Int J Cancer Manag. 2019 April; 12(4):e87773. doi: 10.5812/ijcm.87773.

Published online 2019 April 23. **Research Article**

Myeloid Cell Leukemia-1 (MCL-1) siRNA Therapy Showed Cytotoxic Effect on T Cells Acute Lymphoblastic Leukemia

Shaghayegh Askarian-Amiri [1, 2, **], Farzane Ordoni Aval [2], Abbas Azadmehr [2, *], Morteza Oladnabi [3, 4], Mahjoobeh Jafari Vesiehsari [5] and Mahmoud Hajiahmadi [6]

[1] Student Research Committee, Babol University of Medical Sciences, Babol, Iran
[2] Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran
[3] Gorgan Congenital Malformations Research Center, Golestan University of Medical Sciences, Gorgan, Iran
[4] Ischemic Disorders Research Center, Golestan University of Medical Sciences, Gorgan, Iran
[5] Genetic Department, Babol University of Medical Sciences, Babol, Iran
[6] Department of Biostatistics and Epidemiology, Babol University of Medical Sciences, Babol, Iran

[*] *Corresponding author*: Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran. Email: azadmehr2010@gmail.com
[**] *Corresponding author*: Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran. Email: shaghayegh.askarian71@yahoo.com

Received 2018 December 17; **Revised** 2019 March 12; **Accepted** 2019 April 10.

Abstract

Background: T-lineage acute lymphoblastic leukemia (T-ALL) is a malignant hyperplastic disease of the hematopoietic system. This tumor is the most common tumor in children and adolescents. Myeloid cell leukemia-1 (Mcl-1) is described as a prosurvival protein from the Bcl2 family. It is an important factor in routine cancer treatments. In fact, in different types of cancers, Mcl-1 downregulation can be a potential target.
Objectives: The present study aims to evaluate the cytotoxic effect of MCL-1 siRNA in T-ALL cells.
Methods: The present study evaluated the effects of Mcl-1 small interfering RNAs (siRNAs) on survival in Jurkat cells. Specific Mcl-1 siRNA was transfected and using quantitative real-time PCR, the relative expression of Mcl-1 mRNA was determined. Moreover, cell survival was determined using the colorimetric MTT assay.
Results: The expression of mRNA reduced effectively in a dose-dependent manner at 48 hours after transfection with Mcl-1 siRNA. In addition, Mcl-1 siRNA treatment could significantly reduce tumor cell survival.
Conclusions: Based on the results, downregulation of Mcl-1 by specific siRNAs in T-ALL cells can effectively reduce cell survival. Therefore, Mcl-1 siRNA may be a complementary agent along with standard methods in the treatment of T-ALL.

Keywords: Mcl-1, siRNA, Jurkat Cell Line, T-ALL

1. Background

Acute lymphoblastic leukemia (ALL) is the most frequently encountered cancer and blood malignancy among children. ALL is caused by abnormal development and uncontrolled proliferation of lymphoid progenitor cells or hematopoietic stem cells and may be of a B-lineage (B-ALL) or a T-lineage (T-ALL) in the bone marrow (1). T cell acute lymphoblastic leukemia (T-ALL). Malignant transformation of T-cell precursors results in T-cell ALL (T-ALL), occurring in almost 25% of adults and 15% of children (2).

T-ALL is normally associated with a large tumor showing hyperleukocytosis. In addition, It is associated with mediastinal enlargement and risk of central nervous system involvement (3). Although many treatments have been discovered for T-ALL, it is associated with recurrence in many cases. Furthermore, the development of resistance to chemotherapy drugs is another problem. Therefore, it is necessary to find a new treatment, which can be used besides chemotherapy and radiotherapy to control T-ALL tumors (4).

There are numerous side-effects often related to chemotherapy drugs; therefore, RNA interference (RNAi) can reduce these effects because of its specificity and potency. In this method, appropriate personalized drugs can be developed for a patient so as to increase its effectiveness (5).

RNAi or mechanisms regulating gene expression at the post-transcriptional level are mediated by small non-coding RNA, such as small interfering RNA (siRNA), antisense oligonucleotides (ASOs), piwi-interacting RNA (piR-

NAs), and microRNA (miRNA) (length, 18 - 30 nucleotides) (6). Generally, siRNA is originated from double-strand RNA (dsRNA) with homologous complementary sequences of specific mRNA. The antisense strand attaches to the target mRNA, while double-stranded siRNA unwinds; the specific mRNA is degraded leading to phenotype dysfunction (7). Recently, this technology has been used in gene therapy and gene function research (8). Therefore, oncogenes, mutations in tumor suppressor genes, and besides other genes are involved in the progression of tumors and can be suggested as proper targets for RNAi gene silencing (5).

