Original Article
Running Title: Venetoclax Modulates BIM and BCL-2, Not MCL1, in Leukemia Model
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Venetoclax Drives Significant Shifts in BIM and BCL-2 but not MCL-1 Gene Expression in a Mouse Model of Acute Lymphoblastic Leukemia

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Abstract

Background: T and B acute lymphoblastic leukemia (T, B-ALL) has seen improved survival rates with intensified chemotherapy, but therapy-resistant or refractory ALL remains a significant clinical challenge. This study examined the inhibitory effects of Venetoclax drug on the apoptosis gene to explore its potential as a novel therapeutic approach for treating human T, B-ALL.

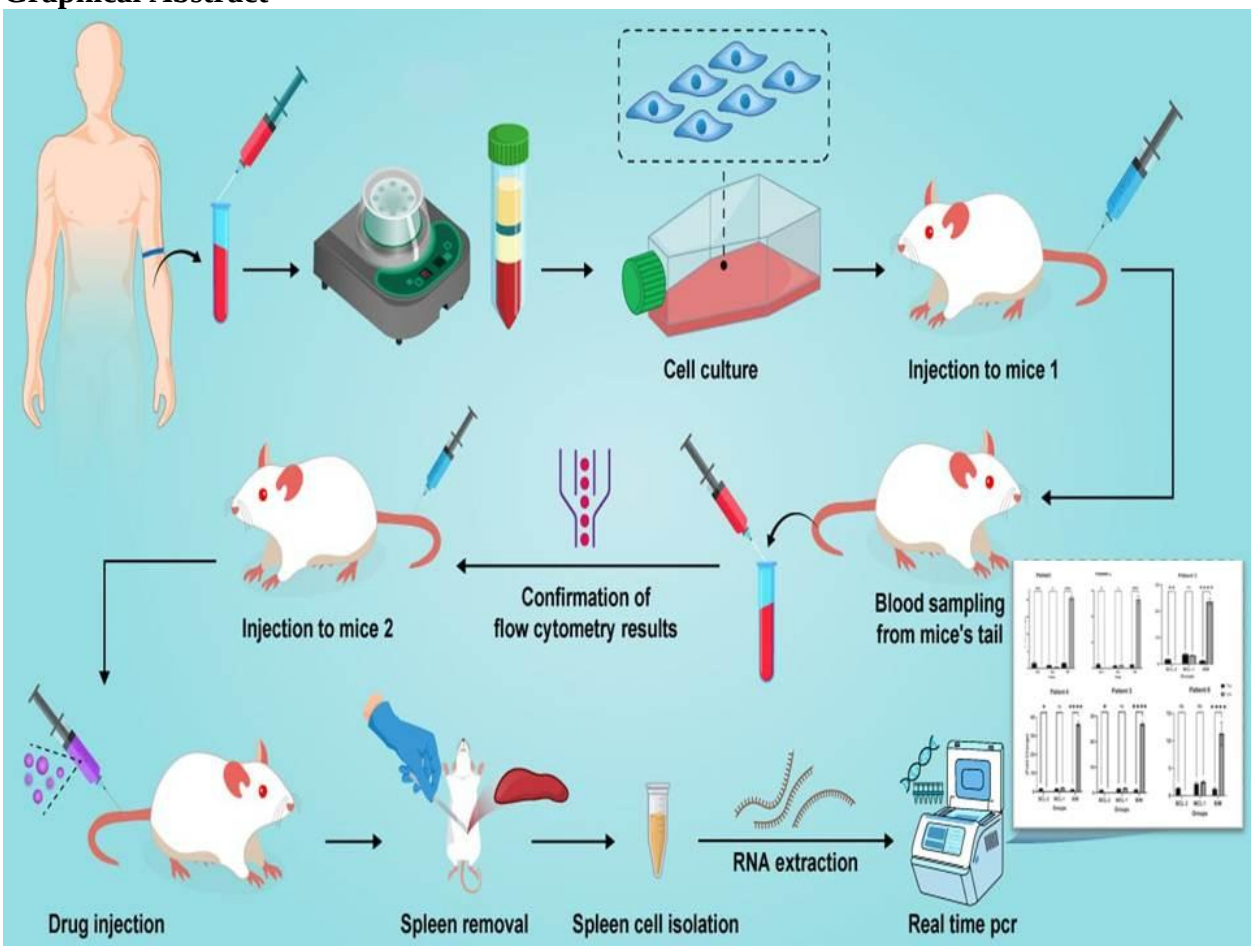
Method: This was a preclinical experimental study. Firstly, samples were collected from a leukemia patient, followed by isolating blast cells that were injected into primary mice. The sample was obtained from mice in 1, 8 and 14 days; the CD45 human was quantified using flow cytometry to confirm the development of leukemia. Spleen cells from the primary mice were isolated and injected into the secondary mice. After 14 days, the Venetoclax drug was administered to the mouse models for 21 days. Subsequently, mouse spleen cells were gathered, and the expression of genes associated with apoptosis was assessed. A two-tailed t-test was performed to compare the expression of apoptotic genes between the control and Venetoclax-treated groups.

