RESEARCH ARTICLE

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Anti-Leukemic Effects of Small Molecule Inhibitor of c-Myc (10058-F4) on Chronic Myeloid Leukemia Cells

Sara Zehtabcheh, Negar Sheikh-Zeineddini, Amir-Mohammad Yousefi, Davood Bashash*

Abstract

Background: As one of the main molecules in BCR-ABL signaling, c-Myc acts as a pivotal key in disease progression and disruption of long-term remission in patients with CML. **Objectives:** To clarify the effects of c-Myc inhibition in CML, we examined the anti-tumor property of a well-known small molecule inhibitor of c-Myc 10058-F4 on K562 cell line. **Methods:** This experimental study was conducted in K562 cell line for evaluation of cytotoxic activity of 10058-F4 using Trypan blue and MTT assays. Flow cytometry and Quantitative RT-PCR analysis were also conducted to determine its mechanism of action. Additionally, Annexin/PI staining was performed for apoptosis assessment. **Results:** The results of Trypan blue and MTT assay demonstrated that inhibition of c-Myc, as shown by suppression of c-Myc expression and its associated genes PP2A, CIP2A, and hTERT, could decrease viability and metabolic activity of K562 cells, respectively. Moreover, a robust elevation in cell population in G1-phase coupled with up-regulation of p21 and p27 expression shows that 10058-F4 could hamper cell proliferation, at least partly, through induction of G1 arrest. Accordingly, we found that 10058-F4 induced apoptosis via increasing Bax and Bad; In contrast, no significant alterations were observed NF-KB pathway-targeted anti-apoptotic genes in the mRNA levels. Notably, disruption of the NF-κB pathway with bortezomib as a common proteasome inhibitor sensitized K562 cells to the cytotoxic effect of 10058-F4, substantiating the fact that the NF-κB axis functions probably attenuate the K562 cells sensitivity to c-Myc inhibition. **Conclusions:** It can be concluded from the results of this study that inhibition of c-Myc induces anti-neoplastic effects on CML-derived K562 cells as well as increases the efficacy of imatinib. For further insight into the safety and effectiveness of 10058-F4 in CML, in vivo studies will be required.

Keywords: c-Myc- 10058-F4- BCR-ABL- chronic myeloid leukemia- NF-κB

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Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder with a translocation involving chromosomes 9 and 22 t(9;22)(q34;q11). A chimeric BCR-ABL oncoprotein is produced via this translocation, which deregulates activity of tyrosine kinase activity [1]. Despite the fact that tyrosine kinase inhibitors (TKIs) have fundamentally changed the way CML is managed, only a fraction of TKI-treated patients achieve long-term remission and some of the patients experience disease progression after initially successful treatment [2]. It has been established the main role of oncogenic fusion protein BCR-ABL in CML pathogenesis is more significant than others pathways. However, recent evidence has stated that treatment issues will remain unresolved until other aberrancies are ignored, in addition to BCR-ABL fusion protein [3]. Through the activity of tyrosine kinase, BCR-ABL could activate many signaling pathways, such as the Ras [4], PI3K/Akt [5], JAK/STAT [6], and NF-κB [7]. Among various molecules responsible for BCR-ABL signaling, c-Myc has a prominent role in regulating of proliferation and survival in malignant cells [8]. C-Myc has a central role in cellular transformation mediated by BCR-ABL and over-expresses when the shift to blast crisis occurs. Moreover, it has been shown that increased c-Myc expression in CML may cause aneuploidy, involving in the progression of disease [9, 10]. Notably, a previous study observed that the mitigating role of c-Myc in the extent of sensitivity of CML cells to the PI3K inhibitor, as a direct oncogenic pathway downstream of BCR-ABL signaling [11].

C-Myc ability for dimerization with MAX to form a productive DNA-binding heterodimer could be interrupted by the small-molecule inhibitor 10058-F4 [12]. It has been demonstrated in several studies that, this inhibitor could act as a promising and potential anti-cancer target in in-vitro or in-vivo investigations [13-16]. Zhang and

*Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran. *For Correspondence: D.bashash@sbmu.ac.ir*

colleagues demonstrated that10058-F4 reduced cell viability in pancreatic ductal adenocarcinoma (PDAC), induced G1/S cell cycle arrest and elevated caspase 3/7 activity [17]. The effect of 10058-F4 could be observed in inhibition of ovarian cancer cells proliferation and growth and intensifying the cytotoxic effects of chemotherapeutic drugs [18]. In the context of combinatorial strategies, Kugimiya et al. illustrated that the combination of 5-fu and 10058-F4 treatment remarkably reduced tumorigenicity in the human colon cancer xenograft murine model [19]. According to previous studies which investigated the impacts of 10058-F4 in hematologic malignancies were promising as well. The results of other studies showed that 10058-F4 could increase the sensitivity of both B and T acute lymphoblastic leukemia (ALL) cell lines to glucocorticoids Lv, Mei et al [20]. According to promising effects of c-Myc inhibition in various types of human malignancies and also given the c-Myc prominent role in CML progression and TKI resistance, our study designed to evaluate whether c-Myc suppression may be a beneficial therapeutic approach in CML or not.