Deficiencies in the apoptotic pathway is a hallmark of cancer cells (9). Myeloid cell leukemia-1 (Mcl-1) from the Bcl2 family is a pro-survival protein. The *Mcl-1* gene, with 3 exons, is located on chromosome 1q21. In 1993, Kozopas et al. discovered *Mcl1* gene during differentiation of ML-1 into monocyte and macrophage. Mcl-1 is majorly involved in cell differentiation, cell death, and cell cycle program (10). This protein is overexpressed in different lymphoid and hematopoietic cancers, as well as solid tumors including CNS, lung, ovarian, colon, breast, melanoma, prostate, and renal tumors (11, 12). Based on the recent reports Mcl-1 is necessary for the survival of hematopoietic and tumor cells (13). Mcl-1 has been recognized as an important factor in common cancer therapies. It can be a potential target in many cancers since its downregulation is adequate to promote cell death in cancer cells (12).

2. Objectives

In the present study, siRNA Mcl-1 was transfected into the Jurkat cell line, which is expressing high levels of Mcl-1, to evaluate the effects of Mcl-1 gene suppression on the survival and cell death in ALL. Apoptosis was induced by silencing Mcl-1 expression in the Jurkat cell line.

3. Methods

3.1. Cell Culture

In this study, Jurkat cell line, which purchased from Pasteur Institute of Tehran (Iran), was kept in RPMI-1640 medium (Sigma-Aldrich, USA), containing 1% antibiotics (including 100 IU/mL of penicillin and 100 μg/mL of streptomycin; Sigma-Aldrich), 10% heat-inactivated fetal bovine serum (FBS; Sigma-Aldrich), 1% sodium pyruvate, and 2 mM glutamine in a humidified 5% CO_2 atmosphere at 37°C. At an initial concentration of 1×10^5 cells/mL, the cells were subcultured when reaching 80% - 90% confluence and then applied in the logarithmic growth phase.

3.2. siRNA Transfection

The negative control (NC) siRNA and Mcl-1 siRNA were supplied by Santa Cruz Biotechnology. The Jurkat cells were cultured in the RPMI-1640 medium (without FBS and antibiotics) in 6-well plates right before transfection at a density of 2×10^6 cells/well. Based on the instructions of the manufacturer, siRNA was used for transfection. In brief, after dilution of the transfection reagent and siRNA in the siRNA transfection medium (Santa Cruz Biotechnology) separately, they were gently mixed. After combining the solutions, they were incubated for 30 minutes to form a complex at ambient temperature.

The complex liquid, containing the reagent and siRNA, was gradually added to each well (containing medium and cells confluence, 80% - 90%) with 800 μL of the optimal medium and incubated in a chamber in a humidified CO_2 atmosphere for 6 hours at 37°C. After adding RPMI-1640 medium (1 mL) containing 20% FBS, without removing the transfection mixture, the cells were incubated under the aforementioned conditions. After 48 hours, the cells were collected for further evaluation.

3.3. RNA Isolation and Q Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Based on the instructions, a total RNA purification mini kit (Yekta Tajhiz Azma, Tehran, Iran) was employed for extracting total RNA from Jurkat cells. RNA (1 μg) was reverse-transcribed into cDNA with 1 μL of Moloney murine leukemia virus reverse transcriptase (M-MLV RT), 1 μL of dNTPs (10 mM each), 4 μL of 5X reaction buffer, 1 μL of random hexamer primer, and 0.6 μL of RNasin (40 U/μL). Afterward, qRT-PCR was performed to amplify 1 μL of cDNA, using SYBR Green-1 dye on an ABI 7300 system (Applied Biosystems, NJ, USA) and a sequence detection system (Corbett Life Science, NSW, Australia). Based on the fluorescent signal the binding of SYBR Green dye to ds DNA reflects the generated amount of ds DNA product during PCR. SYBR Green I dye was used to enhance sensitivity and specificity. PCR specificity was confirmed, using melting curve analysis.