Results: Our findings indicated a decrease in the expression of the B-cell lymphoma 2 (BCL-2) gene, while the expression of the BCL-2-interacting mediator (BIM) of cell death gene exhibited an augmentation. The level of expression of the MCL-1 (Myeloid leukemia 1) gene did not display any significant divergence compared with the control group.

Conclusion: Venetoclax drug shows potential therapeutic potential in B and T-ALL, increasing BIM expression and decreasing BCL-2, but further investigation and clinical trial studies are needed.

Keywords: Precursor T-Cell Lymphoblastic Leukemia-Lymphoma, Precursor B-Cell Lymphoblastic Leukemia-Lymphoma, Apoptosis

Graphical Abstract



Introduction

Acute leukemias are malignant hematopoietic cell diseases caused by the malignant transformation of primary hematopoietic stem cells. The causes are unknown, but many patients have specific chromosomal abnormalities.[1] These diseases are divided into acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL). ALL, which is more common in children, peaks between ages two and five. It is rare in middle age and increases in old age. AML and ALL make up 90% of adult leukemia cases.[2]

Lymphoblastic leukemia, a rare childhood cancer, is characterized by bone marrow failure, pallor, weakness, bleeding, fever, infection, and bone pain due to multiplying blasts in the bone marrow. The disease can also affect other organs, leading to lymphadenopathy, hepatomegaly, splenomegaly, skin infiltrates, meningeal infection, and testicular enlargement. The prognosis depends on the tumor's genetic characteristics, age, white blood cell count (WBC), patient's general clinical condition, and vital organ functioning.[3, 4] Cytogenetic abnormalities in adults may lead to worse prognoses. Advances in treating ALL in the 1990s increased the definitive treatment rate for children by 80%. Long, induction chemotherapy with vincristine, prednisone, and L-asparaginase is used. Complete remission rates in children range from 99%-97%. Relapsed patients have less survival compared with newly diagnosed patients.[5]

ALL is categorized into B-ALL (85%) and T-ALL (15%), with T-ALL patients predominantly male and black, high blood cell counts, and involvement of the central nervous system and mediastinal lymph nodes. About 6,000 cases are diagnosed in the United States (U.S.) annually, with several genetic factors increasing the likelihood of developing ALL. However, patients have also been diagnosed who do not have any genetic factors. In various studies, polymorphic variants in several genes, such as AT-Rich Interaction Domain 5B (*ARID5B*), CCAAT/enhancer binding protein (*CEBPF*), GATA Binding Protein 3 (*GATA3*), and IKAROS Family Zinc Finger 1(*IKZF1*), have been identified, which are related to ALL. Genetic mutation t(12:21) and hyperdiploidy are associated with a good prognosis, and hypodiploidy, Mixed lineage leukemia (MLL) gene rearrangement, breakpoint cluster region protein- Tyrosine-protein kinase ABL1 (BCR-ABL) fusion gene, and cytokine receptor-like factor 2 (CRLF2) rearrangement are associated with a bad prognosis.[6]