Materials and Methods

Cell culture and drug treatment

We cultured the K562 derived from human CML in RPMI 1640 medium with 10% heat-inactivated fetal bovine serum, 100 units per ml of penicillin, and 100 mg per ml of streptomycin. The cells were maintained in a humidified, 37°C incubator with 5% CO2. BCR-ABL expression in proliferated cells was confirmed using qRT-PCR. The appropriate concentrations of 10058-F4 (Selleckchem) were dissolved in sterile dimethyl sulfoxide (DMSO) to make a stock solution. Then, calculated amount of 10058-F4 was diluted in a culture medium to attain concentrations of 50–200 μM. Moreover, autophagy inhibitor chloroquine (CQ) (Sigma, Taufkirchen, Germany), proteasome inhibitor Bortezomib (BTZ) (Selleckchem, Munich, Germany), and Imatinib (Sigma) were dissolved in DMSO and stored at -20 °C. K562 cells were treated with the proper concentrations of the 10058-F4, either alone or in combination with other inhibitors.

Trypan blue assay

The inhibitory effect of 10058-F4 on the viability and cell count of K562 cells was evaluated by Trypan blue staining. Briefly, the cells were seeded at 200×103 cells/well density and incubated in the presence of the designated concentrations of 10058-F4, either alone or in a combined modality. After the indicated time intervals (24, 36 and 48 hours), drug-treated cells were centrifuged, and cell pellets were exposed to 0.4% Trypan blue solution at room temperature. 10μl of the cell suspension was loaded onto the chamber of the Neubauer hemocytometer. The total number of viable (unstained) and nonviable (stained) cells were manually counted with the light microscope.

MTT assay

The metabolic activity of treated cells was measured after incubation with MTT solution to evaluate the anti-leukemic effects of 10058-F4 and its combination with other agents in CML-derived K562 cells. Incubation of cells (5,000 per well) with the indicated concentrations of agents was performed in a 96-well plate. After indicated time intervals, the plate was incubated at 37°C for three hours after adding MTT solution (5 mg/mL in PBS). The resulting formazan was solubilized with DMSO, the optical density was measured in an ELISA reader at570nm.

Flow cytometric analysis of cell cycle distribution

Flow cytometric analysis after 24 h incubation of K562 cells with 10058-F4 was used to analyze Cellular DNA content and cell cycle distribution. 1×10^6 cells were harvested, washed twice with cold PBS, and then fixed in 70% ethanol at -20 °C overnight. To remove ethanol, cells were centrifuged, and re-suspended in a staining solution comprising 1 mg/mL propidium iodide (PI), 0.2 mg/mL RNase, and 0.1% Triton X-100 at 37 °C. After 30 min incubation, the DNA content of the cells was quantified by analysis of flow cytometric histograms, and FlowJo, version 10 was used for analysis.

Assessment of apoptosis using flow cytometry

To investigate whether 10058-F4 cytotoxic effects were exerted through induction of programmed cell death, K562 cells were subjected to flow cytometry analysis. After 24 h, 10058-F4-treated K562 cells were harvested, washed with PBS, and suspended in 100 μL of the incubation buffer. After that, Annexin-V and PI Flous (2 μl per sample) were added and cell suspensions were incubated for 20 min in the dark. Annexin V-positive and PI-negative cells were considered to be in the early apoptotic phase. Cells with positive staining for annexin-V and PI were presented late apoptosis.

RNA extraction, cDNA synthesis, and quantitative real-time PCR

Total RNA from K562 cells was extracted using the RNA Isolation Kit (Roche, Mannheim, Germany). After quantification of the isolated RNA by a Nanodrop instrument (Nanodrop ND-1000 Technologies), 1 μg of RNA from each sample was used to synthesize complementary DNA (cDNA) using the cDNA Synthesis Kit (Takara Bio). Next, to examine the effect of 10058-F4 on the expression of proliferation- and apoptotic-related genes, the cDNAs were subjected to quantitative real-time PCR (qRT-PCR). GAPDH was used for normalization and the fold change values were computed based on 2-ΔΔCT relative expression formula.

Detection of autophagy by acridine orange staining

K562 cells treated with an autophagy inhibitor CQ at different concentrations of (20-60 μM). We investigated whether autophagy suppression correlates with K562 sensitivity to 10058-F4 by treating them with either CQ or 10058-F4 alone or in a combination with each other. After 24 hours, treated cells were collected and then treated with acridine orange (1 g/mL) (Merck, Darmstadt, Germany) for 15 minutes in the dark. Fluorescence microscopy was used to observe the differences in acidity between autophagic lysosomes and the cytoplasm/nucleus (Labomed, Los Angeles). It was observed that autophagic

lysosomes appeared as orange/red fluorescent cytoplasmic vesicles, whereas the nucleus and cytoplasm were green in color.

Calculation of combination index (CI) and dose reduction index (DRI)

we used the classic isobologram equation [CI=(D)1/(Dx)1+(D)2/(Dx)2] to determine the kind of interaction between 10058-F4 and Imatinib (synergistic, additive or antagonistic). While (Dx)1 and (Dx)2 are the concentrations of single agents of Imatinib and 10058-F4 necessary to produce a given level of suppressive effect on viability, (D)1 and (D)2 indicate the concentrations of the agents required to inhibit viability at the same level in combination. The CI values of <1, = 1, and >1 represent synergistic, additive, and antagonistic effects of the drugs respectively. Furthermore, the dose reduction index (DRI) for the drug combination was calculated using the following formula: (DRI)1 = (Dx)1/(D)1, and (DRI)2 = (Dx)2/(D)2.)

