are exceedingly intricate and refined, encompassing a series of molecular events reliant on energy. Presently, investigations have revealed the existence of two primary pathways through which apoptosis occurs: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. Nevertheless, there is presently substantiated proof that these two pathways are interconnected, and the molecules within one way can influence the other (10).

The regulation of mitochondrial membrane permeability is controlled by the Bcl-2 family of proteins, which can exhibit either pro-apoptotic or anti-apoptotic functions. Presently, 25 genes have been identified within the Bcl-2 family. Notable anti-apoptotic proteins in this family encompass Bcl-2, Bcl-x, Bcl-XL, Bcl-XS, Bcl-w, and BAG, while some of the pro-apoptotic proteins include Bcl-10, Bax, Bak, Bid, Bad, Bim, Bik, and Blk. These proteins hold significant importance due to their ability to determine whether the cell will undergo apoptosis or if the process will be terminated. It is postulated that the primary mode of action for the Bcl-2 family of proteins lies in their ability to regulate the release of cytochrome c from the mitochondria by modulating the permeability of the mitochondrial membrane (11-13). By exploiting the functionality of BH3 subset pro-apoptotic proteins in facilitating programmed cellular demise, numerous BH3 mimics have been formulated for employment as therapeutic agents in cancer treatment. These agents interact in a suppressive fashion with the anti-apoptotic proteins BCL-2, BCL-XL, and BCL-w (14).

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Suppression of apoptosis represents a fundamental characteristic of resistance towards anticancer drugs. Consequently, in recent years, anti-apoptotic proteins have emerged as an appealing target for therapeutic intervention in various hematologic malignancies (15).

Venetoclax (also known as ABT-199, GDC-0199, or venclexta™), an innovative and orally administered biological small molecule inhibitor designed to specifically target B-cell lymphoma-2 (BCL-2), exhibits remarkable efficacy and safety profiles in a wide range of hematologic malignancies, particularly chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML) (16).

During the year 2016, the United States Food and Drug Administration (FDA) granted approval for the utilization of Venetoclax in the treatment of patients diagnosed with CLL and harboring a 17p deletion, who have previously undergone at least one alternative therapeutic intervention. Subsequently, in June 2018, the FDA expanded the approval to include CLL or small lymphocytic lymphoma (SLL) patients, irrespective of the presence of 17p deletion, who have received prior therapy. Furthermore, in November 2018, the FDA granted approval for the utilization of Venetoclax in combination with azacitidine, decitabine, or low-dose cytarabine (LDAC) for the treatment of newly diagnosed AML in individuals aged 75 years or older, as well as for individuals with comorbidities but without severe disease. It is important to note that induction chemotherapy is recommended in these cases. Lastly, in May 2019, the utilization of Venetoclax was extended to encompass all adult patients diagnosed with CLL or SLL (17).

.In addition, the European Medicines Agency (EMA) has granted approval to Venetoclax for the therapeutic intervention of patients diagnosed with chronic lymphocytic leukemia (CLL) when used in conjunction with rituximab as a secondary form of treatment. Furthermore, it may also serve as a monotherapy for patients who are not eligible for chemoimmunotherapy due to the inadequacy or ineffectiveness of pathway inhibitors such as ibrutinib and idelalisib (18).

Venetoclax possesses the potential to be an intriguing, relatively small, and profoundly selective orally bioavailable compound designed to specially target the BH3 domain of BCL2. Venetoclax can be classified as a medication that functions similarly to BH3, exhibiting a pronounced affinity for its intended target (19, 20). This particular substance can fit perfectly within the specific location in the BCL2 molecule where the BH3 molecule attaches typically. As a result, it can displace any previous occupant of that location. The proteins known as BIM, which actively promote cell death, become attached to the BCL2 protein. Consequently, the presence of specific proteins, referred to as free BH3-only proteins, can initiate apoptosis, or programmed cell death, in cells. These BH3-only proteins can activate specific proteins, namely BAX and BAK, which are responsible for inducing cell death. Alternatively, these BH3-only proteins can also inhibit other proteins that attempt to prevent cell death, such as MCL-1. Therefore, Venetoclax, through its mechanism of action, facilitates the demise of tumor cells by releasing proteins that trigger cell death (21-23).

