Biochemical and Biophysical Research Communications 461 (2015) 243–248

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Arsenic trioxide synergistically potentiates the cytotoxic effect of fludarabine in chronic lymphocytic leukemia cells by further inactivating the Akt and ERK signaling pathways

Carol Lozano-Santos [a, 1], Irene Amigo-Jiménez [b, 1], Sara Nova-Gurumeta [a], Nuria Pérez-Sanz [a], Angeles García-Pardo [b, *, 2], José A. García-Marco [a, **, 2]

[a] Molecular Cytogenetics Unit, Hematology Department, Hospital Universitario Puerta de Hierro-Majadahonda and IDIPHIM, Madrid, Spain
[b] Cellular and Molecular Medicine Department, Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain

ARTICLE INFO

Article history:
Received 28 March 2015
Available online 11 April 2015

Keywords:
CLL
Arsenic trioxide
Fludarabine
Synergistic effect
Combined therapies
Survival pathways

ABSTRACT

CLL remains an incurable disease, making it crucial to continue searching for new therapies efficient in all CLL cases. We have studied the effect of combining arsenic trioxide (ATO) with fludarabine, a frontline drug in CLL. We have found a synergistic interaction between 1 μM ATO and 5 μM fludarabine that significantly enhanced the cytotoxic effect of the individual drugs. Importantly, ATO sensitized fludarabine-resistant cells to the action of this drug. The mechanism behind this effect included the downregulation of phospho-Akt, phospho-ERK, and the Mcl-1/Bim and Bcl-2/Bax ratios. The combination of ATO and fludarabine partially overcame the survival effect induced by co-culturing CLL cells with stromal cells. Therefore, low concentrations of ATO combined with fludarabine may be an efficient therapeutic strategy in CLL patients.

1. Introduction

Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of $CD5^+$ B-lymphocytes in peripheral blood and lymphoid tissues [1,2]. Albeit being a common type of leukemia, CLL remains uncurable. Current therapies include the use of the purine analogue fludarabine, alone or combined with anti-CD20 monoclonal antibodies or kinase inhibitors [3–5]. Although these treatments control the disease in many cases, patients carrying bad prognostic markers (del17p13, unmutated IgH_V) do not respond well to therapy, making it crucial to continue searching for new compounds, efficient in CLL patients who have indication for treatment.

Arsenic trioxide (ATO) is a successful treatment for acute promyelocytic leukemia and it is being trialed for its possible use in other hematologic and non-hematologic malignancies, generally in combined therapies [6–8]. We and others have shown that ATO, at 2–4 μM concentration or higher, effectively induces *in vitro* apoptosis of CLL cells, including those cases with unfavorable prognosis [9–11]. It is not known whether the combination of low doses of ATO with the frontline therapeutic agent fludarabine, could be an efficient treatment for CLL, particularly in those cases with known resistance to fludarabine. In the present report we show that 1 μM ATO synergistically interacts with fludarabine and enhances the cytotoxic effect of fludarabine on all CLL cases studied. We further show that this is due to the down-regulation of important survival pathways and that the combination of ATO and fludarabine partially overcomes the CLL cell survival effect induced by stroma.

2. Materials and methods

2.1. Patients, cells and cell cultures

Approval was obtained from the Hospital Puerta de Hierro and the CSIC Bioethics Review Boards for these studies. All patients

* Corresponding author. Centro de Investigaciones Biológicas, Ramiro de Maeztu 9, 28040 Madrid, Spain.
** Corresponding author. Hematology Department, Hospital Universitario Puerta de Hierro-Majadahonda, Manuel de Falla 1, 28222 Majadahonda, Madrid, Spain.
E-mail addresses: agarciapardo@cib.csic.es (A. García-Pardo), jagarciam@aehh.org (J.A. García-Marco).
[1] These authors contributed equally to this work.
[2] These two senior authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.04.007
0006-291X/