The PCR assay was carried out using 5 μL of SYBR green reagent, 0.2 μL of passive reference dye (50X), 1 μL of cDNA template, 0.5 μL of each primer, and 2.8 μL of nuclease-free distilled water; the reaction volume was 10 μL. In this study, the primers included: Mcl-1, 5′-GCGACGGCGTAACAAACTG-3′ (forward) and 5′-GAACTCCACAAACCCATCCCAG-3′ (reverse) with an amplified fragment length of 191 bp; and β-actin, 5′-TCCCTGGAGAAGAGCTACG-3′(forward) and 5′-GTAGTTTCGTGGATGCCACA-3′(reverse) with an amplified fragment length of 131 bp. The conditions of cDNA amplification using PCR were as follows: pre-degeneration for 3

minutes at 95°C, degeneration for 30 seconds at 95°C, annealing for 25 seconds at 64°C, extension for 30 seconds at 72°C (40 cycles), and storage at 4°C. Using the $2^{-(\Delta\Delta Ct)}$ method, relative Mcl-1 mRNA expression was determined, with β-actin mRNA expression as the internal control. The PCR reactions were performed in triplicate.

3.4. Cell Cytotoxicity Assay Using MTT

The MTT assay kit (Sigma-Aldrich; USA) was used to determine the cytotoxic effect of Mcl-1 siRNA on the Jurkat cell line. Cells in the logarithmic growth phase were collected and counted. Afterward, the cells (4×10^4 cells/well) were cultured in 96-well plates. Subsequently, transfection was performed according to the above-mentioned method. The groups included different doses of Mcl-1 siRNA (40 - 80 pmol), untreated cells, cells treated with scrambled siRNA (negative control), pure Mcl-1 siRNA, and transfection reagent. The MTT solution (5 mg/mL) was added at 48 hours post- transfection (volume, 20 μL/well). In the process of incubation, water-insoluble formazan crystals were formed within 4 hours at 37°C. Then, 100 μL of the supernatant was carefully removed from the wells, and DMSO (100 μL; 0.5 mg/mL) was added to solubilized crystals. Optical density (OD) was read by a microplate reader (Rayto, China) (Awareness Technology, FL, USA) in each well at 570 nm (reference, 650 nm).

3.5. Data Analysis

Data are presented as mean $\pm$ SEM. Data analysis was performed utilizing GraphPad Prism-5 statistic software (LaJolla, CA, USA) and one-way analysis of variance (ANOVA) was used to determine variations among groups. $P < 0.05$ was statistically accepted to be significant.

4. Results

4.1. Mcl-1 Expression in Jurkat Cell Line Following siRNA Treatment

The control siRNA, tagged with fluorescein (siRNA-FITC), was used to assess transfection efficiency (Figure 1). According to Figures 1 and 2, Mcl-1 siRNA reduced Mcl-1 mRNA both dose- and time-dependently ($P < 0.0001$). The relative Mcl-1 expression was 27.8%, 24.7%, and 37.1% after 24, 48, and 72 hours of transfection, respectively ($P < 0.0001$; Figure 2), while the relative expression of 40, 60, and 80 pmol of Mcl-1 siRNA on Mcl-1 mRNA was 52.9%, 24.3% and 56.2%, respectively ($P < 0.0001$; Figure 3). The optimum knockdown time and concentration were considered respectively as 48 hours and 60 pmol. In addition, the level of β-actin mRNA expression (internal control) was similar in qRT-PCR for all the groups. Transfection with NC siRNA (scrambled control, a sequence without specific degradation of cellular mRNAs) insignificantly affected Mcl-1 expression in comparison with the control group. According to the results, specific siRNA could target Mcl-1 mRNA and reduce Mcl-1 gene expression in Jurkat cells.

4.2. Dose-Dependent Cytotoxic Effects on Jurkat Cells Induced by Mcl-1 Suppression

According to Figure 3, the percentages of survival were 88.33%, 35.05%, and 59.88%, respectively. Cytotoxicity significantly improved with 60 and 80 pmol of Mcl-1 siRNA-transfected cells versus the blank control and scrambled siRNA-transfected cells ($P < 0.0001$). In contrast, compared with the control and non-transfected groups, cell survival slightly reduced with 40 pmol of Mcl-1 siRNA. Moreover, transfection with control transfected siRNA and pure siRNA and transfection reagent had no significant cytotoxic effects on leukemic cells in comparison with the blank control group (Figure 4).

5. Discussion

T-ALL is a malignant hyperplastic disease of the hematopoietic system and it is the most common tumor in children and adolescents (14). Considering the development of chemoresistance in leukemia cells, most patients, exposed to standard treatments, show relapse following the initial treatment or fail to achieve complete remission (15-17). Improvement of treatment methods has caused a significant increase in survival; nevertheless, the survival of adult T-ALL is very low (< 40% for > 60 years). These studies show that non-toxic medicines and new strategies are required to improve therapy and increase survival in T-ALL patients (14). Gene therapy is an effective molecular targeted therapy. In recent years, RNAi technology has been the most common and simple genetic tool (18).