Apoptosis is a programmed cell death triggered by various molecular pathways, including the cell death receptor-dependent pathway, activated by ligands like tumor necrosis factor and CD95/Fas, and the mitochondrial-dependent pathway, a cascade of elements that make the mitochondrial membrane permeable, leading to cell death.[7] The induction of cell death receptors by the ligand causes the accumulation of the death-inducing signal complex (DISC) on the inner side of the plasma membrane, which in turn causes the stimulation and activation of caspases 8 and 10, which further activates the effector caspases 3, 6 and 7. Caspase 8 can also be activated by the protease activity of BH3 interacting domain death agonist (BID), which causes the permeability of the mitochondrial outer membrane and establishes the main link between the extrinsic and intrinsic apoptosis pathways. The extrinsic path also includes ligand-independent death receptors whose intermediates have not been fully characterized.[8, 9] Intrinsic pathways involve intracellular signals like DNA damage and endoplasmic reticulum stresses, leading to mitochondrial outer membrane destruction, releasing apoptotic factors like cytochrome C and cytosol, respectively. The apoptotic protease apoptotic peptidase activating factor 1 **(**apaf-1) and Adenosine triphosphate/ Adenosine diphosphate (ATP/ADP) form the apoptosome complex, which activates caspase nine, and further, this complex activates effective caspases 3, 6, and 7.[10] The Bcl-2 apoptosis regulator (BCL-2) protein family assumes a significant role in the apoptotic response. This assemblage of proteins, which share structural similarities, can be categorized into two groups: proapoptotic and antiapoptotic. These groups engage in mutual interactions. There are two recognized Homology Motifs. Regarding their functionality, members of the BCL-2 family can be classified into three groups: antiapoptotic, proapoptotic proapoptotic effector, and proapoptotic proapoptotic activator. Antiapoptotic proteins like BCL-2, BCL-2-like 1 (BCL2L1), BCL-2 like 2(BCLL2), myeloid cell leukemia (MCL-1), and BCL-2 like 10 (BCL-2L10) act as inhibitors to the activator and effector proteins, thereby impeding apoptosis.[11] The other group comprises proapoptotic proteins, further divided into activator and effector proteins. Activator proteins possess a BH3 domain; for instance, BID, BCL-2-interacting mediator (BIM), BCL-2 associated agonist of cell death (BAD), NOXA, and p53 upregulated modulator of apoptosis (PUMA), while effector proteins exhibit four homologous domains from the BCL-2 family, such as BCL-2 associated X(BAX), BCL-2 antagonist (BAK), and BCL-2 family apoptosis regulator BOK (BOK).[12]

The Bcl-2 family contains a BH motif, and apoptotic activators, which have only one motif, are crucial intermediates in response to cellular stressors like DNA damage.[13] They induce mitochondrial pores, and antiapoptotic proteins obstruct both apoptotic effectors and apoptotic activators through direct interaction. Antiapoptotic proteins bind to the BH3 domain of apoptotic activators, inhibiting their function.[14, 15]

The modified manifestation of the prosurvival proteins belonging to the BCL-2 family (namely, BCL-2, BLC-XL, and MCL1) represents a characteristic feature of malignancies in humans, leading to the initiation of intricate mechanisms within cells to elude apoptosis.[16] The increased expression of these proteins in many cancers allows cell growth and cell survival in the presence of apoptotic signals and chemotherapy strategies.[17] The BCL-2 gene is overexpressed in many follicular lymphomas, chronic lymphocytic leukemia (CLL), and about 25% of B-non-Hodgkin's lymphoma (NHL). BCL-2 is vital in responding to chemotherapy, radiotherapy, and radiation therapy. Therefore, the use of its antisense is considered as an achievement in science. One of the most critical factors that can affect BCL-2 is Antisense oligonucleotides, small molecules detecting the level of BCL-2 and anti-BCL-XL mentioned.[18, 19]

The Venetoclax drug is an essential drug that selectively inhibits the BCL-2 protein, which can cause programmed cell death in malignant cells.[20] The Abotte laboratory first investigated this drug in collaboration with Genentech, and these two companies for the treatment of blood malignancies developed. In April 2016, it was approved by the United States for use in patients with CLL patients with a deletion in chromosome 17 (17del) and patients who had previously received a course of treatment. This drug has a high binding ability to BCL-2 and can also bind to BCL-XL and BCL-w. By binding to BCL-2, proteins binding to BCL-2, such as Bim, are shifted which in turn leads to increased membrane permeability, activation of the apoptotic cascade, and finally, the apoptosis of cancer cells.[21] Moreover, approval has been granted by the European Medicines Agency (EMA) for the use of venetoclax in the therapeutic management of individuals diagnosed with CLL in combination with rituximab as a secondary treatment option. Additionally, it may also function as a sole therapy for patients who are deemed ineligible for chemoimmunotherapy due to the inefficacy or inadequacy of pathway inhibitors like ibrutinib and idelalisib.[22]

Venetoclax demonstrates the capacity to be a compelling, relatively small, and highly selective orally bioavailable compound engineered to target the BH3 domain of BCL-2 specifically. It can be categorized as a therapeutic agent operating akin to BH3, displaying a significant attraction to its target.[23, 24] This compound can precisely occupy the site within the BCL-2 molecule where the BH3 molecule typically binds, displacing any prior site occupant. The proteins identified as BIM, which actively facilitate cellular apoptosis, become affixed to the BCL-2 protein. Consequently, the existence of particular proteins, known as free BH3-only proteins, can initiate apoptosis, or programmed cell death, within cells. These BH3-only proteins can activate specific proteins, namely BAX and BAK, which trigger cell death. Moreover, these BH3-only proteins can also hinder other proteins that endeavor to impede cell death, such as MCL-1. Thus, venetoclax, through its operation, promotes the demise of cancerous cells by liberating proteins that instigate cell death.[25-27]