signed an informed consent before blood was drawn. B-lymphocytes were purified from the 35 CLL samples listed in Table 1 by centrifugation, using Ficoll gradient (Rafer, S.L. Zaragoza, Spain). The resulting B cell population was >90% $CD19^+$, determined on a BD FACScalibur flow cytometer (San Jose, CA, USA). The human stromal cell lines HS-5 (fibroblastoid) and HS-27A (epithelioid) [12] were purchased from ATCC (Manassas, VA, USA) and cultured in RPMI/10% FBS. For CLL-stromal cell co-culture, stromal cells were seeded onto gelatin-coated wells for 4–6 h and stimulated with 15–20 ng/ml TNFα overnight at 37 °C, 5% CO_2. CLL cells were added to the confluent stromal cell monolayers or cultured in suspension for comparison. After 2 h at 37 °C, 1 μM ATO, 5 μM fludarabine, both drugs together, or vehicle was added and cells further incubated for 48 h. CLL cells were gently collected with culture medium for further analyses.

2.2. Antibodies and reagents

Rabbit polyclonal antibodies (RpAbs) to Mcl-1 (sc-819), Bax (sc-526) and mouse monoclonal Ab (mAb) to Bcl-2 (sc-509) were from Santa Cruz Biotechnology (Santa Cruz, CA). RpAb to Bim (559685) was from BD Pharmingen (Franklin Lakes, NJ). Anti-vinculin mAb (V9131) was from Sigma–Aldrich (St. Louis, MO). Anti-phospho-Akt (Thr308) and phospho-ERK1/2 (Thr202/Tyr204) RpAbs were from Cell Signaling Technology Inc. (Beverly, MA). HRP-labelled Abs to rabbit or mouse immunoglobulins were from Dako (Glostrup, Denmark). Arsenic trioxide (ATO) and fludarabine were from Sigma–Aldrich.

2.3. Analysis of cell viability

2×10^5 CLL cells, cultured for 48 h in suspension or with stroma, and with or without drugs, were suspended in 300 μl binding buffer (Immunostep, Salamanca, Spain) containing 1 μl FITC-Annexin V and 1.5 μg/ml propidum iodide (PI). Cell viability was determined using a five-color flow cytometer (FC 500; Beckman Coulter Inc., Fullerton, CA, USA). Data were analyzed using the CompuSyn software (BioSoft, Cambridge, UK), which allows the calculation of the combination index based on the algorithm of Chou and Talalay [13]. Combination index values <1 indicate synergism, values = 1 indicate additivity, and >1, antagonism.

2.4. Western blotting

These assays were performed exactly as previously described [11]. Protein bands were developed using the enhanced chemiluminiscent detection method (GE Healthcare Europe GmbH, Barcelona, Spain) and quantitated using the MultiGauge V3.0 program (Fujifilm Global Lifescience, Düsseldorf, Germany). Protein load was corrected using vinculin as internal standard.

2.5. Mutational analysis

ATM (exons 2–63) and *BIRC3* genes (exons 6–9) were analyzed by High-resolution melting (HRM) analysis using the LightCycler 480 plataform (Roche Diagnostics, Mannheim, Germany) and Sanger sequencing using the BigDye Terminator Chemistry and an

Table 1
Clinical characteristics of CLL patients.