For instance, siRNA, which disrupts the target gene expression, is an overexpressed specific gene in tumor cells. It can inhibit tumor formation, growth, and proliferation (19-22).

Overexpression of some oncogenes regulates cellular proliferation and inhibits apoptosis in different cancers, such as leukemia and breast cancer (23-26).

Apoptosis is controlled by 2 signaling pathways. The extrinsic pathway, which is activated by extracellular death receptors, causes caspase-8 activation, while the intrinsic mitochondrial pathway is stimulated by many signals, leading to mitochondrial outer membrane permeabilization (MOMP), caspase-9 activation, and cytochrome C release to the cytoplasm. These 2 pathways activate the proteolytic cascade and induce cell death. Therefore, MOMP is

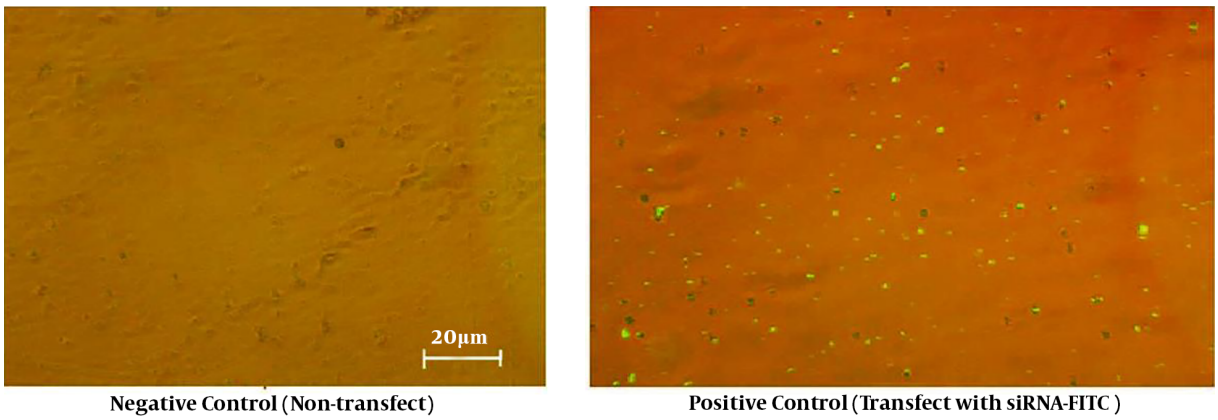

Figure 1. The transfection efficiency of FITC positive cells

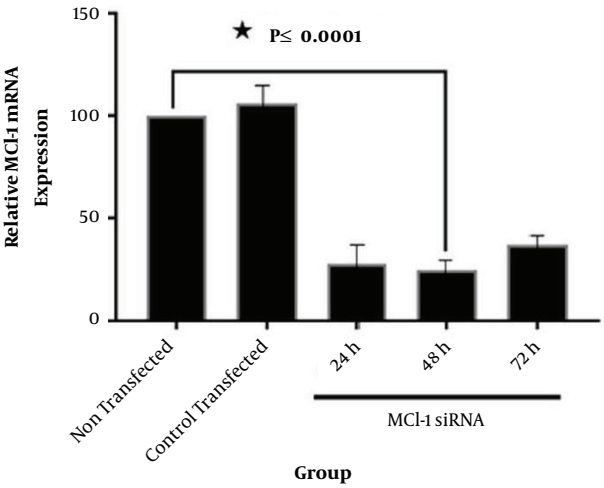

Figure 2. The Jurkat tumor cells transfected with Mcl-1 siRNA at different times. Total RNA was extracted and mRNA was analyzed via qRT-PCR assay at 24, 48, and 72 hours following transfection. The relative mRNA expression was quantified by $2^{-(\Delta\Delta CT)}$ formula (β-actin, internal control) (data presented as mean ± SD; * P < 0.0001 vs. controls).