Venetoclax has been shown to work on its own against different types of leukemia and lymphoma cells in both in vitro and in vivo. It has also shown positive effects in Chronic Lymphoblastic Leukemia CLL (21), non-Hodgkin lymphomas (22), Acute Myelogenous Leukemia (AML) (23), and Multiple Myeloma (MM) (24) patients when used as the only treatment.

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Venetoclax has been tested in multiple clinical trials https://clinicaltrials.gov/ and is approved for treating CLL and AML (24, 25). Still, it has just begun to be tested in patients with acute lymphoblastic leukemia.

In light of the indications of reliance on BCL2 in acute ALL (26), our hypothesis posited that the integration of Venetoclax, a discerning BCL2 inhibitor, could potentially facilitate the targeting of BCL2 to augment apoptotic cell demise of lymphoblasts.

Materials and methods

Patient 1

A 4-year-old male manifested by an increased difficulty in breathing, accompanied by feelings of weakness, fatigue, and fever, which have persisted for a month. The laboratory analysis revealed a white blood cell count of 6.5 × 10^9/L, with 30% of these cells being blast cells. Additionally, the patient's hemoglobin level was measured at 10 g/dL, and their platelet count was 25 × 10^9/L. Further examination of the bone marrow indicated a hyper cellular marrow, with over 60% of the cells being blast cells. Flowcytometry was performed for definitive diagnosis .

Patient 2

A 14 years old male presented with an increased sensation of breathlessness, accompanied by symptoms of weakness, lethargy, and fever that have persisted for a duration of one month. Upon conducting laboratory tests, it was revealed that the individual's white blood cell count was 156.5 × 10^9/L, with 50% of these cells being blasts on differential. Additionally, the individual's hemoglobin level was measured to be 6.1 g/dL, while the platelet count was found to be 26 × 10^9/L. Further analysis of the bone marrow indicated the presence of a hyper cellular marrow, with more than 60% of the cells being blasts.

Isolation and Culture of PBMC

Patient peripheral blood mononuclear cells (PBMCs) were isolated by using Ficoll density gradient centrifugation, according to manufacturer instructions (Ficoll-Lymphodex Innotrain, Germany). Isolated PBMCs were stained with a 0.4% trypan blue solution (DNAbiotech, I.R.Iran) and then enumerated and assessed for viability using a hemocytometer. PBMCs were then cultured in 6-well microtiter plates (6 × 106 cells/well in 3 mL n 3 mL RPMI 1640 media [BioIdea, I.R.Iran]) supplemented with 10% heat-inactivated FBS (DNAbiotech, I.R.Iran) and penicillin/streptomycin solution (BioIdea, I.R.Iran) at 37 °C with 5% CO2 for 72 h.

Colorimetric MTT (tetrazolium) assay

The cells (0.5×10^6 cells/mL) were plated in 96-well plates. The next day, the cells were treated with concentrations of 100, 500, 1000, 2000, 3000, 4000 and 5000 nM of Venetoclax drug for 48 h and analyzed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Briefly, stock MTT (BioIdea, I.R.Iran) solution (10 µL per 100 µL medium) was added to all wells of an assay, and plates were incubated at 37 °C for 4 h. The solid reaction product was dissolved by adding 100 µL of Dimethylsulfoxide solution (DMSO), mixing, and incubating for 2 hours at room temperature in the dark. Absorbance at 570 nm was measured using a Stat Fax 3200 (Awareness Microplate Reader, Awareness, USA) plate reader. Statistical analysis was performed using Graph Pad Prism 9 software and a one-way ANOVA test.

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Treatment of PBMNCs with Venetoclax

PBMNCs were divided into four groups at $5*10^3$/cm2 cell culture flask. Two groups (T-ALL, B-ALL) were treated with Venetoclax (VENCLYXTO , Abbvie) for 48 h. The untreated group was used as a control group.