Patient	Sex/Age	Stage[a]	IGHV status	CD38/ZAP70[b]	TP53	ATM	BIRC3	SF3B1	NOTCH1	11q	Therapy
P1	Male/57	A/0	M	−/ND	ND/del-	UM	UM	UM	UM	del-	No
P2	Female/54	A/0	M	−/ND	WT/del-	UM	UM	UM	UM	del-	No
P3	Male/67	B/II	UM	−/+	WT/del-	UM	M	UM	UM	del-	No
P4	Male/67	A/0	UM	−/+	WT/del-	UM	UM	UM	UM	del-	No
P5	Male/54	A/I	M	−/+	ND/del-	UM	UM	UM	UM	del-	No
P6	Male/43	B/II	UM	−/+	WT/del-	UM	UM	UM	UM	del-	No
P7	Female/53	A/0	UM	−/+	WT/del-	UM	UM	UM	UM	del-	No
P8	Female/52	A/0	M	ND/ND	ND/del-	UM	UM	UM	UM	del-	No
P9	Male/58	A/I	UM	−/+	ND/del-	UM	UM	UM	UM	del+	No
P10	Male/74	A/0	M	+/+	ND/del-	UM	UM	UM	UM	del-	No
P11	Female/42	B/I	M	−/+	WT/del-	UM	UM	UM	UM	del-	No
P12	Female/59	A/0	M	−/+	WT/del-	M	UM	UM	UM	del-	No
P13	Male/73	A/0	UM	−/+	M/del-	M	UM	UM	UM	del-	No
P14	Male/57	B/II	UM	−/+	WT/del-	UM	UM	UM	UM	del-	No
P15	Male/64	B/II	UM	−/ND	M/del-	ND	ND	UM	UM	del-	No
P16	Female/55	B/II	M	−/+	M/del-	ND	ND	UM	UM	del-	No
P17	Male/47	A/0	ND	−/−	WT/del-	ND	ND	UM	UM	del-	No
P18	Male/75	A/I	ND	−/−	ND/del-	ND	ND	UM	UM	del-	No
P19	Female/72	A/0	M	−/+	WT/del-	UM	UM	UM	UM	del-	No
P20	Female/69	B/II	ND	−/+	ND/del-	ND	ND	UM	UM	del+	No
P21	Male/65	A/II	ND	−/ND	ND/del-	ND	ND	ND	ND	del-	No
P22	Female/72	A/0	M	−/+	WT/del-	UM	UM	UM	UM	del-	CH
P23	Male/44	B/I	UM	+/+	M*/del-	UM	UM	UM	UM	del-	F
P24	Female/68	A/0	UM	−/+	WT/del-	UM	UM	UM	UM	del-	CH
P25	Female/67	A/II	UM	+/ND	WT/del-	UM	UM	UM	UM	del-	FC
P26	Female/65	A/I	M	−/−	M/del-	UM	UM	M	UM	del-	Benda
P27	Male/50	A/0	M	−/+	ND/del-	UM	UM	UM	UM	del-	FCR + Rm
P28	Male/68	A/0	M	−/−	WT/del-	UM	UM	M	ND	del-	FC
P29	Female/53	A/0	UM	−/ND	M*/del-	UM	UM	M	UM	del-	FC
P30	Male/46	A/0	UM	+/+	WT/del-	UM	UM	UM	M	del-	FC
P31	Male/49	A/I	UM	+/+	WT/del-	M	UM	UM	M	del+	CH
P32	Male/46	A/II	M	+/+	WT/del-	M	UM	UM	M	del-	FC-Alem
P33	Male/64	B/I	UM	−/ND	WT/del-	M	M	UM	UM	del+	FC
P34	Male/69	B/II	UM	−/+	ND/del-	UM	UM	UM	UM	del+	FCR
P35	Male/65	A/0	UM	+/−	WT/del-	ND	ND	UM	UM	del-	F

IGHV, inmunoglobulin heavy variable gene; WT, wild type; M, Mutated; M*, acquired mutation; UM, unmutated; CH, Chlorambucil; F, Fludarabine; Benda, Bendamustine; FC, Fludarabine + Cyclophosphamide; FCR, Fludarabine + Cyclophosphamide + Rituximab; Rm, Rituximab maintenance; Alem, Alemtuzumab; ND, Not determined.

[a] Clinical stage according to references [1,2].

[b] The coexpression of CD38 and ZAP-70 has clinical prognostic value [1,2].

ABIPrism 3130 automated DNA sequencer (Applied Biosystems, Foster City, CA). NOTCH1 (p.P2515fs*4) and SF3B1 genes (p.K700E) were studied by qbiomarker somatic mutation PCR *assay* (SABiosciences, Qiagen, Hilden, Germany) using 7500 RT-PCR (Applied Biosystems) [14].

2.6. Statistical analyses

Statistical significance of the data was determined using the two-tailed Student's t-test. A p value of ≤ 0.05 was considered significant. Analyses were performed using the GraphPad InStat v3.06 software (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. 1. ATO enhances the cytotoxic effect of fludarabine in CLL cells

To determine whether ATO enhanced the cytotoxic activity of drugs currently used for the treatment of CLL, CLL cells from previously untreated (UT, n = 14) or treated (T, n = 13) patients (Table 1) were incubated with 1 μM ATO alone or combined with increasing doses of fludarabine. After 48 h, cell viability was measured by flow cytometry using Annexin V and PI. The average constitutive viability of these samples was 86% (UT patients) and 84% (T patients) and was normalized to 100. Fig. 1A shows for both cases that ATO alone had a limited effect, reducing viability by 25% and 21%, respectively. Fludarabine alone decreased viability in a dose-dependent manner leading to 50% (UT patients) and 51% (T patients) at 5 μM concentration. The combination of ATO and fludarabine significantly increased their individual effect at all concentrations of fludarabine tested, resulting in 26% (UT patients) and 42% (T patients) at the highest dose of fludarabine tested.

