Biochemistry and Biophysics Reports 15 (2018) 69–75

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

BCL2 inhibitor ABT-199 and JNK inhibitor SP600125 exhibit synergistic cytotoxicity against imatinib-resistant Ph+ ALL cells

Chisato Inoue[a], Sayaka Sobue[a], Yuka Aoyama[a], Naoki Mizutani[a], Yoshiyuki Kawamoto[a], Yuji Nishizawa[a], Masatoshi Ichihara[a], Akihiro Abe[b], Fumihiko Hayakawa[b], Motoshi Suzuki[c], Yoshinori Nozawa[d], Takashi Murate[a,*]

[a] College of Life and Health Sciences, Chubu University, Matsumoto-cho 1200, Kasugai 487-8501, Japan
[b] Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya 461-8560, Japan
[c] Division of Molecular Oncology, Fujita Health University School of Medicine, Toyoake 470-1192, Japan
[d] Tokai Gakuin University, Kakamigahara 504-8511, Japan

ARTICLE INFO

Keywords:
Ph+ ALL
Imatinib-resistance
JNK inhibitor
BCL2 inhibitor
Synergistic action

ABSTRACT

Imatinib (IMT), a specific tyrosine kinase inhibitor (TKI), has drastically changed the treatment strategy for Ph+ ALL (Philadelphia chromosome-positive acute lymphoblastic leukemia). However, TKI resistance remains a serious problem for patient prognosis. Here, a Ph+ ALL cell line NphA2 and the IMT-resistant subline NphA2/STIR were analyzed to identify a potential novel treatment strategy. We also examined other Ph+ ALL cells, MR87 and its IMT-resistant subline, MR87/STIR. IMT induced apoptosis of NphA2 and MR87 but had no effect on resistant sublines. Increased phosphorylated ERK and BCL2, but not BCL-XL, were observed in NphA2/STIR compared with NphA2. NphA2/STIR but not NphA2 was moderately sensitive to U0126, an ERK inhibitor. Interestingly, SP600125, a JNK inhibitor, was potent in cell growth inhibition and apoptosis induction of both parental and IMT-resistant NphA2 and MR87 cells. Moreover, NphA2 and MR87 and their IMT-resistant sublines were sensitive to ABT-199, a specific BCL2 inhibitor. The combination of SP600125 and ABT-199 synergistically suppressed both parental and IMT-resistant cells, including one with T315I mutation, suggesting that Ph+ ALL exhibits high sensitivity to ABT-199 and SP600125 regardless of TKI resistance. This combination might be a possible therapeutic strategy for Ph+ ALL in the future.

1. Introduction

The Philadelphia chromosome (Ph) cytogenic abnormality results in the *BCR-ABL* fusion gene and comprises at least 25% of adult acute lymphoblastic leukemia (ALL) cases and up to 50% in the older population [1]. These leukemia cells are mostly sensitive to conventional cytotoxic drugs, but complete remission is not durable [2,3]. Before the era of molecularly targeted therapy, less than 30% of these patients achieved stable remission, even with aggressive chemotherapy [4]. Although allo-stem cell transplantation represents an effective form of treatment, the long-term overall survival is still not satisfactory.

The *BCR/ABL* fusion gene encodes a tyrosine kinase that plays a critical oncogenic function in Ph+ ALL and chronic myeloid leukemia (CML). The development of BCR/ABL-specific tyrosine kinase inhibitors (TKIs) has drastically changed the treatment of these diseases [5,6]. Treatment with the first TKI, imatinib (IMT), was tolerable with no therapy-related death, and induced complete hematological remission as well as cytogenetic response. TKIs thus represent the first-line treatment of these diseases.

Despite the implementation of TKIs, this disease still shows relapse [7]. One reason for relapse is the acquired resistance against IMT due to point mutation (including T315I) or compound mutations of BCR/ABL. More potent TKIs, including second, third, and new generation TKIs, have been or will be introduced to the clinic; however, resistance against third generation TKIs has already been reported [8]. In addition to resistance issues, some TKIs also produce serious vascular effects [9].

In this study, we examined IMT resistance using NphA2 [10], a Ph+ ALL cell line, as well as MR87 (Ph+ bi-phenotypic AL) [11], along with the IMT-resistant sublines, NphA2/STIR and MR87/STIR. NphA2/STIR cells do not have mutation in the kinase domain of BCR/ABL and do not

Abbreviations: ABL, Abelson kinase; ALL, acute lymphoblastic leukemia; BCR, break clustering region; CML, chronic myeloid leukemia; DST, dasatinib; IMT, imatinib; NRT, nirotinib; Ph+, Philadelphia chromosome positive; TKI, tyrosine kinase inhibitor

* Corresponding author.
E-mail address: murate@isc.chubu.ac.jp (T. Murate).

https://doi.org/10.1016/j.bbrep.2018.07.001
Received 26 March 2018; Received in revised form 28 June 2018; Accepted 2 July 2018
Available online 13 July 2018