Total RNA extraction

Genomic RNA was extracted from peripheral blood mononuclear cells (PBMC) utilizing the RNX-Plus reagent (Favorgen, Taiwan), taken after by DNase I assimilation (sinaclon, I.R Iran), concurring with the user handbook. RNA quality was surveyed utilizing the Smart Nano Micro Volume UV/Vis Spectrophotometer (Canada Smart Tech Inc, Canada) instrument perusing at 230, 260 and 280 nm wavelengths.

cDNA synthesis and quantitative real-time PCR

For reverse transcription of mRNA to cDNA, cDNA Synthesis Kit (SinaClon, I.R Iran) was utilized. BCL-2, MCL1 and BIM expression were measured utilizing the SYBR Green PCR Kit (YTA, Iran). Amplification condition was as follows: initial denaturation at 95 °C for 3 min followed by 40 cycles of denaturation at 95 °C for 5 s, annealing temperature at 60 °C for 5 s and extension temperature at 72 C for 5 s. Beta-actin was chosen as the housekeeping gene. Primer sequences are shown in Table 2 PCR and Primer efficiency was calculated with LinReg PCR software; then expression data were normalized to the geometric mean expression level of the housekeeping gene B-actin and calculated utilizing the Efficiency $PCR^{-\Delta ct}$ and Efficiency PCR $^{-\Delta\Delta ct}$ methods. Each sample was performed in triplicate, and each experiment was repeated thrice. The set of primers for specific genes is displayed in Table 1.

Table 1. List of primer sets used in real-time polymerase chain reaction assays.

	Genes	Primer sequences	
1	*BCL-2*	Forward primer	GGTGGGGTCATGTGTGTGG
		Reverse primer	CGGTTCAGGTACTCAGTCATCC
2	*MCL-1*	Forward primer	GTAATAACACCAGTACGGACGG
		Reverse primer	CCACAAACCCATCCTTGGAAG
3	*BIM*	Forward primer	TAAGTTCTGAGTGTGACCGAGA
		Reverse primer	GCTCTGTCTGTAGGGAGGTAGG
4	*B-ACT*	Forward Primer	CACCATTGGCAATGAGCGGTTC
		Reverse primer	AGGTCTTTGCGGATGTCCACGT

Annexin V–fluorescein isothiocyanate and propidium iodide staining

Cell viability was measured by Annexin V–fluorescein isothiocyanate (FITC) and PI staining (*MabTag GmbH, Germany*). Cells were treated with the indicated concentration of Venetoclax. After the indicated time, cells were washed once in phosphate-buffered saline PBS and were stained with Annexin V–FITC and PI according to the manufacturer's instructions. Samples were acquired on a BD FACSCalibur™ Flow Cytometer (BD biosciences, USA) and analyzed with Flowing Software 2.5.1 (Turku Bioscience Centre) In the process of apoptosis, the external cellular environment is exposed to phosphatidyl serine through lipid scrambling, whereby annexin V can selectively attach to phosphatidyl serine to identify the occurrence of apoptosis in viable cells. To detect the early stage of necrosis, cells were stained using red-fluorescent PI, which is capable of permeating the cell membranes and binding to nucleic acid present within the cells.

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Statistical analysis

Statistical analysis All experiments were performed independently at least three times and the results are presented as mean±standard error of the mean (SEM). Statistical analysis was performed using Student's t test. Statistical significance was defined as $p<0.05$. All results are replicated technically three times.

Results

The results of flow cytometry showed the diagnosis of B and T acute lymphoblastic leukemia in patients 1 and 2, respectively

Patient 1, these blast cells were found to express immature B-cell markers, specifically CD19, and exhibited surface expression of bright HLA-DR, CD10, CD34, and variable CD45. On the other hand, the blast cells showed dim expression of CD20, while testing negative for CD13, CD3, and CD15. Based on these findings, the patient was diagnosed with Pre B-ALL (Figure 1 (a-b) and table 1). Patient 2, these blasts were found to express markers associated with immature T-cells, as evidenced by the presence of cytoplasmic CD3 and nuclear terminal deoxynucleotidyl transferase (TdT), along with the bright surface expression of CD2 and CD5. The expression of CD45 was found to be variable, while sCD3 CD13, CD19, CD20 and CD34 were not expressed. This constellation of findings was indicative of the diagnosis of T-ALL (Figure 1(c-d) and table 2).