The Food and Drug Administration (FDA) has sanctioned Venetoclax for patients diagnosed with CLL carrying a 17p deletion who have undergone at least one prior

therapeutic intervention or for individuals newly diagnosed with AML, in conjunction with azacitidine, decitabine, or low-dose cytarabine in adults over the age of 75, who are not deemed suitable for induction chemotherapy.[28] The cytotoxic activity of ventoclax has been shown in CLL, various types of NHL,[29] acute ALL,[30] AML,[31] chronic myeloid leukemia (CML) and multiple myeloma (MM).[31]

Given the evidence suggesting a dependence on BCL-2 in acute ALL,[32, 33] our hypothesis postulated that the incorporation of venetoclax, a selective BCL-2 inhibitor, could conceivably enhance the ability to target BCL-2 and amplify the apoptotic cell death of lymphoblasts in T-ALL and B-ALL Mouse model.

Materials and Methods

Patients and leukemic cells

This was a preclinical experimental study. First, six children with T-ALL and B-ALL were selected. Patient characteristics are listed in Table 1. Bone marrow samples were collected. Mononuclear cells were purified on a Ficoll density gradient and frozen in liquid nitrogen in 10% dimethyl sulfoxide (DMSO) until required. All cell suspensions must contain >85% leukemic blasts, as confirmed by morphologic and immunophenotype analysis. Before injection, cells were quickly thawed in RPMI 1640 medium containing 10% fetal bovine serum (FBS). After centrifugation at 250 g for 5 minutes at 4 °C, cells in RPMI 1640 containing 10% FBS were resuspended, and the number of viable cells was estimated with 0.2% trypan blue. The cells were centrifuged again, suspended in cold phosphate-buffered saline (PBS) without calcium and magnesium, placed on ice, and inoculated into mice.

Non-Obese diabetic/severe combined immunodeficiency (NOD/SCID) mice

The Ethics Committee in Biomedical Research of the Faculty of Medical Sciences of Tarbiat Modares University approved all animal studies. A total number of 24 female NOD/SCID mice, aged between 5 to 6 weeks, were acquired (North Pasteur Institute Research Center, Iran). The mice were housed in sterile filter cages in a tranquil backflow cabinet. Theywere provided with sterilized chow and acidified water containing 70 mg/L of polymyxin B and 80 mg/L of ciprofloxacin (Arya Pharm, Iran). Before the injection of leukemic cells, no conditioning regimen was implemented. The infusion of 2.5×10^6 mononuclear cells in 100 ml of PBS was performed intravenously through the lateral tail vein into the primary group. To monitor the progress of leukemia, 5 ml a peripheral blood sample was obtained from the lateral tail vein using capillary blood collection tubes in 1, 8 and 14 days and stained with anti-human CD45 antibodies, conjugated with fluorescein isothiocyanate (FITC). After flow cytometry confirmation, spleen cells from the primary mice were isolated and injected into the secondary mice.

Therapeutic intervention and evaluation of induced effects

The induced mouse model received the drug 14 days after cells were injected into secondary mice. One of them did not receive the drug and only received saline, which was considered as the control group (Table 2). The injection dose of Venetoclox was 100mg/kg/day orally for 21 days (Based on AbbVie).[34] After 21 days, the spleen of the second group of mice was separated, and its cells were extracted in the same way as previously described.

Total RNA extraction, cDNA synthesis, and quantitative real-time polymerase chain reaction (RT-PCR)

According to the provided user manual, genomic RNA was isolated using the RNX-Plus reagent (Favorgen, Taiwan)succeeded by DNase I digestion (salon, I.R Iran). RNA

quality was assessed using the Smart Nano-Micro Volume UV/Vis Spectrophotometer (Canada Smart Tech Inc, Canada) apparatus scanning at 230, 260, and 280 nm wavelengths. The mRNA was reverse transcribed to cDNA using the cDNA Synthesis Kit (SinaClon, I.R Iran). BCL-2, MCL1 and BIM expression levels were quantified using the SYBR Green PCR Kit (YTA, Iran). The amplification process entailed an initial denaturation at 95 °C for 3 minutes, succeeded by 40 cycles of denaturation at 95 °C for 5 seconds, annealing at 60 °C for 5 seconds, and extension at 72 °C for 5 seconds. Beta-actin was designated as the internal control gene. The primer sequences are delineated in Table 1 PCR. The Primer efficiency was ascertained by employing the LinReg PCR software. Then, the expression data were normalized against the geometric mean expression level of the housekeeping gene Beta-actin and calculated using the Efficiency PCR−Δct and Efficiency PCR −ΔΔct approaches. Triplicate analyses were performed for each sample, and the experiments were conducted autonomously and replicated thrice. The set of primers for specific genes is displayed in Table 3.