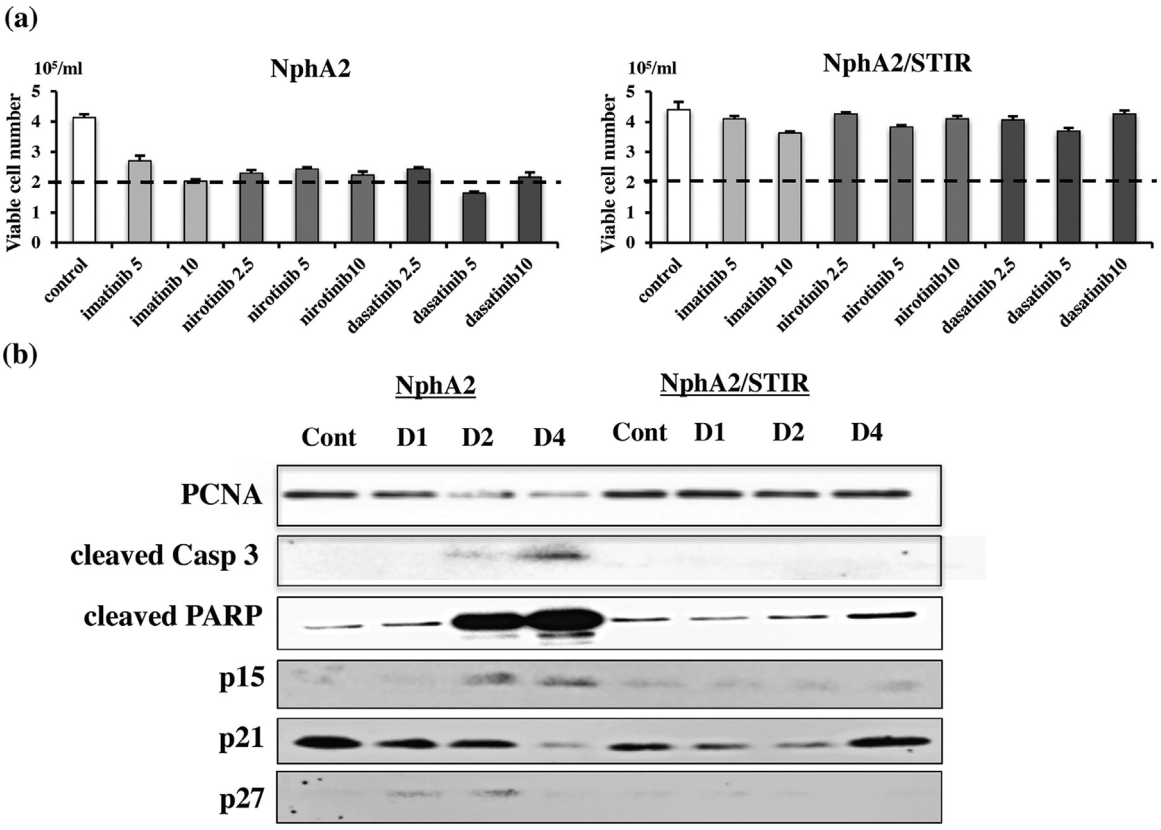

Fig. 1. Characterization of NphA2/STIR cells. (a) Viabilities of NphA2 and NphA2/STIR cells treated with indicated concentrations of IMT, NRT and DST (in μM) for 2 days. Dotted line indicates initial concentration. Cell culture experiments were performed in triplicate. Data are shown as mean ± SD. (b) NphA2 and NphA2/STIR cells were cultured with 10 μM of IMT and collected on day 1, day 2 and day 4 for western blot analysis.

show increased phosphorylated ABL [10]. We used these cells to analyze the BCR/ABL-independent IMT-resistance mechanism and focused on several key signaling and apoptosis pathways.

2. Materials and methods

2.1. Cell lines

NphA2 and its IMT-resistant subline NphA2/STIR were previously reported [10]. K562 cells and the doxorubicin-resistant K562/DNR cells were described before [12]. The MR87 cell line has been reported, and the IMT-resistant subline, MR87/STIR, was established by gradually increasing IMT concentration up to 10 μM during cell culture. TCCY/sr is a Ph+ ALL cell line with T315I mutation [13]. NALM16, Jurkat and U937 cells were reported previously [14]. CML-derived MEGA2 cells were described before [15].

2.2. Reagents

IMT, NRT (nirotinib), and DST (dasatinib) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Cell signaling pathway inhibitors U0126, LY294002, SB203580, and SP600125 were obtained from Calbiochem (San Diego, CA, USA). The BCL2-specific inhibitor ABT-199 (venetoclax), pan-BCL2 family inhibitor ABT-263 (navitoclax), and MCL1 inhibitor A-1210477 were from Abcam (Cambridge, UK). The MCL1 inhibitor marinopyrrole A (moritoclax) was purchased from ChemScene (Monmouth Junction, NJ, USA). The JNK inhibitor JNK-IN-8 was obtained from Sigma (St. Louis, MO, USA).

2.3. Cell viability assays

Cells were plated in triplicate at 2×10^5/ml (in most cases) in 24-well plates and treated with drugs at various concentrations as indicated in the experiments. Cells were cultured for the indicated number of days and then viable cells were counted by trypan blue dye exclusion. Assays were performed in triplicate.

2.4. Western blotting

Western blotting was performed as previously described [12] using the antibodies shown in Supplementary Table 1.

2.5. Isobologram analysis

The isobologram method was performed according to the previous report [14].

2.6. Statistical analysis

Statistical significance was analyzed by Student's *t*-test or one-way ANOVA with Tukey's test for multiple comparisons. All analyses were performed using Prism 6 software (GraphPad; La Jolla, CA, USA).

3. Results

3.1. Characterization of NphA2/STIR cells

A previous study showed that NphA2/STIR exhibits BCR-ABL-independent and RAS/MAPK pathway-dependent IMT resistance [10]. We tested whether NphA2/STIR cells show resistance to other BCR/ABL kinase inhibitors, NRT and DST. Cell viability assays showed that the BCR/ABL-specific TKIs (IMT, NRT, and DST) all effectively suppressed proliferation of parental NphA2 cells (Fig. 1a). However, NphA2/STIR cells were resistant to IMT, as well as NRT and DST. NphA2 cells