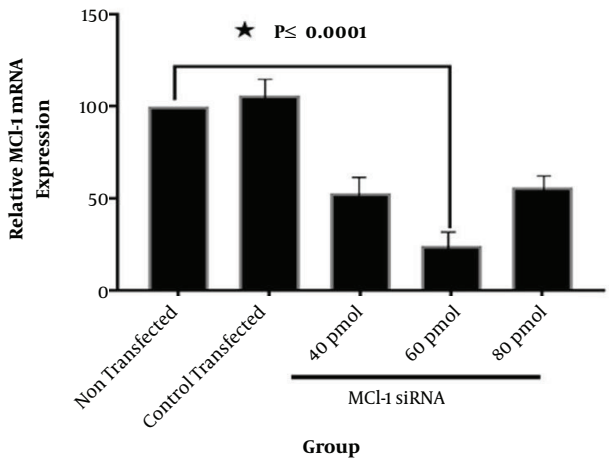

Figure 3. The Jurkat tumor cells transfected with different doses of siRNA (40, 60, and 80 pmol). Total RNA was extracted and mRNA was analyzed via qRT-PCR at 48 hours after transfection. The relative expression of mRNA was determined with $2^{-(\Delta\Delta CT)}$ formula (β-actin, internal control) (data presented as mean ± SD; * P < 0.0001 vs. controls).

majorly involved in the intrinsic pathway and regulated by BCL-2 proteins (27, 28).

The protein-protein interactions between Bcl-2 members can regulate apoptosis (29). Mcl-1 from the antiapoptotic Bcl-2 family has shown overexpression in different cancers, including leukemia (27). On the outer mitochondrial membrane, Mcl-1 neutralized the proapoptotic members of the Bcl-2 family, including Bax, Bim, and Bak; in this way, it could block the mitochondrial pathway apoptosis (30). Researchers have demonstrated that Mcl-1 downregulation can result in apoptosis and prevent tumor cell proliferation (31-33).

The suppressive effects of specific siRNA on Mcl-1 proteins in the Jurkat cell line (T-ALL) were evaluated. In this study, quantification of mRNA by qRT-PCR confirmed that Mcl-1 siRNA transfection could significantly reduce Mcl-1 mRNA expression in Jurkat cells. Karami et al. showed that siRNA knockdown of Mcl-1 notably stimulated apoptosis and prevented proliferation in AML (23). In addition, another study on siRNA MCL-1 found it to be a proper target for CLL and ALL; its silencing could significantly increase the therapeutic effect of rituximab (34). The findings of the present study are consistent with previous research on leukemia. The therapeutic method of this study using siRNA could significantly downregulate Mcl-1 mRNA in Ju-

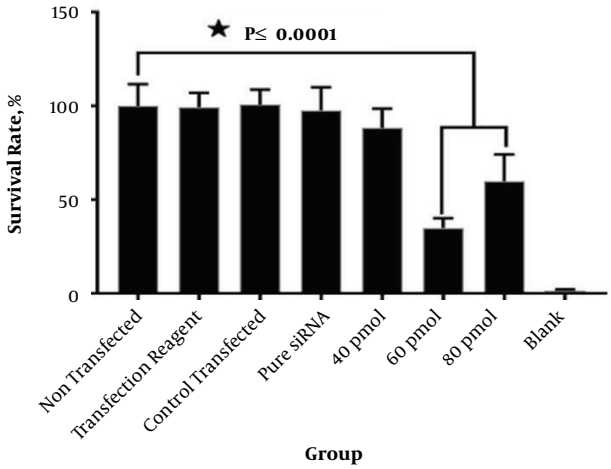

Figure 4. The cytotoxicity of Jurkat tumor cells transfected with Mcl-1 siRNA. Cytotoxicity was examined using MTT assay after 48 hours (data presented as mean ± SD; * P < 0.0001 vs. controls).

rkat cells. Consequently, inhibition of Mcl-1 expression by siRNA, in combination with other cancer treatments (e.g., immunotherapy with antibodies), could enhance the sensitivity of chemotherapy drugs. However, further studies on combination therapy for leukemia are suggested.

According to the MTT assay, Mcl-1 siRNA had a major contribution to the survival of leukemia cells. Based on the result of this study, the negative control scramble siRNA and reagent caused no changes in gene expression and cellular survival. According to a study by Hao et al. On the human gastric cancer cell, SGC-7901, cell proliferation was examined by MTT assay. The results revealed that Bcl-2 expression knockdown by siRNA reduced gastric cancer cell growth (35). Based on the present study, Mcl-1 siRNA hindered Mcl-1 expression as an antiapoptotic agent in lymphocyte leukemia, and after transfection with specific siRNA, leukemia cell survival decreased.

On the other hand, another study by Meng et al. on pediatric patients with A-BLL demonstrated that transfection of Bcl-2-siRNA into leukemic cells significantly increased the apoptosis rate (36).