Statistical analysis

Experimental data were collected from three independent experiments in which treated cells were compared with untreated cell lines as a control group. One-way variance analysis and two-tailed Student's t-tests were used to determine whether differences between experimental variables were significant. Using Dunnett's multiple comparison test, the control group and the treated group were compared. All data are presented as mean ± standard deviation (SD). $P \leq 0.05$ was defined as statistically significant.

Results

A large amount of evidence has indicated the aberrant expression of c-Myc is one of the most prevalent events in hematopoietic malignancies [21]. In chronic myeloid leukemia (CML), the BCR-ABL aberrant kinase activity in CML could induce c-Myc transcription and translation, which correlates with poor treatment response [10]. Given this, it was tempting to investigate the effect of 10058-F4, a potent and highly selective inhibitor of c-Myc, in CML-derived K562 cells. Time-dependent experiments showed that treating cells with inhibitor of the cells with the inhibitor at different time intervals (24, 36, and 48h) reduced K562 cell viability (Figure 1A). The resulting data also demonstrated that 48 hours of treatment decreased the number of treated cells (Figure 1B), which indicate the potent anti-proliferative effect of 10058-F4 on CML cells. This finding was further confirmed by the MTT assay results, showing that 10058-F4 capable of reducing metabolic activity of K562 cells in the similar manner (Figure 1C). To ascertain whether the 10058-F4 anti-leukemic effect is mediated by abrogation of c-Myc expression in K562 cells, we evaluated the expression level of this oncogene. The results of qRT-PCR analysis indicated a noticeable reduction in the c-Myc expression level. In consistent, 10058-F4 was able to decrease the expression levels of PP2A, CIP2A, and hTERT, which are c-Myc-associated genes (Figure 1D).

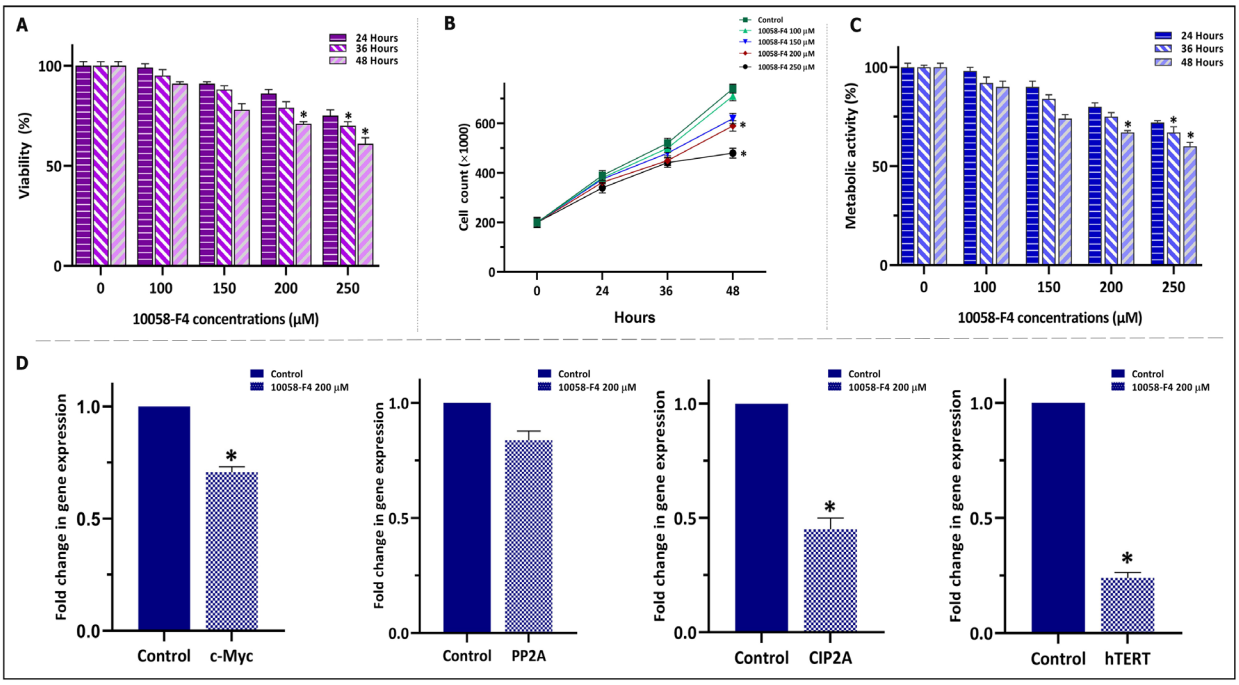

Figure 1. Suppression of c-Myc Using 10058-F4 Reduced the Proliferation and Survival Rate of K562 Cells. (A and B) The result of Trypan blue assay showed that 10058-F4 reduced the viability and proliferative rate of K562 cells. (C) MTT assay revealed that 48 h incubating K562 cells with increasing concentrations of 10058-F4 (100-250 μM) reduced the metabolic activity in a concentration- and time-dependent manner. (D) After exposing the K562 cell line with 10058-F4 at the concentration of 200 μM, the mRNA expression level of c-Myc and its associated genes such as PP2A, CIP2A, and hTERT was decreased. Values are given as mean ± standard deviation of three independent experiments. *$P \leq 0.05$ represents significant changes from untreated control.