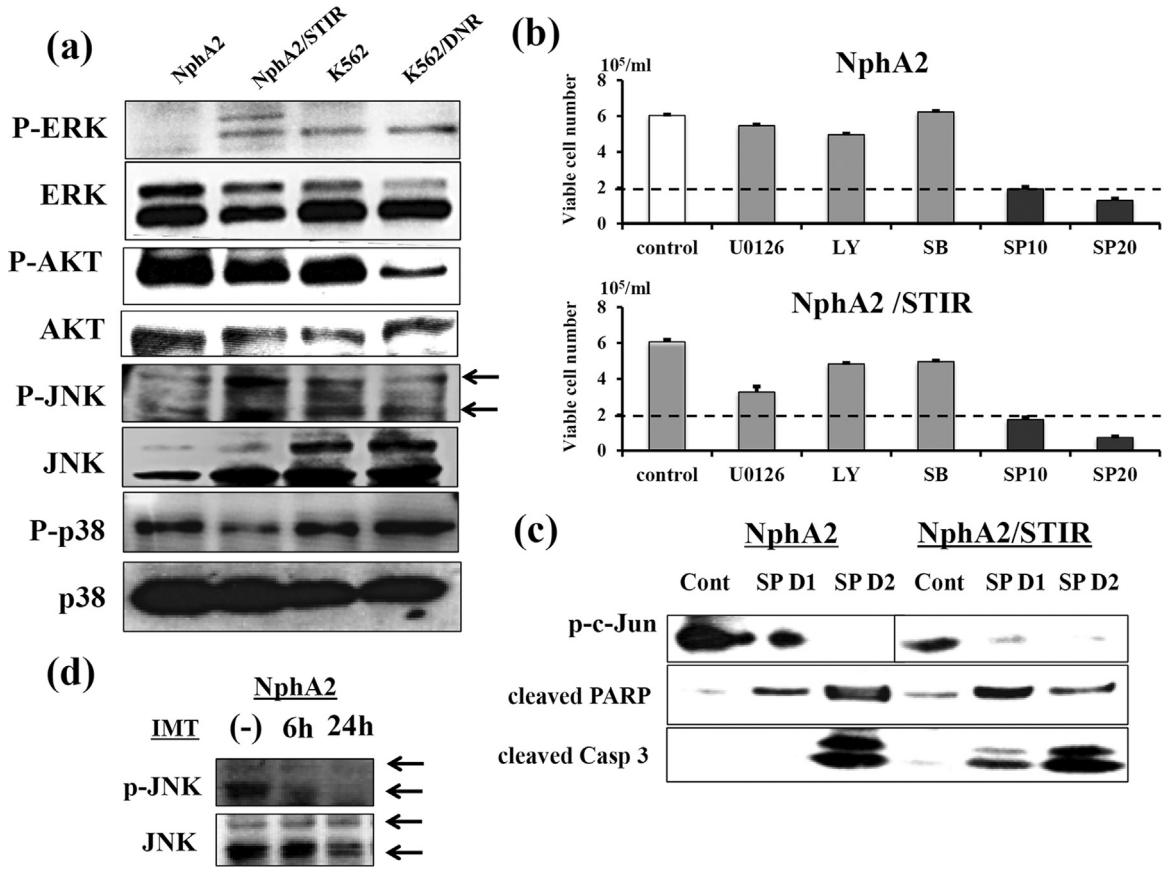

Fig. 2. Effects of cell signaling pathway inhibitors. (a) Western blot analysis of cellular signaling pathway proteins and their phosphorylated forms in the indicated cell lines. (b) Effects of various inhibitors on cellular signaling pathways in NphA2, NphA2/STIR, K562 and K562/DNR cells. Cells were plated at 2×10^5/ml in the presence of U0126 (ERK inhibitor, 10 μM), LY294002 (AKT inhibitor, 10 μM), SB203580 (p38 inhibitor, 25 μM), or SP600125 (JNK inhibitor, 10 and 20 μM). Viable cell number was counted on day 2. Culture experiments were performed in triplicate. Data are shown as mean ± SD. (c) NphA2 and NphA2/STIR cells were cultured with or without SP600125 (20 μM) for 1 or 2 days (D1 and D2, respectively) and then examined for phosphorylated c-jun, cleaved PARP and cleaved caspase 3 expression. Because of the weaker expression of p-c-Jun of NphA2/STIR, p-c-Jun Western blotting of NphA2/STIR needed longer exposure than that of NphA2. (d) Effect of IMT on phosphorylated JNK of NphA2 cells were analyzed. Six and 24 h after IMT treatment (10 μM), cells were collected and phosphorylated JNK and total JNK expression were examined by the Western blotting. Arrowhead denotes JNK1 (lower) and JNK2 (upper), respectively.

showed decreased PCNA, increased cleaved PARP and cleaved caspase-3, indicating a gradual induction of apoptosis from day 2 to day 4 (Fig. 1b). Both p15 and p27, but not p21, were increased by IMT in NphA2 cells but not in NphA2/STIR cells, suggesting an involvement of p15 and p27 and cell cycle arrest in IMT-induced decrease in cell viability. P16 was not detected in these cells (data not shown).

We also examined the sensitivities of NphA2 and NphA2/STIR cells to conventional anti-cancer drugs (Supplementary Fig. 1). These two cell lines showed similar sensitivity patterns, although NphA2/STIR were slightly more resistant to daunorubicin, AraC, paclitaxel and camptothecin than NphA, suggesting that the TKI resistance of NphA2/STIR was relatively BCR/ABL-TKI specific.

We established MR87/STIR cells from parental MR87 [11] by gradually increasing IMT concentration in cell culture. MR87/STIR cells showed resistance to high concentration of IMT, NRT and DST (Supplementary Fig. 2). We also examined K562, a CML-derived erythroleukemia cell line, and the daunorubicin-resistant subline K562/ADR. Both K562 and K562/DNR cells were sensitive against all three TKIs, suggesting a different mechanism of daunorubicin-resistance in K562/ADR cells compared with IMT-resistance mechanism in NphA2/STIR or MR87/STIR cells.

3.2. Analysis of cellular signaling pathways

We next examined several cellular signaling pathways. As shown previously [10], ERK was activated to a higher extent in NphA2/STIR cells than NphA2 cells (Fig. 2a). U0126, an ERK inhibitor, inhibited proliferation of NphA2/STIR cells more than NphA2 cells (Fig. 2b). AKT was activated to similar levels in both NphA2 and NphA2/STIR cells. Interestingly, SP600125, a JNK inhibitor, not only inhibited cell proliferation but also induced cell death of these two cell lines, as well as K562 and K562/DNR cells (Fig. 2b and Supplementary Fig. 2c). Decreased c-Jun phosphorylation, a target of JNK, by SP600125 (20 μM) was confirmed in both NphA2 and NphA2/STIR cells (Fig. 2c), and SP600125 induced cleaved PARP and cleaved caspase-3, suggesting apoptosis induction in both cell lines (Fig. 2c). Moreover, JNK-IN-8, a novel JNK inhibitor with high specificity [16], exhibited similar cytotoxicity in NphA2/STIR cells to that of SP600125 (Supplementary Fig. 3). We examined whether JNK is located downstream of BCR/ABL. Fig. 2d showed that IMT treatment of NphA2 suppressed p-JNK in a time-dependent way.

Supplementary Fig. 4 showed cell signaling proteins in MR87 cells and IMT-resistant MR87/STIR cells. The expression patterns of these cells were similar except p-ERK (high in MR87 and low in MR87/STIR). MR87 and MR87/STIR cells were also sensitive to SP600125 (Supplementary Fig. 5a).