Ethical considerations

According to educational and research regulations, the ethical code for working with animals was obtained from the Ethics Committee of Tarbiat Modares University (Ethics ID: IR.MODARES.AEC.1402.018). Efforts were made to minimize animal suffering, including the use of humane methods and adherence to ethical guidelines, throughout the study.

Statistical analysis

Data were analyzed using GraphPad Prism (Version 8.4.3). Gene expression levels (BCL-2, BIM, and MCL-1). For statistical analysis of gene expression between control and treatment groups, a two-tailed t-test was performed to assess significant differences, assuming normal distribution of the data and equal variance between the groups. The flow cytometry results were analyzed using Flowing Software (version 2.5.1). Statistical significance was defined as a p-value of less than 0.05, and the use of triplicate analyses was found adequate to ensure reliable results while optimizing resource use. All data are presented as the mean ± standard error of the mean (SEM).

Result

Monitoring leukemic progression in vivo

If the human CD45+ cells in the peripheral blood of mice were 50%, the transplant was considered successful (Figure 1). After 14 days, the primary recipient mice were killed by spinal cord cutting, then the mice were placed in 70% alcohol to sterilize them, and then the mice's spleen was removed. To establish the mouse model, 2×10^5 cells were administered to the mice belonging to the second group. These particular mice were subsequently designated as leukemic mice.

Effect of venetoclax on BIM and BCL-2 gene expression in T-ALL and B-ALL mouse models

The levels of BCL-2, MCL-1, and BIM expression were assessed in spleen cells from the T-ALL and B-ALL murine models. It was observed that BCL-2 is under-expressed in the experimental group compared with the control group. At the same time, BIM is over-expressed in the experimental group compared with the control group (with a *P*-value of 0.05). However, no significant difference existed between the MCL1 levels in the experimental and control groups. The lack of change in mcl-1 could be due to the intrinsic biological variability and the limited statistical power of the triplicate analyses, which may be too small to detect effect sizes (Figure 2).

Discussion

In this study, we investigated the effect of Venetoclox drug as an inhibitor in mouse models of ALL and its effect on the expression of apoptotic genes. To do so, first, B-ALL and T-ALL mouse models were created using blast cells from ALL patients. Then, the Venetoclax drug was injected, and finally, BCL-2, MCL-1, and BIM apoptotic genes were investigated.

The results showed a decrease in BCL-2 gene expression and an increase in BIM gene expression, but no significant change in MCL-1 gene expression was observed in both B and T ALL mouse models. MCL-1 expression did not show significant changes due to its rapid and transient regulation in response to specific stress signals, which may require longer or more specific conditions, and its lower sensitivity to certain treatments or stressors, which may explain its less pronounced changes.

Peirs et al. investigated the effect of venetoclax on T-ALL patients' cell lines and blast cells. They first examined the BCL-2 gene expression in these cells. In the next step, the cells with high BCL-2 gene expression were treated with venetoclax. This study highlighted BCL-2 as a captivating molecular objective in distinct subtypes of human T-ALL that may be effectively used through Venetoclax.[30] Our findings agreed with their findings, demonstrating a reduction in BCL-2 gene expression in the T-ALL mouse model.

Pariury et al.have proposed a treatment approach for hypodiploid B-ALL in vivo that demonstrates synergistic effects. This approach involves the concurrent inhibition of BCL-2 (venetoclax) and CDK9 (dinaciclib). This combination has proven to be remarkably efficient in eradicating leukemic blast cells from internal organs. As a result, it overcomes the persistency of these blasts, which was observed when Venetoclax was used as a standalone therapy.[35]