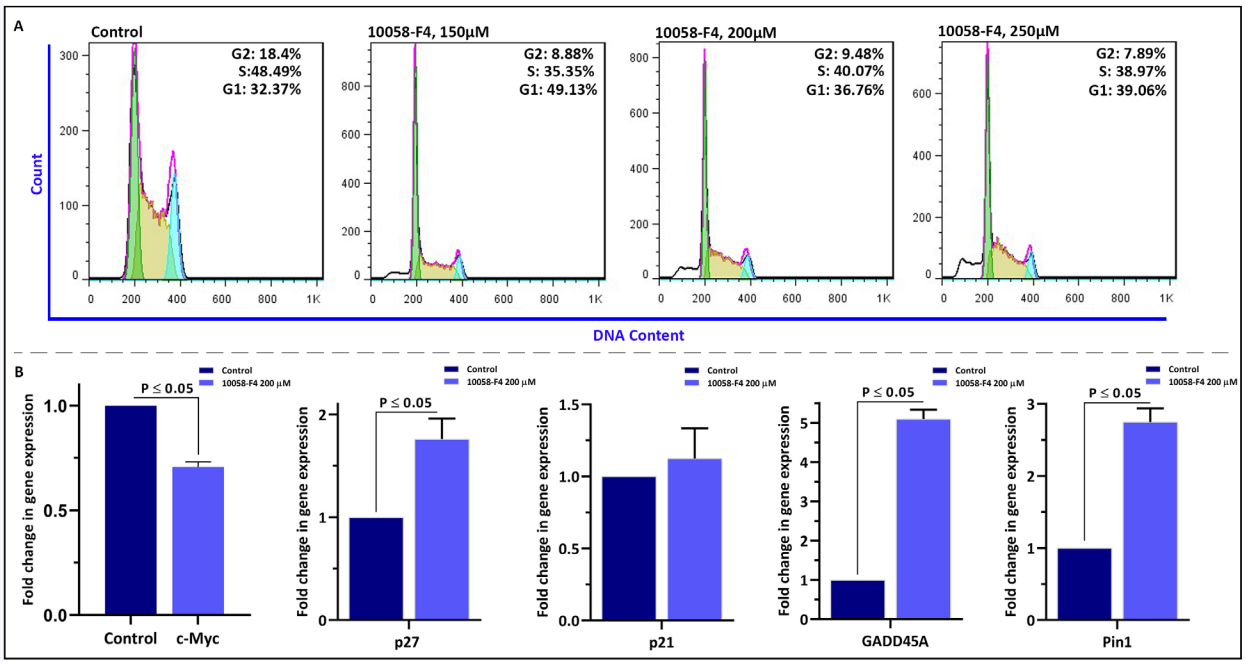

Figure 2. Effect of 10058-F4 on the Distribution of CML Cells in Different Phases of the Cell Cycle. (A) Treatment of the K562 cells with 10058-F4 resulted in a significant elevation in the proportion of cells in G1 phase and decrease in the population of the cells in both S and G2/M phases of the cell cycle. (B) The results of qRT-PCR also showed that 10058-F4 a significant increase in the main regulators of cell cycle genes such as p27, p21, GADD45A and Pin-1. Values are given as mean ± standard deviation of three independent experiments. *P ≤ 0.05 represents significant changes from untreated control.

10058-F4 decreased the proliferative capacity of K562 cells through G1 cell cycle accumulation

The role of c-Myc in regulating cell proliferation and cell cycle progression has been well-established in several reports. PI staining revealed that c-Myc inhibition resulted in increasing the quantity of cells in the G1 phase. Moreover, we found that the number of cells in both S and G2/M phase was decreased (Figure 2A); while 18.4% of the untreated cells were accumulated at G2/M phase, this number declined to 7.89% in K562 cells treated with 250 µM. Expression of gene that involved in regulating cell transition from G1 were examined to explore molecular mechanisms by which 10058-F4 disturbs K562 cell cycle progression. As represented in Figure 2B, quantitative