3.3. Effects of BCL2 family inhibitors

We next examined BCL2 family proteins. BCL2 levels in NphA2/

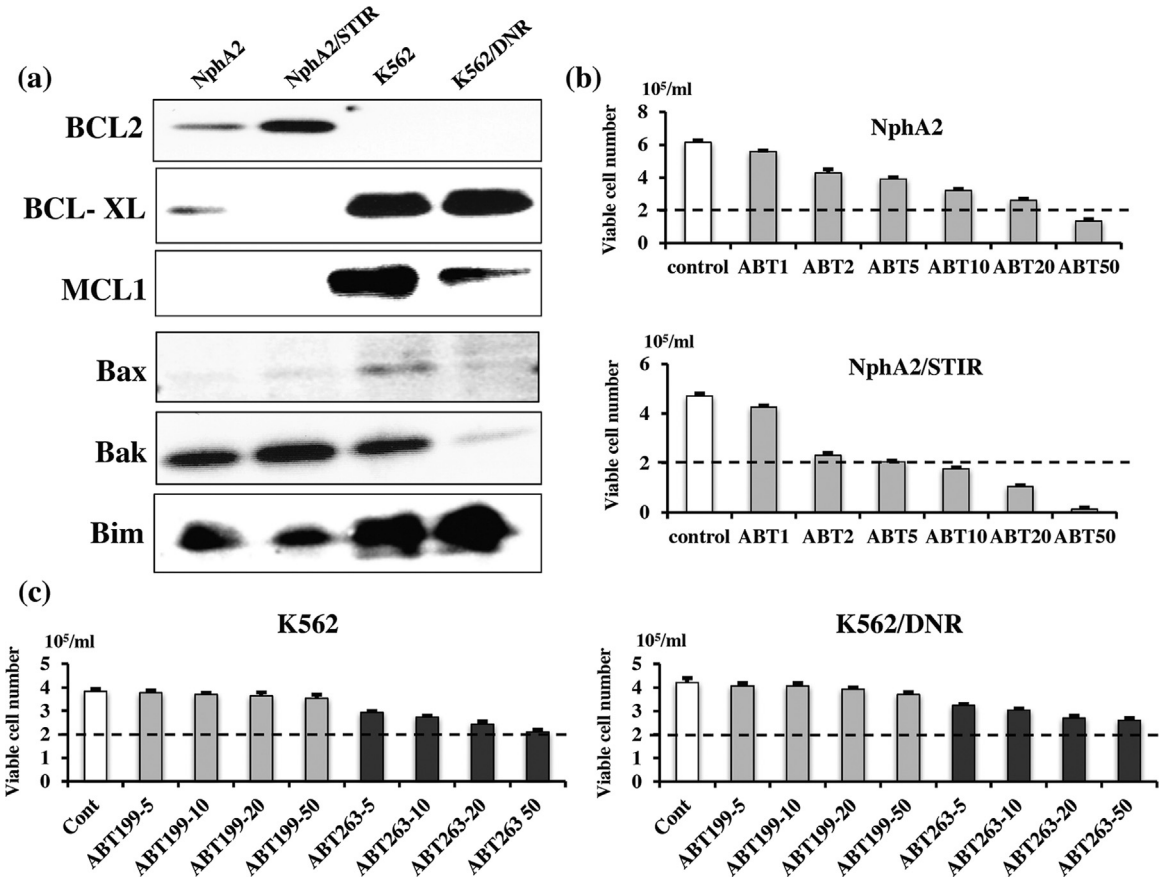

Fig. 3. Effects of BCL2 family inhibitors. (a) BCL2 family protein expression was examined in NphA2, NphA2/STIR, K562, and K562/DNR cell lines by western blotting. (b) Effects of the BCL2-specific inhibitor ABT-199 (1, 2, 5, 10, 20 and 50 nM) on NphA2 and NphA2/STIR cell viability. Cells were plated at 2×10^5/ml and viable cell number was counted on day 2. Culture experiments were performed in triplicate. Data are shown as mean ± SD. (c) Effects of the indicated concentrations of ABT-199 (in nM) and pan-BCL2 inhibitor ABT-263 (in nM) on the viability of K562 and K562/DNR cells. Experiments were performed in triplicate. Data are shown as mean ± SD.

STIR cells were higher than that of NphA2 cells, whereas K562 and K562/DNR cells did not show significant BCL2 expression (Fig. 3a). Interestingly, K562 and K562/DNR cells expressed much higher BCL-XL and MCL1 than NphA2 and NphA2/STIR cells. We did not detect survivin in any of the cell lines in our experimental conditions (data not shown). The expression profiles of apoptosis-related proteins BCL2, BCL-XL and MCL1 in MR87 and MR87/STIR cells were similar to those in NphA2 and NphA2/SITR cells (Supplementary Fig. 4).

We next examined the effect of the BCL2-specific inhibitor ABT-199 (venetoclax) on cell viability (Fig. 3b). ABT-199 inhibited cell proliferation of NphA2 and NphA2/STIR cells in a dose-dependent manner, and at higher concentrations, it rapidly induced cell death. In contrast, K562 and K562/DNR cells were not sensitive to ABT-199, but were moderately sensitive to ABT-263 (navitoclax), an inhibitor of both BCL2 and BCL-XL (Fig. 3c). The mild effect of ABT-263 in K562 and K562/DNR cells suggests the involvement of other survival mechanisms, such as those mediated by MCL1, which is not sensitive to ABT-263 and ABT-199. MR87 and MR87/STIR cells also showed high sensitivity against both ABT-199 and ABT-263 (Supplementary Fig. 5b). Further analysis of other leukemia cell lines NALM16, Jurkat, and U927 revealed that NALM16 cells were sensitive to ABT-199, whereas Jurkat and U937 cells were resistant to both ABT-199 and ABT-263 (Supplementary Fig. 6a). Interestingly, Jurkat and U937 cells exhibited high sensitivity to marinopyrrole A (maritoclax), a MCL1 inhibitor, suggesting a MCL1-dependent survival pathway in these cells (Supplementary Fig. 6b). We also examined another MCL1 inhibitor, A1210477, and found similar suppressive effects against K562, K562/DNR, Jurkat, U937 and MEG-A2 cell viability (data not shown).

3.4. Combined effects of ABT199, IMT and SP600125

We next examined the effects of combining ABT199 with IMT or SP600125 in NphA2 and NphA2/STIR cells and found a significant reduction in cell viability compared with each reagent alone in NphA2 cells (Fig. 4a). SP600125 (10 μM) combined with ABT199 was also effective in suppressing growth and inducing death in both NPhA2 and NphA2/STIR cells. ABT199 (20 nM) alone strongly suppressed viability of both cell lines. Similar data were obtained with JNK-IN-8 combined with ABT199 in NphA2/STIR cells (Supplementary Fig. 3).

We next determined whether the combination of ABT-199 and SP600125 was synergistic or additive. The isobologram illustrating IC50 doses of ABT-199 and SP600125 combination revealed a shift to the left against the line connecting the IC50s of SP600125 (13 nM) and ABT-199 (8.5 μM), indicating a synergistic effect. The combination of IMT + ABT-199 also showed the synergistic effect but not the combination of IMT + SP600125, although high concentration of IMT (100 μM) is needed to reach IC50 in NphA2/STIR cells (Fig. 4b). It is interesting that both ABT-199 + Sp600125, and IMT + ABT-199 produced synergistic effect while IMT + SP600125 did not show such promising effect, suggesting the relationship between each signaling pathway of NphA2/STIR cells.

Western blotting of cleaved PARP and cleaved caspase-3 of NphA2/STIR cells treated with various inhibitors and their combination (Fig. 4c) supports the strong induction of apoptosis in response to some of these inhibitors.