Another study in 2013 showed that using Bcl-2 siRNA could significantly increase apoptosis and mortality rate in the gastric cancer cell, BGC823. In addition, they indicated that by treatment with siRNA Bcl-2, a notable increase was observed in the sensitivity of cancer cells to X-ray (37). It is likely that downregulation of Mcl-1 can reduce resistance to X-ray irradiation in the Jurkat cell line. Therefore, further studies are recommended to identify the effects of antiapoptotic proteins on resistance to chemotherapy or radiotherapy.

Moreover, a recent study in 2013 on breast cancer indicated that miR-26a, which is a potential tumor suppressor, inhibited tumor migration, proliferation, and triggered apoptosis via Mcl-1 targeting (38). Similarly, based on the present study, Mcl-1 expression by siRNA could be suppressed and reduce cell survival in leukemia cells. If this method affects tumor migration, further research on tumor migration is needed.

5.1. Conclusions

Collectively, the present study showed that downregulation of Mcl-1 by specific siRNAs can give rise to death in T-ALL cells. Likewise, this study demonstrated that specific siRNAs can be suggested as an effective complementary therapeutic agent for the treatment and management of T-ALL.

Acknowledgments

We would like to thank the Cellular and Molecular Biology Research Center, Health Research Institute, and also Student Research Committee, Babol University of Medical Sciences, Babol, Iran for financial support of this project. Hereby, we would like to thank Dr. Alireza Rafiei and Mr. Kardan at Cellular and Molecular Biology Research Center from Mazandaran (Sari) University of Medical Sciences. Moreover, we would like to extend our thanks to Dr. Ali Akbar Moghadamnia, Dr. Sadegh Sedaghat and Pedram Torabian for their comments in part of laboratory and proposal stages of this study.

Footnotes

Authors' Contribution: It is not declared by the authors.

Conflict of Interests: The authors declared that there is no conflict of interests regarding the publication of this paper.

Financial Disclosure: It is not declared by the authors.

Funding/Support: The authors declared that there is no funding/support regarding this study.

References

1. Mullighan CG. New strategies in acute lymphoblastic leukemia: Translating advances in genomics into clinical practice. *Clin Cancer Res*. 2011;**17**(3):396–400. doi: 10.1158/1078-0432.CCR-10-1203. [PubMed: 21149616].
2. Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. *N Engl J Med*. 2004;**350**(15):1535–48. doi: 10.1056/NEJMra023001. [PubMed: 15071128].
3. Ferrando AA. The role of NOTCH1 signaling in T-ALL. *Hematology Am Soc Hematol Educ Program*. 2009:353–61. doi: 10.1182/asheducation-2009.1.353. [PubMed: 20008221]. [PubMed Central: PMC2847371].