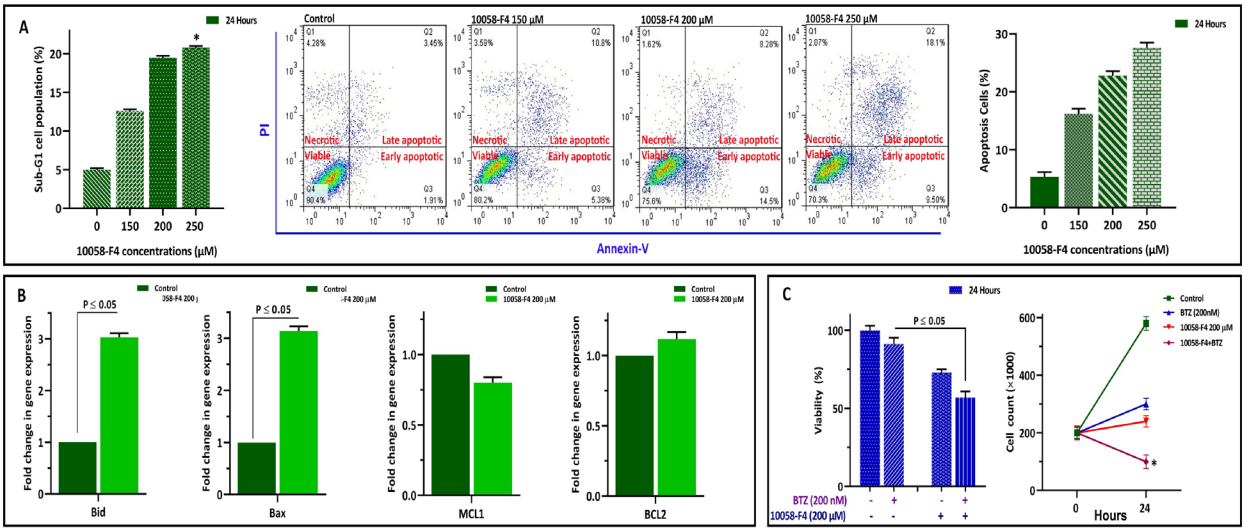

Figure 3. The Apoptotic Effect of 10058-F4 on K562 Cells is Mediated through the up-regulation of pro-apoptotic Genes. (A) The percentage of Annexin-V/PI double-positive inhibitor-treated cells was increased in response to drug treatment after 24 hours, as compared with the untreated group. (B) Treatment of K562 cells with c-Myc inhibitor raised the mRNA expression level of pro-apoptotic genes but no significant alteration in the mRNA levels of anti-apoptotic target genes of the NF-κB pathway, such as Bcl-2 and MCL-1. (C) Suppression of the NF-κB signaling using a well-known proteasome inhibitor (Bortezomib) enhanced 10058-F4-induced cytotoxicity as compared to either agent alone. Values are given as mean ± standard deviation of three independent experiments. *P ≤ 0.05 represents significant changes from untreated control cells.

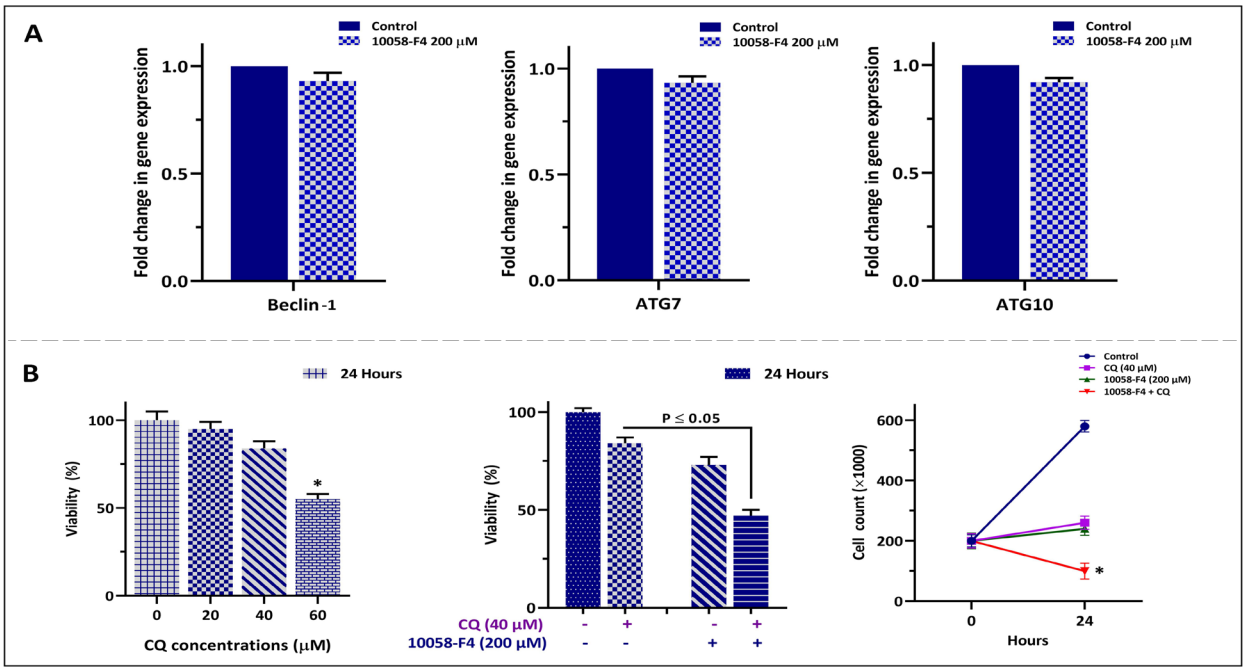

Figure 4. Autophagy Inhibition could Reinforce the Anti-Leukemic Property of 10058-F4 in CML Cells. (A) The single agent of 10058-F4 could slightly down-regulate the mRNA expression of autophagy-related genes (Beclin-1, ATG-7, and ATG-10). (B) Viability and cell count of K562 cells were decreased upon treatment with increasing concentrations of chloroquine (CQ) in a concentration dependent manner. Co-treatment of K562 cells with 10058-F4 and non-cytotoxic concentration of CQ (40 µM) could induce a greater cytotoxicity in comparison to either agent alone. Values are given as mean ± SD of three independent experiments and statistically significant values of *p < .05 are determined compared to untreated control cells.