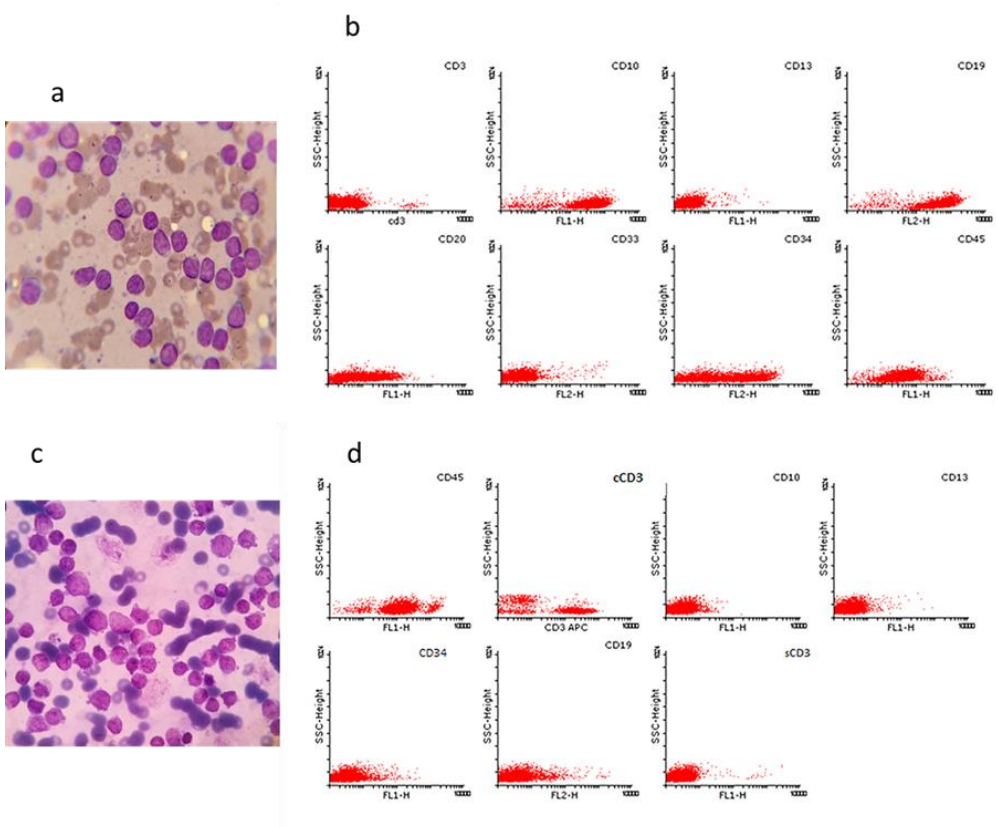


Fig. 1. Peripheral blood smear and flow cytometry results. Blasts in peripheral blood smear and flow cytometry results(bright CD19, CD10, CD34, negative CD3,CD13,CD20,CD33,Variable CD45) indicate B acute lymphoid leukemia(a,b) and Blasts in peripheral blood smear and flow cytometry results (Variable CD45,bright cCD3, negative CD10, CD34, CD19 and sCD3) indicate T acute lymphoid leukemia(c, d).

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Table 2. Patient profile

	Patient 1	**Patient 1**
Symptoms	increased difficulty in breathing,feelings of weakness, fatigue, and fever	
Age	4-year-old	14-year-old
Gender	Male	Male
Hematological parameter	WBC: 6.5 × 10^9/L Hb: 10 g/dL PLT: 25 × 10^9/L	WBC: 156.5 × 10^9/L Hb: 6.1 g/dL PLT: 26 × 10^9/L
Flow cytometry results	CD19, HLA-DR, CD10, CD34 bright, variable CD45, CD20 dim and negative for CD13, CD3, and CD15.	CD2 and CD5 bright, CD45 variable,sCD3 CD13, CD19, CD20 CD34 were not expressed
Diagnosis	B-ALL	T-ALL

MTT Result

Venetoclax drug has the 2600 nM and 690 nM Half-maximal inhibitory concentration (IC50) on T and B blastic, respectively. Graphs (a) and (b) show the viability of T cells and graphs (c) and (d) show the viability of B cells in different drug concentrations. The results are shown in Table 3.