Norman J. et al. conducted a review of the outcomes witnessed in 36 patients who were administered a combination of venetoclax (Ven) + Navitoclax (Nav) along with chemotherapy to treat ALL or lymphoblastic lymphoma (LLy). It has been observed that the treatment with Ven+Nav in conjunction with chemotherapy has exhibited a significant level of tolerance. The initial efficacy of Ven+Nav has demonstrated promising outcomes in this specific patient population, who have already undergone extensive treatment regimens like stem cell transplantation or chimeric antigen receptor T-cell therapy. A notable percentage of patients achieved complete remission, complete remission with incomplete hematologic remission, or complete remission with partial hematologic remission. Furthermore, a considerable proportion of patients, notably 56%, exhibited at least unresectable residual disease.[36] Their findings can be regarded as consistent with our findings. It is important to note that Nav + Ven combined treatment was employed in their study, and its results were derived from studies carried out on ALL and LLy patients. In addition, study was on investigating apoptosis genes. In a similar study, the viability of employing venetoclax and low-dose navitoclax in conjunction with customary chemotherapy regimens is demonstrated in individuals afflicted with B-ALL, T-ALL, and LL to simultaneously suppress BCL-2 and BCL-XL while achieving a harmonious equilibrium between maximizing effectiveness and clinical tolerability. One shortcoming of this investigation lies in its designation as a solitary arm phase I study, necessitating supplementary investigations with direct comparator arms to corroborate these observations.[37] This findings of this study have limitations, including the use of murine models, potential off-target effects, and insufficient treatment duration. These factors

may limit the generalizability of Venetoclax's effectiveness in clinical settings. Additionally, biological variability in mouse models, such as age, weight, and genetic background, could influence individual responses. Acknowledging these limitations is crucial for understanding the context of the study and guiding future research.

In summary, the targeted suppression of BCL-2 is emerging as a promising and novel therapeutic strategy, both independently and in synergy with other chemotherapeutic drugs, for patients afflicted with T and B ALL. It is imperative to conduct further research to assess the influence of Venetoclax on the expression of genes related to programmed cell death through clinical trials, as well as the amalgamation of Venetoclax with other medications in the management of T and B ALL, to gain a more profound understanding of the safety and efficacy of these therapeutic regimens.

Although this study demonstrated the therapeutic potential of Venetoclax in T and B-ALL using preclinical models and patient-derived cells, limitations include interspecies differences, a focus on a limited number of apoptotic genes, and the lack of combination therapy assessments. Future studies should address these by conducting broader pathway analyses, evaluating venetoclax in combination with other treatments, and performing clinical trials to confirm efficacy and safety.

Conclusion

The study results showed an increase in BIM expression and a decrease in BCL-2, suggesting the Venetoclax drug as a potential therapeutic drug in B and T-ALL. However, further investigations are warranted to scrutinize the impact of the Venetoclax drug both independently and in conjunction with other medications and agents such as chemotherapy. Future clinical trial studies are also recommended.

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Authors' Contribution

K.T.: Conceptualization, data curation, formal analysis, investigation, software, visualization, writing theoriginal draft; **S.A.:** Conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, writing-review, and editing. All authors have read and approved the final manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest

None declared.

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Table 1. Patient profile

	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**
Symptoms	Weakness, Fatigue, Fever	Prolonged fever (>2 weeks), Fatigue	Weakness, Fatigue	Weakness, Anorexia, Weight loss	Prolonged fever (>2 weeks), Weight loss	Weight loss, Fatigue, Fever
Age	4-year-old	14-year-old	6-year-old	8-year-old	7-year-old	9-year-old
Gender	Male	Male	Female	Female	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	WBC: 8.5×10^9/L Hb: 11 g/dL PLT: 50×10^9/L	WBC: 15.1×10^9/L Hb: 8.3 g/dL PLT: 26×10^9/L	WBC: 7.9×10^9/L Hb: 9.1 g/dL PLT: 52×10^9/L	WBC: 43.2×10^9/L Hb: 7.1 g/dL PLT: 12×10^9/L
Flowcytometry Results	CD19, HLA-DR, CD10, CD34 bright, variable CD45, CD20 dim and negative for CD13, CD3, and CD15.	CD2 and CD5 bright, CD45 variables,CD3 CD13, CD19, CD20 CD34 were not expressed	CD19, HLA-DR, CD10, CD34, CD45 bright, CD20 dim and negative for CD13, CD3, and CD15.	CD3 and CD5, CD45, CD34 bright, CD13, CD19, CD20 were not expressed	CD19, HLA-DR, CD10, CD34 bright, variable CD45, CD20 dim and negative for CD13, CD3, and CD15.	CD2 and CD3, CD34 bright, CD45 variable, CD13, CD19, CD20 were not expressed
Diagnosis	B-ALL	T-ALL	B-ALL	T-ALL	B-ALL	T-ALL

ALL: Acute lymphoblastic leukemia; WBC: White blood cell count; Hb: Hemoglobin; PLT: Platelets; CD: Cluster of differentiation; B-ALL: B- Acute lymphoblastic leukemia; T-ALL: T- Acute lymphoblastic leukemia; dim: diminished.