RT-PCR was used to confirm that 10058-F4 inhibited the cell cycle where we found elevated expression level of p27 and p21 mRNA. According to reports, GADD45A in connection with p21 could influence cell

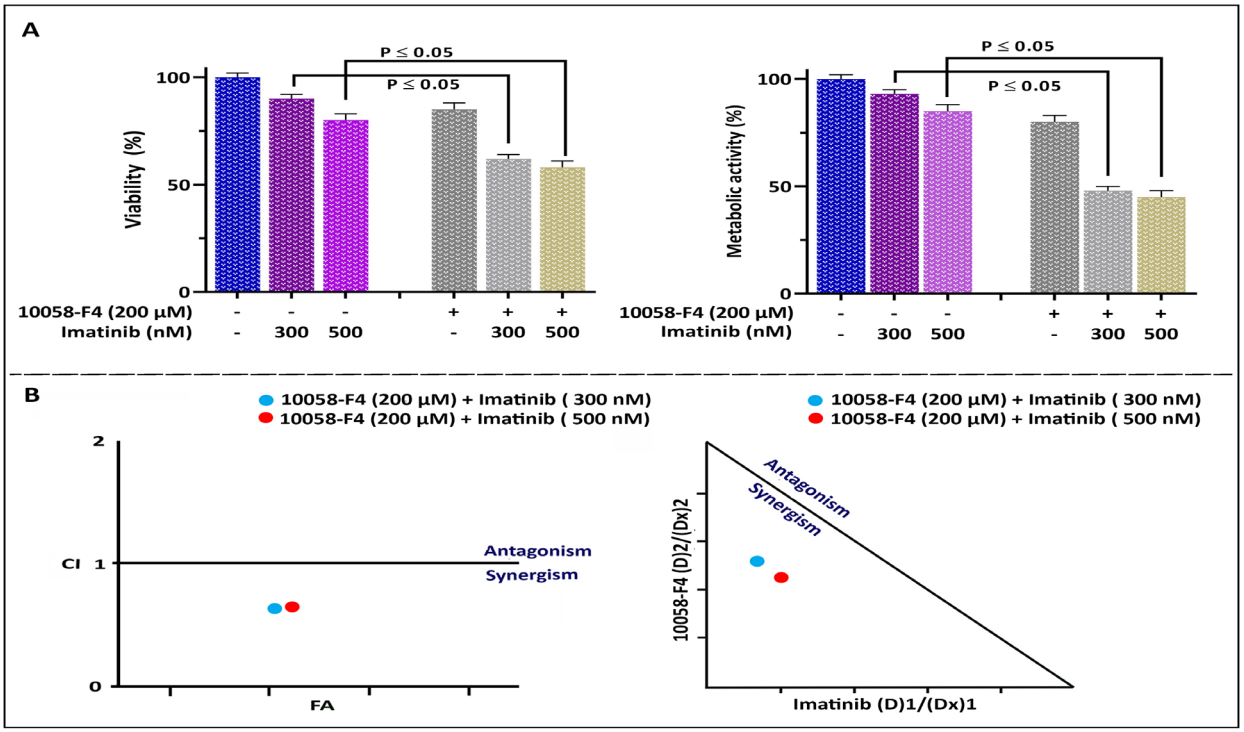

Figure 5. Synergistic Effects of 10058-F4 and Imatinib. (A) Trypan blue and MTT assays were applied to evaluate the viability and metabolic activity in 10058-F4-plus-Imatinib co-treated K562 cells. The results showed that 10058-F4 could enhance the sensitivity of K562 cells to Imatinib. (B) The results of both combination index (CI) and isobologram demonestrated the synergistic effect between 10058-F4 and Imatinib. Values are given as mean ± SD of three independent experiments and statistically significant values of *p < .05 are determined compared to untreated control cells.