We next analyzed whether the interaction of ATO and fludarabine was synergistic or additive by calculating the combination index using the CompuSyn software. In samples from UT patients, the interaction of both drugs was additive for 1 μM fludarabine and synergistic for the higher concentrations of 3 and 5 μM (Fig. 1B). However, in samples from T patients, the combination of ATO with 1 or 3 μM fludarabine was synergistic while the combination with 5 μM fludarabine was additive (Fig. 1B), reflecting the differential response of the two groups of patients.

To then determine whether ATO was able to sensitize fludarabine-resistant cells to the action of this drug, we selected samples from both UT (P3, P5, P9, P10, P11) and T patients (P23, P26, P27, P29, P31) that responded poorly to fludarabine. Fig. 1C shows that fludarabine alone was highly inefficient as at the highest concentration cell viability remained at 81% and 85%, respectively, for UT and T samples. Likewise, 1 μM ATO alone rendered cell viability values of 77% and 86%, respectively, for UT and T samples. Combination of several doses of fludarabine with 1 μM ATO significantly decreased cell viability at all points tested, reaching 36% (UT samples) and 69% (T samples) at 5 μM fludarabine (Fig. 1C). For T samples, the most efficient combination was 1 μM ATO/1 μM fludarabine. The interaction of ATO and fludarabine in these groups of patients followed the same synergistic or additive pattern described above, except that for UT samples, the combination of 1 μM ATO/1 μM fludarabine was also synergistic (Fig. 1D). Altogether these results indicated that low concentrations of ATO potentiated the effect of fludarabine, both in UT and T samples, leading to increased cell death.

3.2. The combination of ATO and fludarabine down-regulates survival pathways in CLL cells

To gain some insights on the mechanisms accounting for the synergistic cytotoxic effect of ATO and fludarabine, we analyzed the possible regulation of well-known molecules involved in CLL survival. CLL cells from several UT patients were treated with either 1 μM ATO, 5 μM fludarabine or both drugs together. After 48 h, cells were lysed and analyzed by Western blotting. Fig. 2 shows the results of two representative samples and the quantitation of all samples studied. As observed, the phosho-Akt kinase was not down-regulated by ATO or fludarabine alone, but it was reduced 1.9-fold by combining both drugs (Fig. 2A, B). Likewise, ATO or fludarabine alone decreased phospho-ERK levels 1.5-fold, but the combination of both drugs led to a significant 4.2-fold reduction (Fig. 2A, B).

We also analyzed the regulation of Bcl-2 family proteins, which are major players in survival/apoptosis [15]. The anti-apoptotic proteins Mcl-1 and Bcl-2 were clearly down-regulated when ATO and fludarabine were used together, although the effect was not statistically significant in the case of Mcl-1, compared to the effect of fludarabine alone (Fig. 2C). The pro-apoptotic members Bim and Bax were not modulated by ATO or fludarabine alone or combined (Fig. 2C). Because regulation of cell apoptosis/survival by Bcl-2 family proteins is determined by the balance of anti-apoptotic and pro-apoptotic members [15], we also measured the ratios of well-known partners of this family. Fig. 2D shows that the Mcl-1/Bim and Bcl-2/Bax ratios were significantly down-regulated by the combination of ATO and fludarabine, compared to the effect of the individual drugs. These results therefore indicated that ATO enhanced the effect of fludarabine by further inactivating kinases and Bcl-2 proteins involved in CLL cell survival.