Table 2. Induced mouse models

Treated	Control
Mouse model induced with T-ALL patient cells, and received drug	Mouse model induced with T-ALL patient cells that did not receive treatment and did not receive drugs (control)
Mouse model induced with B-ALL patient cells, and received drug	Mouse model induced with B-ALL patient cells that did not receive treatment and did not receive drug (control)

ALL: Acute lymphoblastic leukemia; B-ALL: B- Acute lymphoblastic leukemia; T-ALL: T- Acute lymphoblastic leukemia

Table 3. List of primer sets used in real-time polymerase chain reaction assays for BCL-2, MCL1, BIM and the housekeeping gene, B-actin

	Genes' name	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

BCL-2: B-cell lymphoma 2; MCL-1: Myeloid cell leukemia 1; BIM: Bcl-2-interacting mediator of cell death; B-ACT: Beta-Actin

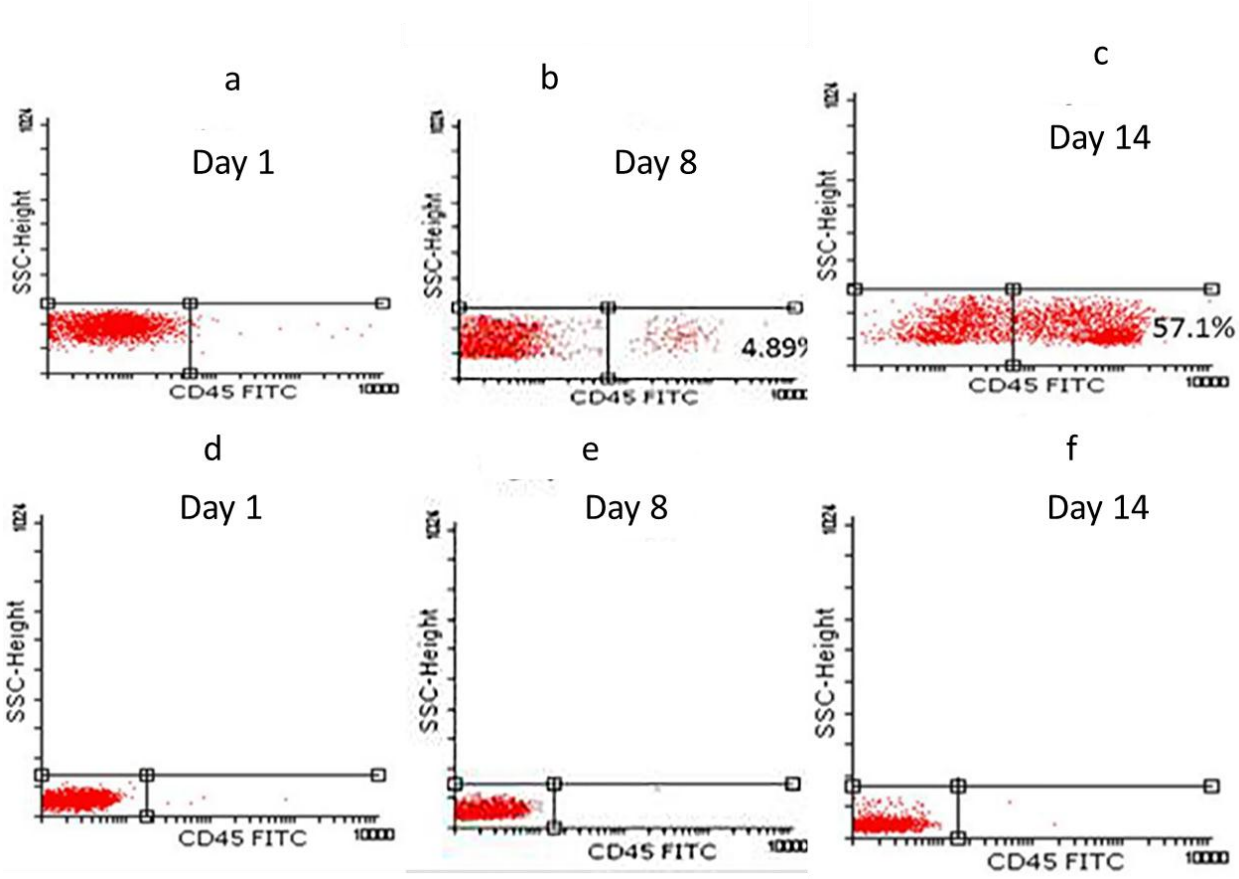

Figure 1. Flowcytometry analysis of blood samples from primary mice on days 1, 8, and 14 after injection of human leukemic cells. Panels a, b, and c show the experimental group, while d, e, and f show the controls. An increase of more than 50% in human CD45 expression on day 14 was considered as the establishment of the mouse model.