K562 and K562/DNR cells did not exhibit high sensitivity to ABT-199 and ABT-263. However, maritoclax inhibited cell proliferation of

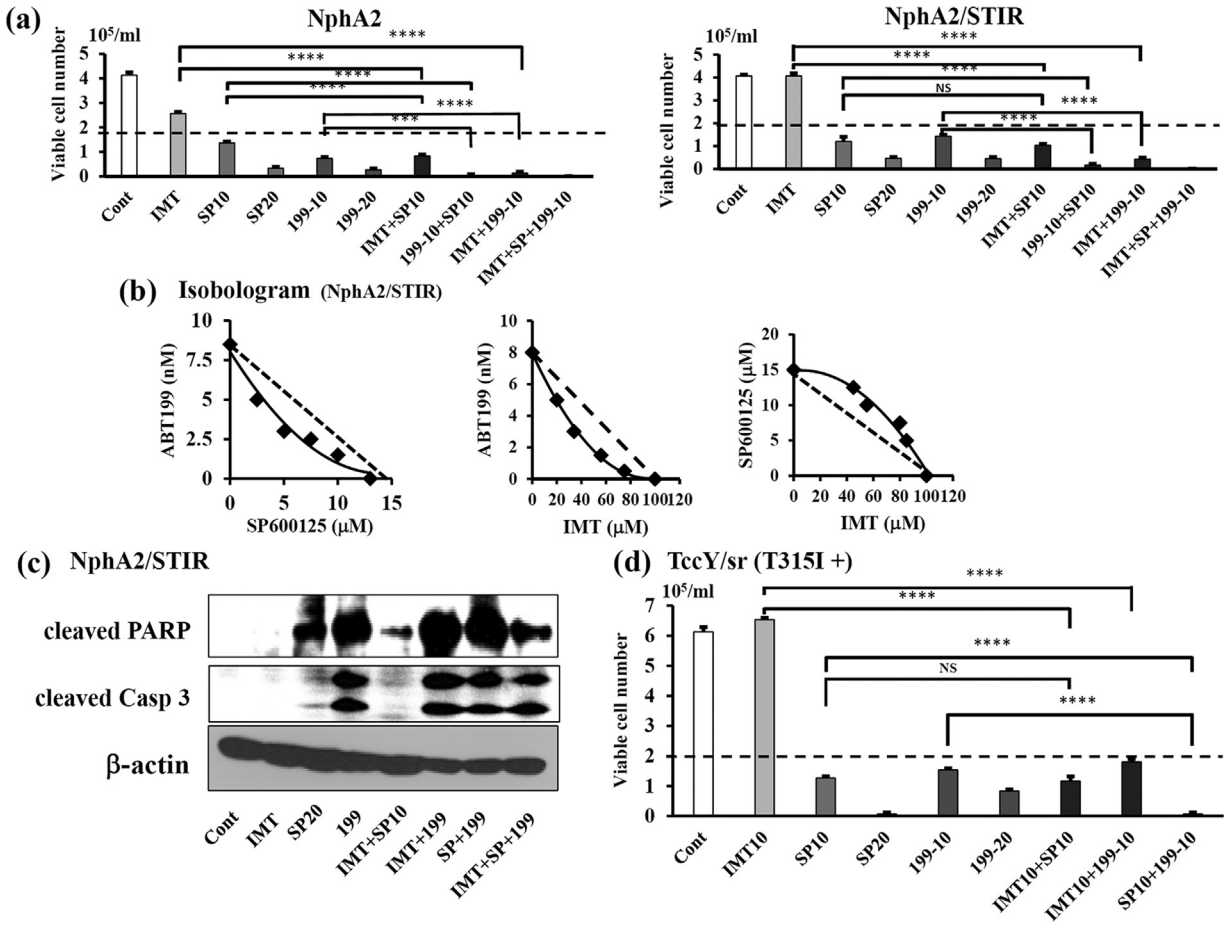

Fig. 4. Combined effects of ABT-199 with SP600125 or IMT. (a) NphA2 cells were treated with IMT (10 μM), SP10 and SP20 (SP600125, 10 and 20 μM), ABT199-10 and 199-20 (ABT-199, 10 and 20 nM), alone or in combination, for 2 days. Cells were plated at 2×10^5/ml. Experiments were performed in triplicate. Data are shown as mean ± SD. $^{***}p < 0.01$, $^{****}p < 0.0001$, NS: not significant. (b) Isoborogram analysis. In the left part, dotted line connects the IC50 of SP600125 (13 μM) and ABT-199 (8.8 nM). The solid squares denote IC50 with the combination of various doses of SP600125 and ABT-199. In the similar way, the relationship between IMT and ABT-199 (middle) as well as IMT and SP600125 (right) were analyzed and the results were illustrated. Dotted line connects IC50 concentration of respective reagent. (c) Western blotting (cleaved PARP, cleaved caspase 3, and β-actin) of NPhA2/STIR cells after 24 h treatment with the indicated inhibitors. (d) TccY/sr (T315I Ph+ ALL) cells were treated with IMT (10 μM), SP10 and SP20 (SP600125, 10 and 20 μM), ABT199-10 and 199–20 (ABT-199, 10 and 20 nM), alone or in combination, for 2 days. Cells were plated in triplicate at 2×10^5/ml. Data are shown as mean ± SD. $^{****}p < 0.0001$, NS: not significant.

K562 and K562/DNR cells, suggesting their MCL1-dependency (Supplementary Fig. 7), as observed in Jurkat and U937 cells. The combination of SP600125 and maritoclax resulted in remarkable cytotoxicity in both K562 and K562/DNR cells.

We also observed a strong effect of SP600125 and ABT199 in highly TKI-resistant Ph+ ALL cells with T315-mutation, TccY/sr (Fig. 4d), indicating these inhibitors are effective in these cells.

4. Discussion

BCR/ABL is derived from a chromosome translocation, t(9:22) and is the major cause of CML and Ph+ ALL due to constitutive ABL kinase activity. The development of BCR/ABL-specific TKIs predicted wide clinical application of these TKIs and the potential cure of BCR/ABL-related diseases. However, TKI resistance is frequently observed and results in worse patient prognosis. Most TKI resistance has been linked to the BCR/ABL protein; however, some studies have reported a BCR/ABL-independent resistance to TKI [17].

ERK inhibition induced growth inhibition of NphA2/STIR cells, consistent with our previous report [10]. The same paper showed EphB4 activation (phosphorylation) and Ras activation (by the RAS activity assay) of NPhA2/STIR, suggesting that the enhanced activation of MEK-MAPK pathway is downstream of RAS pathway. It has recently been reported that EphB4 promotes RAS/MEK/ERK pathway in MCF-7 breast cancer cells [18].

Concerning another important signaling pathway, JAK/STAT, phosphorylated STAT3 was increased, whereas phosphorylated STAT5 was decreased [10]. Furthermore, inhibition of the ATK pathway and the JAK/STAT pathway did not produce significant changes of cell proliferation of NPhA2 and NPhA2/STIR cells, suggesting that these pathways did not play a major role [10].