Table 3. IC50 values of the indicated compounds Venetoclax drug in the T-ALL and Pre B-ALL.

	Cells	**IC50 (nM)**
Venetoclax	T-ALL	2600
	B-ALL	690

Venetoclax increased BIM gene expression and decreased BCL-2 gene expression in T-ALL and B-ALL cells

The expression levels of BCL2, MCL-1 and BIM were measured in treated T-ALL cells with Venetoclax 48 h after treatment. BCL2 is down-regulated in the sample group (in comparison to a control group) and BIM is UP-regulated in the sample group (in comparison to the control group) (P-value: 0.05), but the MCL1 sample group is not different to the control group (Figure 3).

Viability and apoptosis of T-ALL and B-ALL blast cells after Venetoclax treatment

A significant increase in apoptosis was observed in T-ALL blast cells treated with Venetoclax compared to the control group ($p<0.05$) (Figure 4B,D) and an increase in necrosis was also observed, which was not significant. The significant increase in apoptosis compared to the control group was seen in the B-ALL blast cells treated with Venetoclax ($p<0.05$) and the increase in necrosis in these cells was not significant (Figure 4A, C).

The development of resistance to drugs at the cellular level is a significant factor that contributes to the ultimate ineffectiveness of chemotherapy. Consequently, the precise evaluation of cellular drug resistance in primary clinical samples is significant in the medical field. The inhibition of apoptosis, as facilitated by

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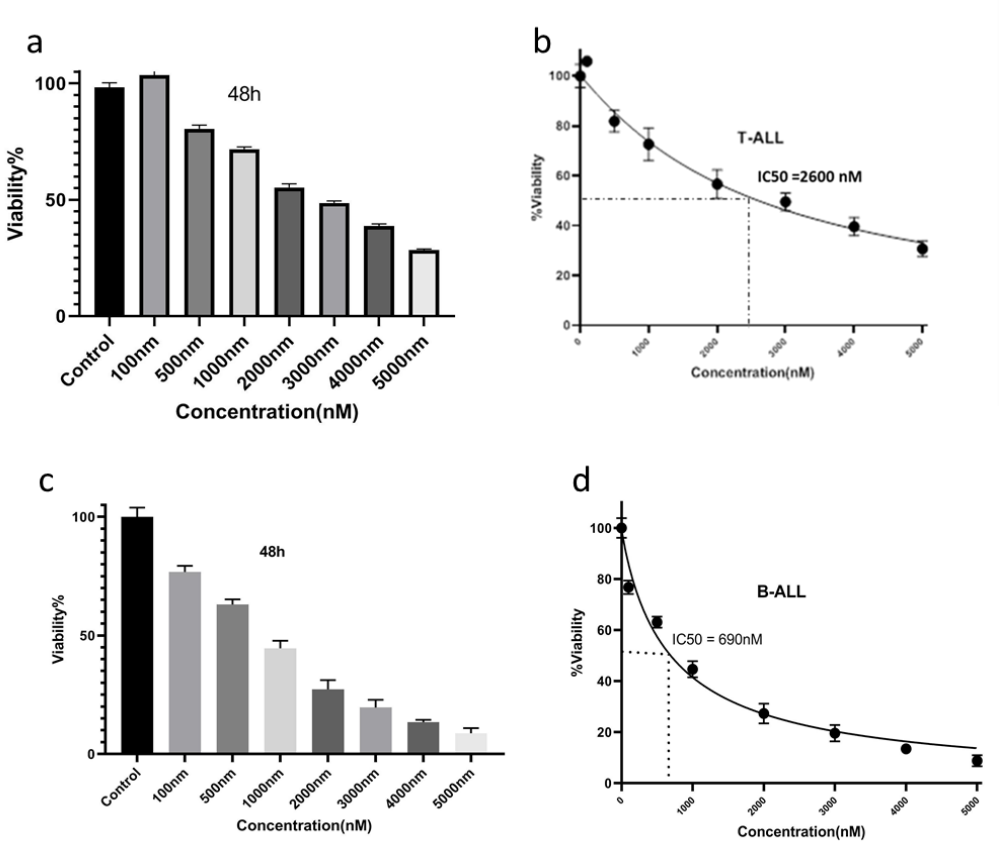