CD45 FITC: Cluster of differentiation 45 fluorescein isothiocyanate

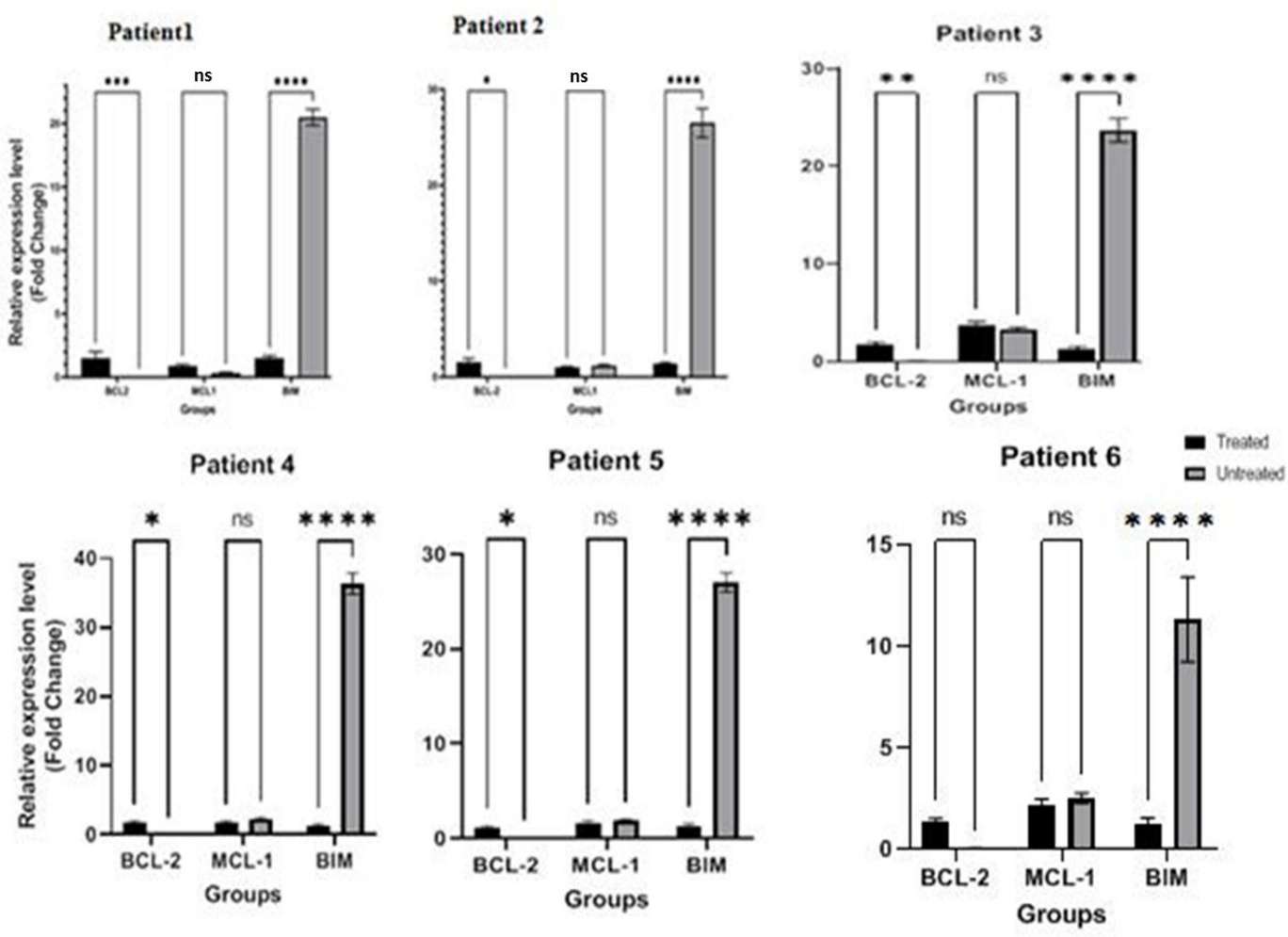

Figure 2. Gene expression analysis of BCL-2, MCL-1, and BIM in induced mouse models. BCL-2 expression significantly decreased in models 1–5 (in order from one to five, ***, *, **, *, *), but not in model 6. No significant change was observed in MCL-1 expression.

BIM expression significantly increased in all models (****, ****, ****, ****, ****, ****). Statistical significance was defined as a p-value of less than 0.05. BCL-2: B-cell lymphoma 2; MCL-1: Myeloid cell leukemia 1; BIM: Bcl-2-interacting mediator of cell death; ns: Not significant