However, the effect of the JNK inhibitor SP600125 on IMT-resistant Ph+ ALL cells has not been studied. We showed cytotoxicity of SP600125 in Ph+ ALL as well as IMT-resistant sublines. JNK activation has been reported to exert various effects, either apoptosis induction or enhancing cell survival and proliferation [19]. BCR/ABL activates JNK and requires Jun for transformation [20], and c-jun gene expression in BCR/ABL-transformed cells corresponds with increased JNK activity [21]. Moreover, It has been reported that JNK1 is crucial in the transformation of B lymphoblasts by BCR/ABL in vitro [22]. Actually, BCR/ABL causes JNK-dependent increase of BCL2 protein expression. Recent study also reported the effect of JNK suppression on imatinib sensitivity [23]. IMT suppressed JNK activation in a time dependent manner of NphA2 cells (Fig. 2d), suggesting that in NphA2 cells, JNK was downstream of BCR/ABL. In contrast, JNK inhibition was reported to induce cycle blockade and apoptosis in some tumor cell types [24,25].

SP600125 was reported to enhance apoptosis of some tumor cells [26,27]. We also showed cytotoxicity of the JNK inhibitor JNK-IN-8, which exhibits higher specificity and a different JNK inhibition mechanism (Supplementary Fig. 3). However, after the initial characterization of SP600125 [28], a heterogeneous action of SP600125 was reported [29]. Therefore, further analysis is needed to identify the target of SP600125 in our cells.

The BCL-2 family represents the key regulators of the intrinsic apoptotic pathway [30] and consists of anti-apoptotic and pro-apoptotic members [31]. Navitoclax (ABT-263), an orally bioavailable small molecule with a high affinity for both BCL2 and BCL-XL, exhibited excellent antitumor efficacy, mainly in lymphoid malignancy [32]. Due to the dose-limiting toxicity of thrombocytopenia by navitoclax, the BCL2-selective inhibitor ABT-199 (venetoclax) was pursued [33]. Clinical efficiency of ABT-199 has been reported in lymphoid malignancies [34–36]. ABT-199 enhances IMT-induced cell death in CML progenitors [37]. The FDA recently approved ABT-199 for some subtypes of chronic lymphocyte leukemia. However, the efficacy of ABT-199 in Ph+ ALL has not been studied extensively [38], and its effect on IMT-resistant cells has not been reported.

A previous study showed that ABT-199 significantly reduced the number of normal myeloid progenitors with an IC50 of 20 nM [37]. Thus, ABT-199 below 20 nM is the practical range to analyze its cytotoxicity against leukemia cells. The most interesting point of our work is that Ph+ ALL or Ph+ AL cells are very sensitive to ABT-199 used at 20 nM, regardless of IMT sensitivity.

In contrast, CML-derived K562 cells were resistant to ABT-199 and moderately sensitive to ABT-263. Although MCL1 inhibitors, including marinopyrrole A, have other targets than MCL1 [39], marinopyrrole A inhibited K562 and K562/DNR cells as well as Jurkat and U937 cells (Supplementary Figs. 5b and 6). Moreover, our experimental data using another MCL1-specific inhibitor, A1210477, confirmed the MCL1-dependency of these several leukemia cell lines including CML-derived cell lines, K562, K562/DNR and MEGA2 (data not shown).

Some studies reported that non-Hodgkin lymphoma cells exhibited BCL2-dependence on their survival, whereas others showed MCL1- or BCL-XL-dependence [40]. Therefore, the expression profile of BCL2 family proteins may determine the survival of clinical cancer and leukemia cells and help predict the therapeutic utility of respective BCL2 family inhibitors. This issue was recently reviewed and BH3 profiling is one useful strategy to select drugs for clinical usage [41].

Together, our present results suggest that the combination of ABT-199 and SP600125 synergistically suppressed cell proliferation and induced apoptosis of Ph+ ALL cells regardless of TKI resistance, including cells with the T315I BCR/ABL mutation. Our findings also suggest that an appropriate BCl2 family inhibitor and JNK inhibitor combination can be envisaged for the future treatment of acute leukemia.

Funding

This work was supported by the Japan Society for the Promotion of Science, Grant-in-Aid for Scientific Research (C) (17K09025).

Appendix A. Transparency document

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbrep.2018.07.001.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbrep.2018.07.001.