4. Chiaretti S, Foa R. T-cell acute lymphoblastic leukemia. *Haematologica*. 2009;**94**(2):160–2. doi: 10.3324/haematol.2008.004150. [PubMed: 19181788]. [PubMed Central: PMC2635412].
5. Bora RS, Gupta D, Mukkur TK, Saini KS. RNA interference therapeutics for cancer: Challenges and opportunities (review). *Mol Med Rep*. 2012;**6**(1):9–15. doi: 10.3892/mmr.2012.871. [PubMed: 22576734].
6. Li Y, Ji P, Jin P. Probing the microRNA pathway with small molecules. *Bioorg Med Chem*. 2013;**21**(20):6119–23. doi: 10.1016/j.bmc.2013.05.030. [PubMed: 23791866]. [PubMed Central: PMC3789859].
7. Timmons L, Court DL, Fire A. Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in Caenorhabditis elegans. *Gene*. 2001;**263**(1-2):103–12. doi: 10.1016/S0378-1119(00)00579-5. [PubMed: 11223248].
8. Lu ZJ, Mathews DH. Efficient siRNA selection using hybridization thermodynamics. *Nucleic Acids Res*. 2008;**36**(2):640–7. doi: 10.1093/nar/gkm920. [PubMed: 18073195]. [PubMed Central: PMC2241856].
9. Zhang H, Guttikonda S, Roberts L, Uziel T, Semizarov D, Elmore SW, et al. Mcl-1 is critical for survival in a subgroup of non-small-cell lung cancer cell lines. *Oncogene*. 2011;**30**(16):1963–8. doi: 10.1038/onc.2010.559. [PubMed: 21132008].
10. Kozopas KM, Yang T, Buchan HL, Zhou P, Craig RW. MCL1, a gene expressed in programmed myeloid cell differentiation, has sequence similarity to BCL2. *Proc Natl Acad Sci USA*. 1993;**90**(8):3516–20. doi: 10.1073/pnas.90.8.3516. [PubMed: 7682708]. [PubMed Central: PMC46331].
11. Placzek WJ, Wei J, Kitada S, Zhai D, Reed JC, Pellecchia M. A survey of the anti-apoptotic Bcl-2 subfamily expression in cancer types provides a platform to predict the efficacy of Bcl-2 antagonists in cancer therapy. *Cell Death Dis*. 2010;**1**. e40. doi: 10.1038/cddis.2010.18. [PubMed: 21364647]. [PubMed Central: PMC3032312].
12. Akgul C. Mcl-1 is a potential therapeutic target in multiple types of cancer. *Cell Mol Life Sci*. 2009;**66**(8):1326–36. doi: 10.1007/s00018-008-8637-6. [PubMed: 19099185].
13. Brunelle JK, Ryan J, Yecies D, Opferman JT, Letai A. MCL-1-dependent leukemia cells are more sensitive to chemotherapy than BCL-2-dependent counterparts. *J Cell Biol*. 2009;**187**(3):429–42. doi: 10.1083/jcb.200904049. [PubMed: 19948485]. [PubMed Central: PMC2779245].
14. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. *N Engl J Med*. 2006;**354**(2):166–78. doi: 10.1056/NEJMra052603. [PubMed: 16407512].
15. Smits EL, Berneman ZN, Van Tendeloo VF. Immunotherapy of acute myeloid leukemia: Current approaches. *Oncologist*. 2009;**14**(3):240–52. doi: 10.1634/theoncologist.2008-0165. [PubMed: 19289488].
16. Xiao J, Yin S, Li Y, Xie S, Nie D, Ma L, et al. SKP2 siRNA inhibits the degradation of P27kip1 and down-regulates the expression of MRP in HL-60/A cells. *Acta Biochim Biophys Sin (Shanghai)*. 2009;**41**(8):699–708. doi: 10.1093/abbs/gmp058. [PubMed: 19657571].
17. Szer J. The prevalent predicament of relapsed acute myeloid leukemia. *Hematology Am Soc Hematol Educ Program*. 2012;**2012**:43–8. doi: 10.1182/asheducation-2012.1.43. [PubMed: 23233559].
18. Fujita Y, Kuwano K, Ochiya T. Development of small RNA delivery systems for lung cancer therapy. *Int J Mol Sci*. 2015;**16**(3):5254–70. doi: 10.3390/ijms16035254. [PubMed: 25756380]. [PubMed Central: PMC4394474].
19. Yang M, Mattes J. Discovery, biology and therapeutic potential of RNA interference, microRNA and antagomirs. *Pharmacol Ther*. 2008;**117**(1):94–104. doi: 10.1016/j.pharmthera.2007.08.004. [PubMed: 17928059].
20. Shan G. RNA interference as a gene knockdown technique. *Int J Biochem Cell Biol*. 2010;**42**(8):1243–51. doi: 10.1016/j.biocel.2009.04.023. [PubMed: 19442757].
21. Devi GR. siRNA-based approaches in cancer therapy. *Cancer Gene Ther*. 2006;**13**(9):819–29. doi: 10.1038/sj.cgt.7700931. [PubMed: 16424918].
22. Ordoni Aval F, Askarian Amiri S, Azadmehr A, Oladnabi M, Saadat P, Ebrahimi H, et al. Gene silencing of TGFbetaRII can inhibit glioblastoma cell growth. *Asian Pac J Cancer Prev*. 2018;**19**(9):2681–6. doi: 10.22034/APJCP.2018.19.9.2681. [PubMed: 30256570]. [PubMed Central: PMC6249455].