Fig. 2. MTT assay results showing cell viability under different Venetoclax drug concentrations over 48 h. Results are represented as a mean ± SD. T-ALL (a-b) B-ALL (c-d)

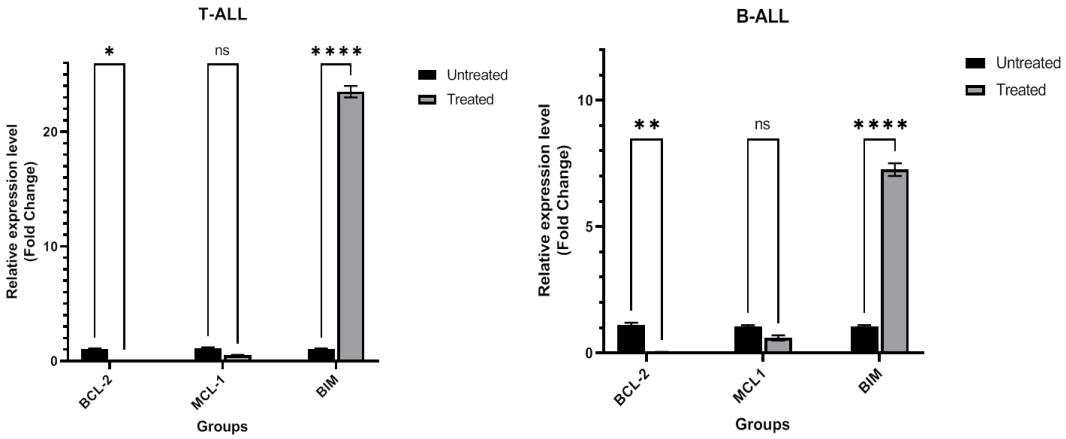


Fig. 3. **A**. BCL2 is DOWN-regulated in the sample group (compared to the control group) by a mean factor of 0.044 (S.E. range is 0.030 - 0.069).The BCL2 sample group is different from to the control group. P (H1) =0.000. The MCL1 sample group is not different from to the control group. P (H1) =0.306, the BIM is UP-regulated in the sample group (compared to the control group) by a mean factor of 23.068 (S.E. range is 17.110 - 31.129).the BIM sample group is different from to control group. P (H1) =0.000. **B**. the BCL2 is DOWN-regulated in the sample group (in compared to control group) by a mean factor of 0.030 (S.E. range is 0.020 - 0.050).the BCL2 sample group is different from to the control group. P (H1) =0.033. The MCL1 sample group is not different from to control group. P (H1) =0.092. The BIM is UP-regulated in the sample group (compared to control group) by a mean factor of 7.440 (S.E. range is 4.307 - 13.032).the BIM sample group is different from to the control group. P (H1) =0.034.