3.3. The combination of ATO and fludarabine partially overcomes the CLL drug-resistance effect induced by stromal cells

Given the relevant role of the microenvironment in inducing survival pathways and drug resistance in CLL cells [16,17], we studied whether the synergistic combination of 1 μM ATO and 5 μM fludarabine overcame this resistance. CLL cells were cultured alone or in the presence of HS-5 or HS-27A stromal cells and treated with ATO, fludarabine or both drugs together. After 48 h CLL cell viability was determined by flow cytometry. The viability of control, untreated, cells in all cases was normalized to 100. In the absence of stroma, the combination of both drugs significantly enhanced the cytotoxic effect of ATO or fludarabine alone, reducing the average viability to 28% (Fig. 3A). Co-culturing with stromal cells clearly protected CLL cells to the individual action of ATO or fludarabine, rendering viability values of 80–90% for both types of stromal cells (Fig. 3B and C). Combining both drugs significantly reduced these percentages to 70% (HS-5 cells) and 74% (HS-27A cells), indicating that the synergistic interaction of ATO and fludarabine partially overcame the survival effect induced by stroma.

4. Discussion

CLL remains an incurable disease and current therapies and clinical trials mostly rely on the use of combined therapies [1–4]. ATO may be a good compound to use in these therapies, due to its demonstrated effectiveness in all CLL cases, regardless of their prognostic markers [9–11]. Indeed, a previous study showed that ATO, at 2–4 μM, preferentially induced apoptosis in CLL samples from patients with unfavorable prognosis, including those that were resistant to fludarabine [9]. Using a different strategy, consisting in combining ATO and fludarabine, we now show for the first time that 1 μM ATO was synergistic with certain concentrations of fludarabine and sufficient to sensitize fludarabine-resistant CLL cells to this drug. Interestingly, samples from previously untreated or treated patients responded well to this drug combination, although the effect on treated patients was moderate. Another