23. Karami H, Baradaran B, Esfahani A, Sakhinia M, Sakhinia E. Therapeutic effects of myeloid cell leukemia-1 siRNA on human acute myeloid leukemia cells. *Adv Pharm Bull*. 2014;**4**(3):243–8. doi: 10.5681/apb.2014.035. [PubMed: 24754007]. [PubMed Central: PMC3992959].
24. Zaffaroni N, Daidone MG. Survivin expression and resistance to anticancer treatments: Perspectives for new therapeutic interventions. *Drug Resist Updat*. 2002;**5**(2):65–72. doi: 10.1016/S1368-7646(02)00049-3. [PubMed: 12135582].
25. Gritsko T, Williams A, Turkson J, Kaneko S, Bowman T, Huang M, et al. Persistent activation of stat3 signaling induces survivin gene expression and confers resistance to apoptosis in human breast cancer cells. *Clin Cancer Res*. 2006;**12**(1):11–9. doi: 10.1158/1078-0432.CCR-04-1752. [PubMed: 16397018].
26. Alotaibi MR, Hassan ZK, Al-Rejaie SS, Alshammari MA, Almutairi MM, Alhoshani AR, et al. Characterization of apoptosis in a breast cancer cell line after IL-10 silencing. *Asian Pac J Cancer Prev*. 2018;**19**(3):777–83. doi: 10.22034/APJCP.2018.19.3.777. [PubMed: 29582634]. [PubMed Central: PMC5980855].
27. Warr MR, Shore GC. Unique biology of Mcl-1: Therapeutic opportunities in cancer. *Curr Mol Med*. 2008;**8**(2):138–47. doi: 10.2174/156652408783769580. [PubMed: 18336294].
28. Sprick MR, Walczak H. The interplay between the Bcl-2 family and death receptor-mediated apoptosis. *Biochim Biophys Acta*. 2004;**1644**(2-3):125–32. doi: 10.1016/j.bbamcr.2003.11.002. [PubMed: 14996497].
29. Juin P, Geneste O, Gautier F, Depil S, Campone M. Decoding and unlocking the BCL-2 dependency of cancer cells. *Nat Rev Cancer*. 2013;**13**(7):455–65. doi: 10.1038/nrc3538. [PubMed: 23783119].
30. Akagi H, Higuchi H, Sumimoto H, Igarashi T, Kabashima A, Mizuguchi H, et al. Suppression of myeloid cell leukemia-1 (Mcl-1) enhances chemotherapy-associated apoptosis in gastric cancer cells. *Gastric Cancer*. 2013;**16**(1):100–10. doi: 10.1007/s10120-012-0153-6. [PubMed: 22527182].
31. Chetoui N, Sylla K, Gagnon-Houde JV, Alcaide-Loridan C, Charron D, Al-Daccak R, et al. Down-regulation of mcl-1 by small interfering RNA sensitizes resistant melanoma cells to fas-mediated apoptosis. *Mol Cancer Res*. 2008;**6**(1):42–52. doi: 10.1158/1541-7786.MCR-07-0080. [PubMed: 18234961].
32. Skoda C, Erovic BM, Wachek V, Vormittag L, Wrba F, Martinek H, et al. Down-regulation of Mcl-1 with antisense technology alters the effect of various cytotoxic agents used in treatment of squamous cell carcinoma of the head and neck. *Oncol Rep*. 2008;**19**(6):1499–503. [PubMed: 18497956].
33. Karami H, Baradaran B, Esfehani A, Sakhinia M, Sakhinia E. Down-regulation of Mcl-1 by small interference RNA induces apoptosis and sensitizes HL-60 leukemia cells to etoposide. *Asian Pac J Cancer Prev*. 2014;**15**(2):629–35. doi: 10.7314/APJCP.2014.15.2.629. [PubMed: 24568469].
34. Hussain SR, Cheney CM, Johnson AJ, Lin TS, Grever MR, Caligiuri MA, et al. Mcl-1 is a relevant therapeutic target in acute and chronic lymphoid malignancies: Down-regulation enhances rituximab-mediated apoptosis and complement-dependent cytotoxicity. *Clin Cancer Res*. 2007;**13**(7):2144–50. doi: 10.1158/1078-0432.CCR-06-2294. [PubMed: 17404098].
35. Hao JH, Gu QL, Liu BY, Li JF, Chen XH, Ji YB, et al. Inhibition of the proliferation of human gastric cancer cells SGC-7901 in vitro and in vivo using Bcl-2 siRNA. *Chin Med J (Engl)*. 2007;**120**(23):2105–11. doi: 10.1097/00029330-200712010-00008. [PubMed: 18167184].
36. Meng WB, Liu JP, Wang XW, E LH. Effect of Bcl-2-siRNA on proliferation and apoptosis of pediatric acute B lymphoblastic leukemia (A-

BLL) cells. *Genet Mol Res*. 2015;**14**(4):12427–36. doi: 10.4238/2015.October.16.9. [PubMed: 26505392].

37. Liu HT, Lu CL. Effect of silencing Bcl-2 expression by small interfering RNA on radiosensitivity of gastric cancer BGC823 cells. *Asian Pac J Trop Med*. 2013;**6**(1):49–52. doi: 10.1016/S1995-7645(12)60199-0. [PubMed: 23317885].
38. Gao J, Li L, Wu M, Liu M, Xie X, Guo J, et al. MiR-26a inhibits proliferation and migration of breast cancer through repression of MCL-1. *PLoS One*. 2013;**8**(6). e65138. doi: 10.1371/journal.pone.0065138. [PubMed: 23750239]. [PubMed Central: PMC3672200].