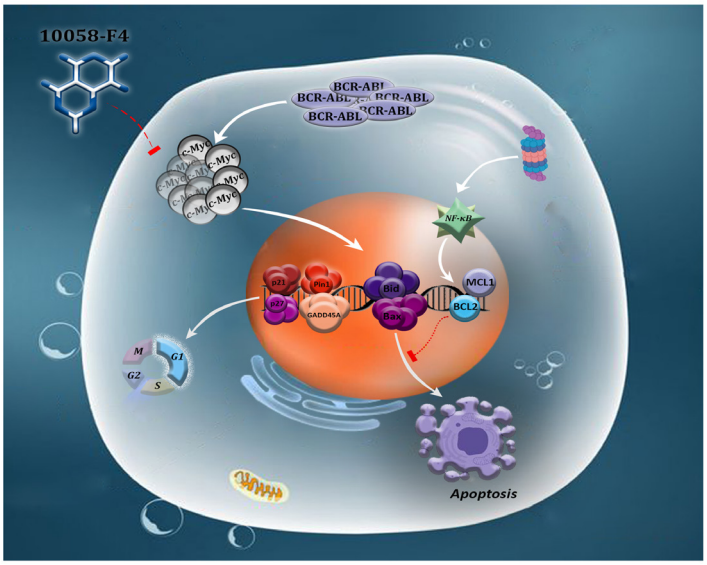

Figure 6. Schematic Representation Proposed for the Plausible Mechanisms of Action of 10058-F4 in Chronic Myeloid Leukemia (CML) Cells. Abrogation of c-Myc using small molecule 10058-F4 altered the expression levels of cell cycle-related target genes, resulting in decreased proliferation rate due to the stimulation of G1 arrest. We also found that 10058-F4 induced apoptosis via increasing Bax and Bad. While no significant changes were noted in the mRNA levels of anti-apoptotic target genes of the NF-κB pathway, suppression of this signaling pathway using a well-known proteasome inhibitor Bortezomib enhanced 10058-F4 cytotoxicity; proposing that the activation of the NF-κB axis probably attenuates the sensitivity of K562 cells to c-Myc inhibition.

Table 1. Combination Index (CI) and Dose Reduction Index (DRI) for Drug Combination by 10058-F4 and Imatinib

10058-F4		Imatinib		CI
Concentration (μM)	DRI	Concentration (nM)	DRI	
200	2	300	7.23151	0.63828
200	2.26316	500	4.97346	0.64293

cycle by arresting in G1/S and G2/M transitions through interacting with c-Myc [22]. GADD45A mRNA level rose in response to 10058-F4 treatment according to the results of c-Myc and p21. Pin1 has been also shown to promote the transcriptional activation of p21 in response to DNA damage [23]. In harmony, we found that 10058-F4 could up-regulate Pin-1 mRNA expression (Figure 2B), shedding light on the molecular mechanisms by which 10058-F4 decreased K562 proliferation, by inducing G1 cell cycle arrest, at least in part.

C-Myc suppression in K562 cells reduced the survival of the cells by inducing apoptotic cell death

The results of DNA content analysis also showed that 10058-F4 could elevate sub-G1 proportion, which is considered an important hallmark of apoptotic cells and indicates the pro-apoptotic ability of the inhibitor in K562 leukemic cells. Flow cytometry analysis was performed to confirm that the drug's cytotoxic effects resulted from the induction of apoptosis. As is consistent with the elevated sub-G1, cells treated with c-Myc inhibitors displayed an increased in cell expressing Annexin-V/PI double-positive in comparison with control group (Figure 3A). In the light of the apoptotic property of the agent, the qRT-PCR analysis was applied to investigate the plausible alteration of crucial genes involved in propagating cell death. While 10058-F4 induced apoptosis by altering the expressions of death promoters mainly via increasing Bax and Bad, there was not remarkable changes in mRNA levels of anti-apoptotic target genes of the nuclear factor (NF)-κB pathway including Bcl-2 and MCL-1 (Figure 3B). As compared with either agent alone, suppression of NF-κB signaling using proteasome inhibitor (Bortezomib) could boosted 10058-F4-induced cytotoxicity (Figure 3C); marking that the inhibitory effect of 10058-F4 in CML-derived K562 cells was probably overshadowed, at least partially, through survival signals that are transmitted from NF-κB.

Superior cytotoxicity of 10058-F4 in combination with autophagy inhibitor

The correlation between c-Myc overexpression and autophagy in multiple types of cancer cells has been studied. It must be noted, however, that the results of many studies are inconsistent; although several reports indicated that the higher expression of c-Myc could suppress the autophagy system [24], a compelling body of evidence demonstrated that autophagy serves as the main mechanism to provide energy for cancer cell proliferation in human disorders with involvement of c-Myc [25, 26]. Although qRT-PCR results showed that K562 treatment with 10058-F4 could moderately down-regulate the mRNA genes expression involved in autophagy (Beclin-1, ATG-7, and ATG-10), its impact on the mRNA level of

these genes was statistically insignificant in K562 cell line; proposing that the presence of autophagy may hinder cytotoxic effect of c-Myc inhibition in CML cells (Figure 4A). As a confirmation of our hypothesis, we then treated K562 cells with either the autophagy inhibitor chloroquine (CQ) alone or with 10058-F4 combined. As shown in Figure 4B, the blocking of autophagy not only reduced cell survival in a monotherapy strategy, but also resulted in effective cytotoxicity in cells treated with 10058-F4; Providing insight into the possibility that autophagy activation may diminish the anti-leukemic effects of 10058-F4 K562 cells.

Inhibition of c-Myc by 10058-F4 could sensitize K562 cells to Imatinib

It is more than several decades that the name of CML has been integrated with Imatinib, famous tyrosine kinase inhibitor largely used as the first-line management of CML. To determine whether inhibition of c-Myc could potentiate the anti-leukemic property of Imatinib, we have investigated the effects of combining the drugs 10058-F4 and Imatinib on K562 cells. The combination of both drugs prevented cell survival and metabolic activity more effectively than either drug alone (Figure 5A). Additionally, to investigate what extent 10058-F4 could enhance the sensitivity of the cells to Imatinib, we calculated the combination index (CI) values based on the Chou–Talalay combination index. Based on the results of a fraction-affect (FA) versus CI analysis, all points fall below the line of additive effects. As agreed to the CI analysis, the isobologram analysis also represented the synergistic cytotoxicity when Imatinib combined with 10058-F4 (Figure 5B). A summary of the combination index (CI) and dose reduction index (DRI) for drug combinations by 10058-F4 and Imatinib is given in Table 1.