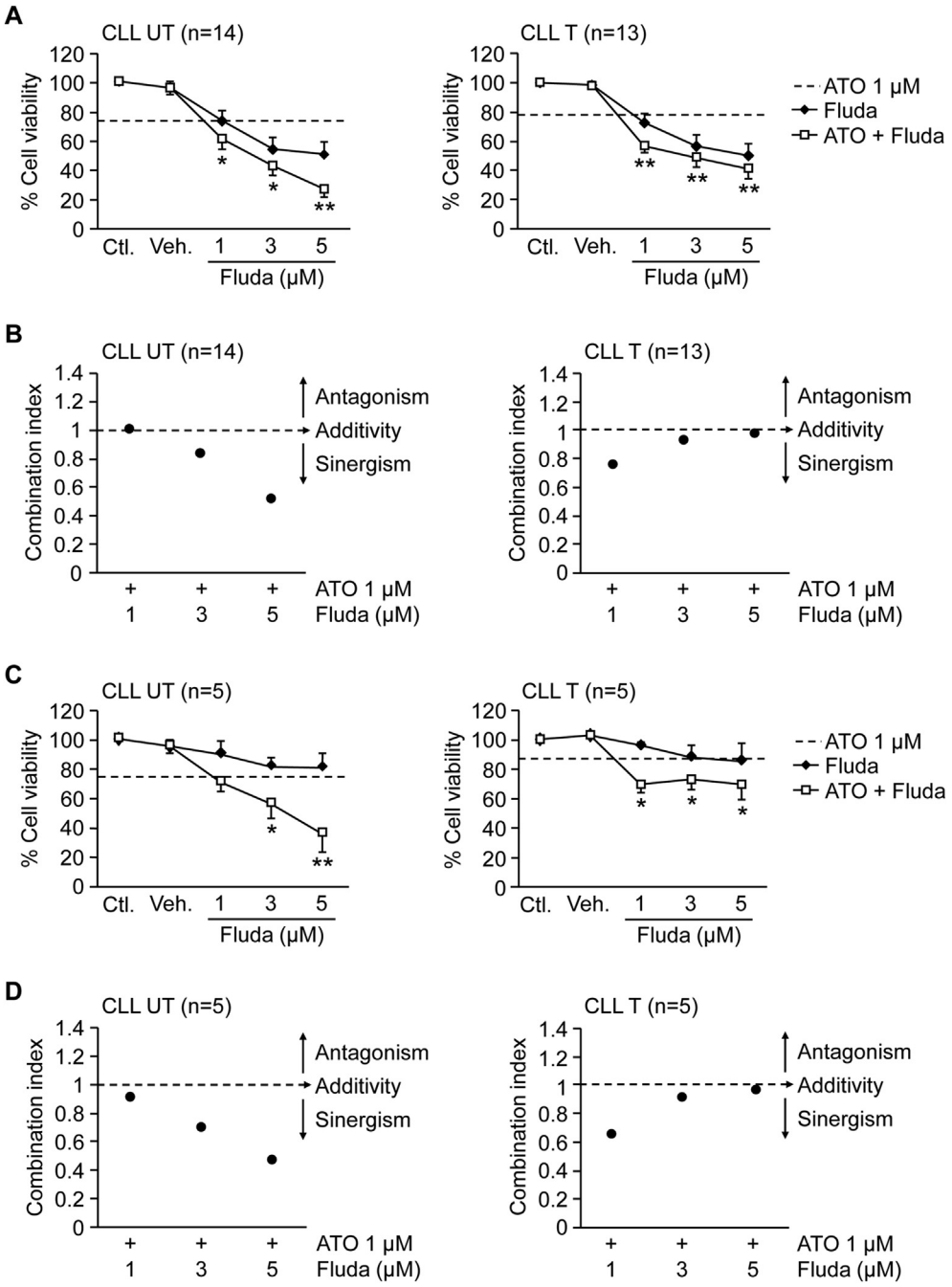

Fig. 1. Increased cytotoxicity against CLL cells by the combination of ATO and fludarabine. (A) 2×10^5 CLL cells from untreated (UT) or treated (T) patients were incubated with medium (control, Ctl), vehicle (Veh) or 1 μM ATO plus the indicated concentrations of fludarabine (Fluda). After 48 h, cell viability was determined by flow cytometry. Cell viability in the presence of 1 μM ATO alone (UT: 75%; T: 79%) is indicated by the dotted line. (B) Combination index values were calculated using the CompuSyn software. Graphs for UT and T samples represent the means of the indicated number of CLL samples, each with duplicate determinations. (C) CLL cells from UT and T patients that responded poorly to fludarabine were selected and treated with 1 μM ATO and the indicated concentrations of fludarabine. After 48 h cell viability was measured as explained. (D) Combination index analyses of the samples studied in (C) were calculated as above. *, $p \leq 0.05$; **, $p \leq 0.01$.

difference between the two groups of patients was the dose of ATO at which synergism with fludarabine was observed. These differences probably reflect the more advanced disease (genetic modifications, clonal evolution, etc.) in the case of treated patients. In agreement with this, we mostly observed NOTCH1 and SF3B1 mutations, relevant for CLL pathology [18], within the group of treated samples (see Table 1).

Our results are in agreement with previous *in vitro* studies in which ATO was tested in combined therapies in several malignancies. These include the combination with interferon-α for aggressive T-cell leukemia/lymphoma [19], or with phytosphingosine for myeloid leukemia cells [20]. ATO has also been tested in combination with kinase inhibitors, oxidative stress modulators and chemotherapeutic drugs, generally with good results [21,22]. Perhaps the best established is the combination of ATO and all-trans retinoic acid, already in clinical trials and shown to be extremely effective for the treatment of acute promyelocytic leukemia [23,24]. In line with these studies, we now report that the