References

[1] O.G. Ottmann, H. Pfeifer, First-line treatment of Philadelphia chromosome-positive acute lymphoblastic leukaemia in adults, , Curr. Opin. Oncol. 21 (Suppl. 1) (2009) S43–S46.
[2] M. Malagola, C. Papayannidis, M. Baccarani, Tyrosine kinase inhibitors in Ph+ acute lymphoblastic leukaemia: facts and perspectives, Ann. Hematol. 95 (2016) 681–693.
[3] J.I. Sive, G. Buck, A. Fielding, H.M. Lazarus, M.R. Litzow, S. Luger, D.I. Marks, A. McMillan, A.V. Moorman, S.M. Richards, J.M. Rowe, M.S. Tallman, A.H. Goldstone, Outcomes in older adults with acute lymphoblastic leukaemia (ALL): results from the international MRC UKALL XII/ECOG2993 trial, Br. J. Haematol. 157 (2012) 463–471.
[4] L. Annino, A. Ferrari, M. Cedrone, F. Giona, F. Lo Coco, G. Meloni, W. Arcese, F. Mandelli, Adult Philadelphia-chromosome-positive acute lymphoblastic leukemia: experience of treatments during a ten-year period, Leukemia 8 (1994) 664–667.
[5] B.J. Druker, C.L. Sawyers, H. Kantarjian, D.J. Resta, S.F. Reese, J.M. Ford, R. Capdeville, M. Talpaz, Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome, N. Engl. J. Med. 344 (2001) 1038–1042.
[6] A.K. Fielding, Treatment of Philadelphia chromosome-positive acute lymphoblastic leukemia in adults: a broader range of options, improved outcomes, and more therapeutic dilemmas, Am. Soc. Clin. Oncol. Educ. Book (2015) e352–e359.
[7] M. Vignetti, P. Fazi, G. Cimino, G. Martinelli, F. Di Raimondo, F. Ferrara, G. Meloni, A. Ambrosetti, G. Quarta, L. Pagano, G. Rege-Cambrin, L. Elia, R. Bertieri, L. Annino, R. Foa, M. Baccarani, F. Mandelli, Imatinib plus steroids induces complete remissions and prolonged survival in elderly Philadelphia chromosome-positive patients with acute lymphoblastic leukemia without additional chemotherapy: results of the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) LAL0201-B protocol, Blood 109 (2007) 3676–3678.
[8] M.S. Zabriskie, C.A. Eide, S.K. Tantravahi, N.A. Vellore, J. Estrada, F.E. Nicolini, H.J. Khoury, R.A. Larson, M. Konopleva, J.E. Cortes, H. Kantarjian, E.J. Jabbour, S.M. Kornblau, J.H. Lipton, D. Rea, L. Stenke, G. Barbany, T. Lange, J.C. Hernandez-Boluda, G.J. Ossenkoppele, R.D. Press, C. Chuah, S.L. Goldberg, M. Wetzler, F.X. Mahon, G. Etienne, M. Baccarani, S. Soverini, G. Rosti, P. Rousselot, R. Friedman, M. Deininger, K.R. Reynolds, W.L. Heaton, A.M. Eiring, A.D. Pomicter, J.S. Khorashad, T.W. Kelley, R. Baron, B.J. Druker, M.W. Deininger, T. O'Hare, BCR-ABL1 compound mutations combining key kinase domain positions confer clinical resistance to ponatinib in Ph chromosome-positive leukemia, Cancer Cell 26 (2014) 428–442.
[9] L. Saini, J. Brandwein, New treatment strategies for Philadelphia chromosome-positive acute lymphoblastic leukemia, Curr. Hematol. Malig. Rep. 12 (2017) 136–142.
[10] M. Suzuki, A. Abe, S. Imagama, Y. Nomura, R. Tanizaki, Y. Minami, F. Hayakawa, Y. Ito, A. Katsumi, K. Yamamoto, N. Emi, H. Kiyoi, T. Naoe, BCR-ABL-independent, R.A.S. MAPK, pathway-dependent form of imatinib resistance in Ph-positive acute lymphoblastic leukemia cell line with activation of EphB4, Eur. J. Haematol. 84 (2010) 229–238.
[11] J. Okamura, S. Yamada, E. Ishii, T. Hara, H. Takahira, J. Nishimura, K. Yumura, K. Kawa-Ha, K. Takase, Y. Enomoto, et al., A novel leukemia cell line, MR-87, with positive Philadelphia chromosome and negative breakpoint cluster region rearrangement coexpressing myeloid and early B-cell markers, Blood 72 (1988) 1261–1268.
[12] Y. Nishida, N. Mizutani, M. Inoue, Y. Omori, K. Tamiya-Koizumi, A. Takagi, T. Kojima, M. Suzuki, Y. Nozawa, Y. Minami, K. Ohnishi, T. Naoe, T. Murate, Phosphorylated Sp1 is the regulator of DNA-PKcs and DNA ligase IV transcription of daunorubicin-resistant leukemia cell lines, Biochim. Biophys. Acta 2014 (1839) 265–274.
[13] Y. Shiotsu, H. Kiyoi, Y. Ishikawa, R. Tanizaki, M. Shimizu, H. Umehara, K. Ishii, Y. Mori, K. Ozeki, Y. Minami, A. Abe, H. Maeda, T. Akiyama, Y. Kanda, Y. Sato, S. Akinaga, T. Naoe, KW-2449, a novel multikinase inhibitor, suppresses the growth of leukemia cells with FLT3 mutations or T315I-mutated BCR/ABL translocation, Blood 114 (2009) 1607–1617.
[14] S. Sobue, S. Nemoto, M. Murakami, H. Ito, A. Kimura, S. Gao, A. Furuhata, A. Takagi, T. Kojima, M. Nakamura, Y. Ito, M. Suzuki, Y. Banno, Y. Nozawa, T. Murate, Implications of sphingosine kinase 1 expression level for the cellular sphingolipid rheostat: relevance as a marker for daunorubicin sensitivity of leukemia cells, Int. J. Hematol. 87 (2008) 266–275.
[15] A. Abe, N. Emi, H. Kato, K. Adachi, T. Murate, S. Saga, M. Ogura, T. Kojima, M. Tanimoto, N. Morishita, et al., Establishment and characterization of an immature human megakaryoblastic cell line, MEG-A2, Leukemia 9 (1995) 341–349.
[16] T. Zhang, F. Inesta-Vaquera, M. Niepel, J. Zhang, S.B. Ficarro, T. Machleidt, T. Xie, J.A. Marto, N. Kim, T. Sim, J.D. Laughlin, H. Park, P.V. LoGrasso, M. Patricelli, T.K. Nomanbhoy, P.K. Sorger, D.R. Alessi, N.S. Gray, Discovery of potent and selective covalent inhibitors of JNK, Chem. Biol. 19 (2012) 140–154.
[17] C.V. Ichim, Kinase-independent mechanisms of resistance of leukemia stem cells to tyrosine kinase inhibitors, Stem Cells Transl. Med. 3 (2014) 405–415.
[18] Z. Xiao, R. Carrasco, K. Kinneer, D. Sabol, B. Jallal, S. Coats, D.A. Tice, EphB4 promotes or suppresses Ras/MEK/ERK pathway in a context-dependent manner: implications for EphB4 as a cancer target, Cancer Biol. Ther. 13 (2012) 630–637.
[19] A.M. Bode, Z. Dong, The functional contrariety of JNK, Mol. Carcinog. 46 (2007) 591–598.
[20] A.B. Raitano, J.R. Halpern, T.M. Hambuch, C.L. Sawyers, The Bcr-Abl leukemia