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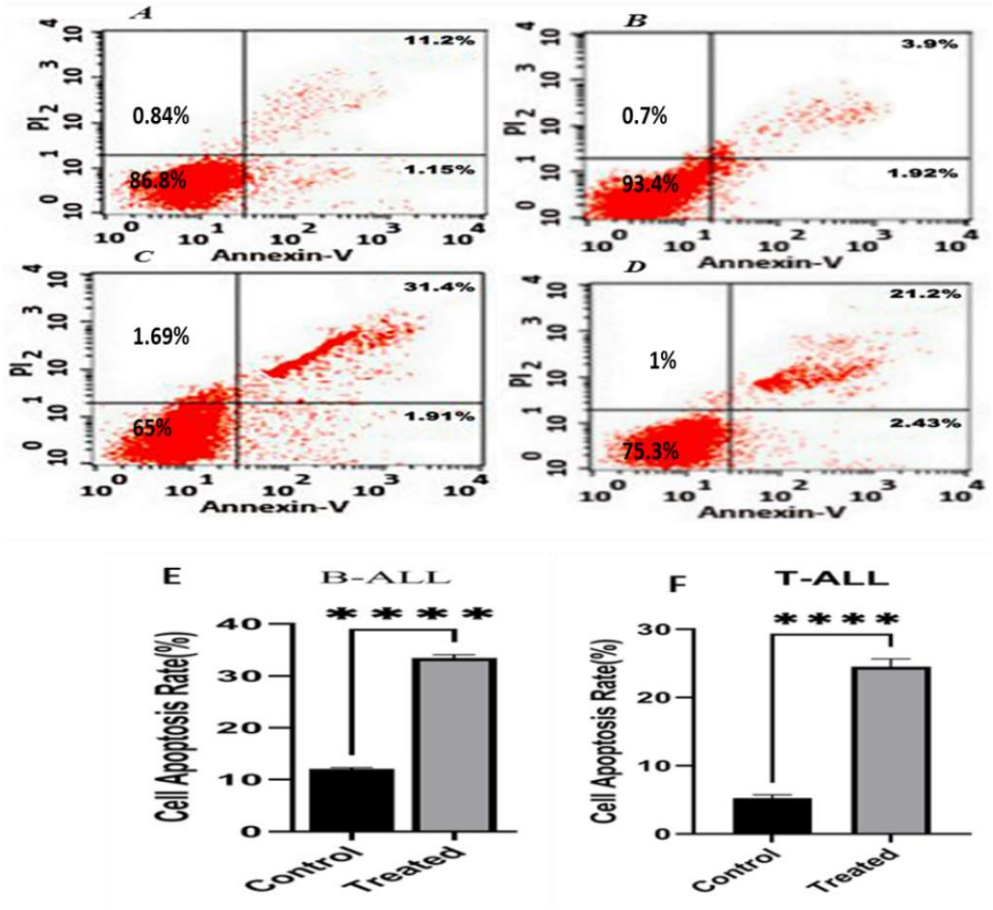


Fig. 4. Cellular apoptosis assay with FCM. (A to D) Flow cytometry analysis with Annexin V-PI staining was performed to evaluate the percentage of apoptotic cells in Venetoclax-induced B-ALL and T-ALL blast cells. The percentage of apoptotic cells in the B-ALL and T-ALL blast cells groups were significantly increased compared with that of controls. (E-F) Analysis on cell apoptosis results of B-ALL and T-ALL blast cells. Data are mean ± SD for relative apoptosis normalized to control cells for three independent experiments. * $p < 0.05$ as determined by Student's t-test, versus Controls group. A, B. B-ALL and T-ALL control group respectively, C,D. B-ALL and T-ALL treated group, respectively.

the expression of BCL-2, has been demonstrated to play a pivotal role in the sustenance of leukemic cells (27).We investigated the effect of Venetoclax drug on the expression of family BCL-2 genes involved in pro-apoptotic and anti-apoptotic cancer cells derived from patients with T and B-ALL.

Evidence has been indicated that cells in ALL samples display reliance on BCL-2 by demonstrating the inhibition of BCL-2 and the eradication of leukemic cells using ABT-737 (28). Also, Chonghaile *et al*. documented that the maturation state of cancer cells can determine the specific anti-apoptotic protein upon which it depends for survival (29). Piers et al. demonstrated high expression of BCL-2 in immature T-ALL and observed in vitro and in vivo sensitivity to Venetoclax that correlated with elevated levels of BCL-2 (30).

Our results showed a significant decrease in BCL-2 gene expression and a significant increase in BIM gene expression. No significant correlation was observed in the expression of the MCL-1 gene in T and B ALL. One of the main limitations of our work was the small number of patient samples and the lack of

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information about their genetic changes. In addition, our results were the result of in vitro studies and these studies can be performed in mouse models and in vivo as well Stephen et al. to survey the common causes of Venetoclax resistance, inspected hereditary changes and the level of expression of BCL 2 genes in 7 Venetoclax-resistant cell lines. There appeared to be an increase in the expression of BCL-XL and MCL-1 anti-apoptotic genes together or alone, in addition to a decrease in the expression of pre-apoptosis genes (31). Therefore, it can be concluded that the Venetoclax drug leads to an increase in apoptosis in cancer cells by reducing the expression of anti-apoptotic genes and increasing the expression of pro-apoptotic genes, which was consistent with our results.