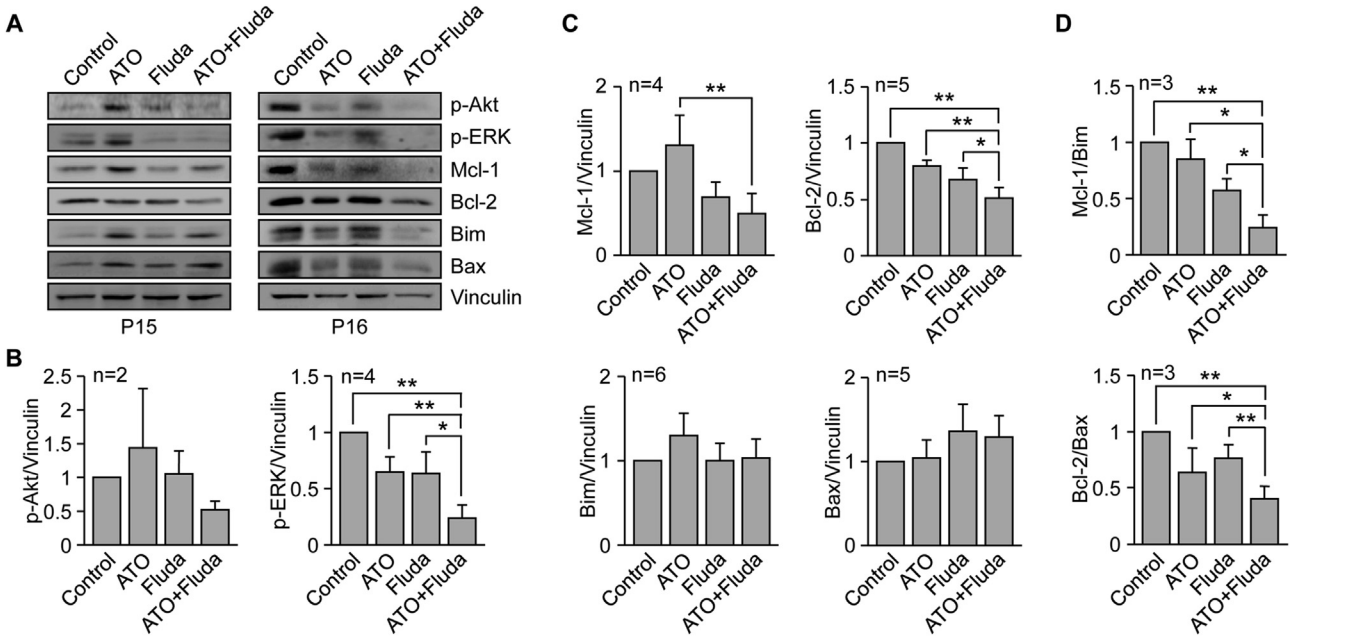

Fig. 2. Survival pathways affected by the combination ATO-fludarabine in CLL cells. (A) 10×10^6 CLL cells from two representative samples were treated with or without 1 μM ATO, 5 μM fludarabine or both drugs together for 48 h. Cells were lysed and lysates analyzed by Western blotting, using vinculin as internal standard. (B) Relative expression levels of phospho-Akt and phospho-ERK, quantitated by chemiluminiscence after normalizing control values to 1. (C) Quantitation of the protein bands corresponding to the Bcl-2 family proteins shown in (A). (D) Quantitation of the indicated ratios. *, $p \leq 0.05$; **, $p \leq 0.01$.

novel combination of very low concentrations of ATO and fludarabine may constitute a promising and effective alternative for the treatment of either naïve or relapsed/refractory patients with CLL.

Analysis of the possible mechanisms accounting for the enhanced cytotoxic effect of ATO + fludarabine revealed the involvement of the Akt and ERK pathways. Indeed, phosphorylation of both kinases was significantly down-regulated when both drugs were used together. Akt has been linked to the action of ATO since several reports have shown that inhibitors of this kinase increase the cytotoxic effect of ATO in myeloid and other cell types [25–27]. In the case of CLL, we have shown that 3 μM ATO downregulated phospho-Akt, facilitating apoptosis [10]. Although the regulation of ERK by ATO appears to be more complex [28], it is clear from our present results that 1 μM ATO combined with fludarabine significantly dephosphorylates ERK. Therefore, the observed enhancement of CLL cell apoptosis when both drugs are present is likely due to the down-modulation of the Akt and ERK survival pathways.

Additional mechanistic insights were obtained by studying the possible regulation of some Bcl-2 family members known to be important for CLL cell survival [29]. Our results show that the expression of the anti-apoptotic proteins Mcl-1 and Bcl-2 clearly decreased upon treatment with ATO and fludarabine. Interesting, Mcl-1 (and possibly Bcl-2) is a downstream target of Akt [30]. Additionally, the ratio with their corresponding pro-apoptotic partners Bim and Bax, was also downregulated by the combination of ATO and fludarabine. These results strongly suggest that the enhanced cytotoxicity observed upon combining ATO and