oncogene activates Jun kinase and requires Jun for transformation, Proc. Natl. Acad. Sci. USA 92 (1995) 11746–11750.
[21] G.S. Burgess, E.A. Williamson, L.D. Cripe, S. Litz-Jackson, J.A. Bhatt, K. Stanley, M.J. Stewart, A.S. Kraft, H. Nakshatri, H.S. Boswell, Regulation of the c-jun gene in p210 BCR-ABL transformed cells corresponds with activity of JNK, the c-jun N-terminal kinase, Blood 92 (1998) 2450–2460.
[22] P. Hess, G. Pihan, C.L. Sawyers, R.A. Flavell, R.J. Davis, Survival signaling mediated by c-Jun NH(2)-terminal kinase in transformed B lymphoblasts, Nat. Genet. 32 (2002) 201–205.
[23] C.C. Niu, C. Zhao, X.L. Zhang, J. Pan, C. Zhao, W.R. Wu, Z.Q. Li, T. Liu, Z. Yang, W.K. Si, Wnt5a enhances the response of CML cells to Imatinib Mesylate through JNK activation and gamma-catenin inhibition, Leuk. Res. 37 (2013) 1532–1537.
[24] C. Kuntzen, N. Sonuc, E.N. De Toni, C. Opelz, S.R. Mucha, A.L. Gerbes, S.T. Eichhorst, Inhibition of c-Jun-N-terminal-kinase sensitizes tumor cells to CD95-induced apoptosis and induces G2/M cell cycle arrest, Cancer Res. 65 (2005) 6780–6788.
[25] O. Potapova, M. Gorospe, F. Bost, N.M. Dean, W.A. Gaarde, D. Mercola, N.J. Holbrook, c-Jun N-terminal kinase is essential for growth of human T98G glioblastoma cells, J. Biol. Chem. 275 (2000) 24767–24775.
[26] S.H. Kook, Y.O. Son, Y.S. Jang, K.Y. Lee, S.A. Lee, B.S. Kim, H.J. Lee, J.C. Lee, Inhibition of c-Jun N-terminal kinase sensitizes tumor cells to flavonoid-induced apoptosis through down-regulation of JunD, Toxicol. Appl. Pharmacol. 227 (2008) 468–476.
[27] Y. Liu, J. Feng, M. Zhao, J. Wu, J. Fan, Q. Wen, J. Xu, J. Zhang, S. Fu, B. Wang, Y. Lu, K. Xiong, L. Xiang, Y. Zhang, L. Yang, JNK pathway inhibition enhances chemotherapeutic sensitivity to Adriamycin in nasopharyngeal carcinoma cells, Oncol. Lett. 14 (2017) 1790–1794.
[28] B.L. Bennett, D.T. Sasaki, B.W. Murray, E.C. O'Leary, S.T. Sakata, W. Xu, J.C. Leisten, A. Motiwala, S. Pierce, Y. Satoh, S.S. Bhagwat, A.M. Manning, D.W. Anderson, SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase, Proc. Natl. Acad. Sci. USA 98 (2001) 13681–13686.
[29] P. Champelovier, M. El Atifi-Borel, J.P. Issartel, J. Boutonnat, F. Berger, D. Seigneurin, Inhibition of c-Jun N-terminal kinase by SP600125: a cDNA microarray analysis, Cancer Genom. Proteom. 7 (2010) 87–92.
[30] P.E. Czabotar, G. Lessene, A. Strasser, J.M. Adams, Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy, Nat. Rev. Mol. Cell Biol. 15 (2014) 49–63.
[31] F. Llambi, T. Moldoveanu, S.W. Tait, L. Bouchier-Hayes, J. Temirov, L.L. McCormick, C.P. Dillon, D.R. Green, A unified model of mammalian BCL-2 protein family interactions at the mitochondria, Mol. Cell 44 (2011) 517–531.
[32] C. Tse, A.R. Shoemaker, J. Adickes, M.G. Anderson, J. Chen, S. Jin, E.F. Johnson, K.C. Marsh, M.J. Mitten, P. Nimmer, L. Roberts, S.K. Tahir, Y. Xiao, X. Yang, H. Zhang, S. Fesik, S.H. Rosenberg, S.W. Elmore, ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor, Cancer Res. 68 (2008) 3421–3428.
[33] A.J. Souers, J.D. Leverson, E.R. Boghaert, S.L. Ackler, N.D. Catron, J. Chen, B.D. Dayton, H. Ding, S.H. Enschede, W.J. Fairbrother, D.C. Huang, S.G. Hymowitz, S. Jin, S.L. Khaw, P.J. Kovar, L.T. Lam, J. Lee, H.L. Maecker, K.C. Marsh, K.D. Mason, M.J. Mitten, P.M. Nimmer, A. Oleksijew, C.H. Park, C.M. Park, D.C. Phillips, A.W. Roberts, D. Sampath, J.F. Seymour, M.L. Smith, G.M. Sullivan, S.K. Tahir, C. Tse, M.D. Wendt, Y. Xiao, J.C. Xue, H. Zhang, R.A. Humerickhouse, S.H. Rosenberg, S.W. Elmore, ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets, Nat. Med. 19 (2013) 202–208.
[34] J.M. Benito, L. Godfrey, K. Kojima, L. Hogdal, M. Wunderlich, H. Geng, I. Marzo, K.G. Harutyunyan, L. Golfman, P. North, J. Kerry, E. Ballabio, T.N. Chonghaile, O. Gonzalo, Y. Qiu, I. Jeremias, L. Debose, E. O'Brien, H. Ma, P. Zhou, R. Jacamo, E. Park, K.R. Coombes, N. Zhang, D.A. Thomas, S. O'Brien, H.M. Kantarjian, J.D. Leverson, S.M. Kornblau, M. Andreeff, M. Muschen, P.A. Zweidler-McKay, J.C. Mulloy, A. Letai, T.A. Milne, M. Konopleva, MLL-rearranged acute lymphoblastic leukemias activate BCL-2 through H3K79 methylation and are sensitive to the BCL-2-specific antagonist ABT-199, Cell Rep. 13 (2015) 2715–2727.
[35] S. Cang, C. Iragavarapu, J. Savooji, Y. Song, D. Liu, ABT-199 (venetoclax) and BCL-2 inhibitors in clinical development, J. Hematol. Oncol. 8 (2015) 129.
[36] V.M. Patel, K. Balakrishnan, M. Douglas, T. Tibbitts, E.Y. Xu, J.L. Kutok, M. Ayers, A. Sarkar, R. Guerrieri, W.G. Wierda, S. O'Brien, N. Jain, H.M. Stern, V. Gandhi, Duvelisib treatment is associated with altered expression of apoptotic regulators that helps in sensitization of chronic lymphocytic leukemia cells to venetoclax (ABT-199), Leukemia (2017).
[37] T.K. Ko, C.T. Chuah, J.W. Huang, K.P. Ng, S.T. Ong, The BCL2 inhibitor ABT-199 significantly enhances imatinib-induced cell death in chronic myeloid leukemia progenitors, Oncotarget 5 (2014) 9033–9038.
[38] J.T. Leonard, J.S. Rowley, C.A. Eide, E. Traer, B. Hayes-Lattin, M. Loriaux, S.E. Spurgeon, B.J. Druker, J.W. Tyner, B.H. Chang, Targeting BCL-2 and ABL/LYN in Philadelphia chromosome-positive acute lymphoblastic leukemia, Sci. Transl. Med. 8 (2016) 354ra114.
[39] M. Vogler, H.S. Walter, M.J.S. Dyer, Targeting anti-apoptotic BCL2 family proteins in haematological malignancies – from pathogenesis to treatment, Br. J. Haematol. 178 (2017) 364–379.
[40] M. Klanova, L. Andera, J. Brazina, J. Svadlenka, S. Benesova, J. Soukup, D. Prukova, D. Vejmelkova, R. Jaksa, K. Helman, P. Vockova, L. Lateckova, J. Molinsky, B.C. Maswabi, M. Alam, R. Kodet, R. Pytlik, M. Trneny, P. Klener, Targeting of BCL2 family proteins with ABT-199 and homoharringtonine reveals BCL2- and MCL1-dependent subgroups of diffuse large B-cell lymphoma, Clin. Cancer Res. 22 (2016) 1138–1149.
[41] M.S. Davids, Targeting BCL-2 in B-cell lymphomas, Blood 130 (2017) 1081–1088.