Discussion

Developing different tyrosine kinase inhibitors (TKIs) generations has intensely changed the prospect of treatment strategies for CML patients [27]; however, BCR-ABL protein's new mutations in the residual leukemic cells are a crucial problem that cause a refractory response and resistance to TKIs[28]. Some pathways are activated by BCR-ABL that leading to a firm preserve oncogenic processes in CML by inducing the transcription factors. BCR-ABL activation leads to inducing transcription factors that maintain oncogenic processes in CML through a number of pathways. In this framework, multiple studies have highlighted that expression of c-Myc not only is elevated in CML blast crisis but also has a correlation with poor response to TKIs [10]. Protein phosphatase 2A (PP2A) activity is impaired by CIP2A, resulting in the stabilization of c-Myc [29]. Accordingly, it has been observed that high expression of CIP2A is related to high BCR-ABL tyrosine kinase activity and increased expression of c-Myc [30]. According to this study, c-Myc abrogation using 10058-F4 was demonstrated to dramatically decrease the survival and proliferative capacities of K562 cells as evidenced by a lower c-Myc expression and associated genes, including PP2A, CIP2A, and hTERT. Intriguingly, when Imatinib was used in combination with 10058-F4 in a synergistic manner, our results showed that the K562 cells' viabilities has a significant reduction, suggesting that c-Myc overexpression, at least partly, may overshadow anti-leukemic effect of Imatinib. It is known that c-Myc is regulated highly throughout the cell cycle, and c-Myc deregulation can change the regulation of the cell cycle in order to facilitate the progression from G1 to S [31]. Investigating the molecular mechanisms of 10058-F4 revealed that c-Myc inhibition could affect K562 cell cycle progression through p21- and p27-mediated G1 phase accumulation. Our findings were accordant with the study supervised by Ghaffarnia et al. [18], who suggested that 10058-F4 causes arrest of the G1 cell cycle in 2008C13 ovarian cancer cells, as well as elevated expression of some genes associated with the cell cycle.

Other studies have showed that c-Myc could change the balance between pro- and anti-apoptotic members of the Bcl-2 family, thereby producing effect on the intrinsic apoptosis pathway [32]. Accordingly, we found that 10058-F4 induced apoptosis by altering the expressions of death promoters mainly via increasing Bax and Bad; however, the mRNA levels of anti-apoptotic target genes of the NF-κB pathway, Bcl-2 and MCL-1, had no outstanding changes. Additionally, it has been found that blocking the NF-κB pathway with bortezomib, a proteasome inhibitor, could sensitize K562 cells to the cytotoxic effect of 10058-F4, substantiating the fact that the NF-κB axis activation probably attenuates the K562 cells sensitivity to c-Myc inhibition (Figure 6). Unlike the results of our study, Sayyadi et al. reported that 10058-F4 could induce apoptosis in acute promyelocytic leukemia (APL)-derived NB4 cells through suppressing NF-κB pathway; probably proposing that BCR-ABL-mediated over-activation of the NF-κB pathway, either directly or via activation of the PI3K cascade, may be in charge of less sensitivity of CML cells to c-Myc inhibitors [33]. In agreement, the results of our previous study showed while 10058-F4 failed to suppress NF-κB in pre-B ALL-derived Nalm-6 cells, the combination of pan-PI3K and c-Myc inhibitors could inhibit the NF-κB pathway; strengthening that the overactivation of PI3K could diminish, at least in part, the effectiveness of c-Myc inhibition [34, 35].

Various studies reported that apoptosis and autophagy pathway have an indispensable role in protecting against cellular damage and cancer development. Autophagy could either trigger cell death or induce a resistance phenotype which depends on cancer cell types. Notably, the correlation between c-Myc overexpression and autophagy has been investigated in various cancer cell types; however, in most of the cases, there are conflicting results. Interestingly, our result showed that the blockade of autophagy using chloroquine (CQ) could reduce the survival of CML cells in a monotherapy experiment, also exerted superior cytotoxicity in 10058-F4-treated cells; enlightening the possibility that autophagy may serve as a mechanism to provide energy for CML cell proliferation. According to our findings, autophagy inhibition has therapeutic potential for eliminating the CML cells when

they are resistant to TKIs [36]. As a conclusion, it has been demonstrated in this study that inhibition of c-Myc by 10058-F4 has cytotoxic effects on CML-derived K562 cells as well as enhanced Imatinib efficacy, suggesting the potential for its therapeutic application either alone or in combination of other treatments; however, further investigation such as in-vivo studies is needed to determine the efficacy of c-Myc inhibition in patients suffering from CML.

Author Contribution Statement

AAuthors D.B, N.SZ. were involved in planning and supervising the work, S.Z, AM.Y were involved in laboratory experiments, data analysis and writing the manuscript.

Acknowledgements

Ethical Statement

This study does not involve human participants or animals and therefore does not require approval from an institutional review board or ethics committee.

Availability of data

All data supporting the findings of this study are provided within the manuscript and its supplementary materials.

Conflict of interest

The authors declare that they have no conflict of interest.

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