Zhaodong Li *et al*. studied of the concurrent impact of MCL-1 inhibitor along with Venetoclax in T-ALL cell line, they to begin with explored drug resistance to Venetoclax and MCL-1 inhibitor, and out of 11 chosen cell lines, two Venetoclax-resistant cell lines were determined. The results of MCL-1 and BCL-2 levels showed no relationship between their levels and drug resistance. At that point, the synergistic impact of two drugs on cell apoptosis was examined. The results showed a significant diminish in viability in cells treated with both inhibitors compared to one of them in both cell lines (32).These results are consistent with our findings; however, in our study, there was no significant difference in MCL-1 gene expression in the treated cells compared to the control group. Also, Felix et al. investigated the synchronous impact of Venetoclax as a specific inhibitor of BCL-2, S63845 as an inhibitor of MCL-1 and A-1331852 as an inhibitor of BCL-XL in Pre B ALL cell lines and cells derived from Patients. Analysis of results showed heterogeneous sensitivity to each of these inhibitors in ALL cell lines. Interestingly, a few leukemia resistant to Venetoclax were delicate to specific MCL-1 and/or BCL-XL antagonists, proposing utilitarian cross-substitution. A utilitarian, investigation by BH3-profiling and investigation of protein complexes appeared that ALL cells treated with Venetoclax were subordinate to MCL-1 and BCL-XL, recommending that MCL-1 or BCL-XL is an Achilles heel (33).

Pariury *et al*. indicated a synergistic treatment strategy for hypodiploid B-ALL in vivo, achieved through the simultaneous inhibition of BCL-2 (Venetoclax) and CDK9 (dinaciclib). The utilization of this combination proved to be highly effective in eliminating leukemic blast cells from internal organs, thereby overcoming the persistence of these blasts, which was observed when Venetoclax was administered as a standalone therapy (34).

Gibson et al. retrospectively reviewed the safety and efficacy of Venetoclax for the treatment of ALL/LBL in the pediatric and young adult populations. The purpose of this study is to provide evidence that Venetoclax is safe and effective to use in pediatric patients with ALL/LBL and should be considered in both the relapsed and upfront settings. Their findings provide support for considering Venetoclax-based treatment regimens in pediatric patients with relapsed/refractory ALL/LBL, as well as investigating its potential as an initial therapy for T-cell ALL/LBL (35). Although our results also showed the efficacy of Ventoclax in patients, our results were related to laboratory studies and the investigation of changes in genes related to apoptosis, but their results were related to the safety of the drug in patients. In addition, the number of patients in their study was more than our study.

Norman J *et al*. reviewed outcomes for 36 patients with ALL or lymphoblastic lymphoma (LLy) treated with Venetoclax (Ven) + Navitoclax (Nav) and chemotherapy. The combination of Ven+Nav and

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chemotherapy exhibits a high level of tolerability, as evidenced by the minimal occurrence of discontinuations or dosage reductions due to adverse events in patients with relapsed/refractory acute lymphoblastic leukemia or lymphoma. The initial effectiveness of Ven+Nav shows promise in this population of patients who have undergone extensive prior treatments, including stem cell transplantation or chimeric antigen receptor T-cell therapy, with a substantial proportion achieving complete remission, complete remission with incomplete hematologic recovery, or complete remission with partial hematologic recovery. Furthermore, a noteworthy 56% of patients demonstrated undetectable minimal residual disease. Ongoing investigations into correlative biomarkers will be presented in due course (36). Their results can be considered in agreement with our results, but in their study, they used the combination of Nav+Ven, and their results were the result of studies on ALL and LLy patients. Also, in our study, we investigated apoptosis genes.

We also investigated the effect of Venetoclax drug on the apoptosis of cells treated with Venetoclax compared to untreated cells, and a significant increase in apoptosis was observed in treated cells compared to the control group.

In summary, with the accumulation of additional data, selectively inhibiting BCL-2 presents itself as a promising and novel therapeutic approach, both as a standalone treatment and in conjunction with other chemotherapeutic agents, for patients diagnosed with T and B ALL. Prospective studies must be conducted to further assess of the Venetoclax in the expression of apoptotic genes in the form of in vivo studies and combination of Venetoclax with other agents in the treatment of T and B ALL to obtain a more comprehensive understanding of the safety and efficacy of these therapeutic combinations.

Conflict of interest

The authors declare no conflict of interest

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