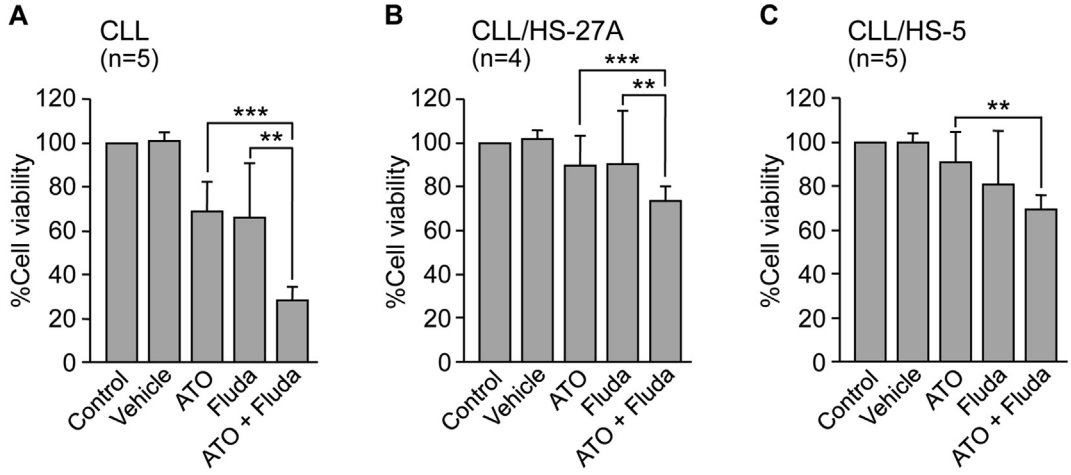

Fig. 3. The combination of ATO and fludarabine partially overcomes the survival effect of stroma. 2×10^5 CLL cells were incubated in suspension or with stromal cells, with or without 1 μM ATO, 5 μM fludarabine or both drugs together. After 48 h, CLL cells were recovered and their viability mesured by flow cytometry. Cell viability of control, untreated, cells in each case was normalized to 100. *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

fludarabine involves down-regulation of crucial survival pathways. Importantly, modulation of these pathways was not achieved by the individual drugs, further demonstrating the synergistic action of ATO and fludarabine.

A crucial component to take into account when studying the action of drugs in CLL and other cells is the effect of the microenvironment, which activate survival signals on the malignant cells that favor drug resistance [16,17]. Using co-cultures of CLL and stromal cells we show in this report that the combination of ATO and fludarabine partially overcomes the survival effect of the stroma. Although these conditions would have to be further studied so that the stroma effect can be completely suppressed, our results are very encouraging, considering the low concentration of ATO employed in our study. In summary, our current findings indicate that ATO in combination with fludarabine may be an efficient and novel therapeutic strategy for all cases of CLL, including those with unfavorable prognosis.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by grants SAF2012-31613 (AGP) and RTICC (Red Temática de Investigación Cooperativa en Cáncer) RD12/0036/0061 (AGP), from the Ministry of Economy and Competitiveness (MINECO), Spain; S2010/BMD-2314-Neoplasbim (AGP) from the Comunidad de Madrid/European Union; and by a grant from the Fundación para la Investigación Biomédica Hospital Universitario Puerta de Hierro, Madrid (JAGM). CLS was supported by a grant from Roche Pharma and IAJ was supported by the Junta de Ampliación de Estudios program, JAEPre2010-00607, CSIC/EU, and by MINECO.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.04.007.

References

[1] L. Pleyer, A. Egle, T.N. Hartmann, R. Greil, Molecular and cellular mechanisms of CLL: novel therapeutic approaches, Nat. Rev. Clin. Oncol. 6 (2009) 405–418.
[2] T. Zenz, D. Mertens, R. Küppers, et al., From pathogenesis to treatment of chronic lymphocytic leukaemia, Nat. Rev. Cancer 10 (2010) 37–50.
[3] M. Hallek, Signaling the end of chronic lymphocytic leukemia: new frontline treatment strategies, Blood 122 (2013) 3723–3734.
[4] F.T. Awan, J.C. Byrd, New strategies in chronic lymphocytic leukemia: shifting treatment paradigms, Clin. Cancer Res. 20 (2014) 5869–5874.
[5] C. Chung, R. Lee, Ibrutinib, obinutuzumab, idelalisib, and beyond: review of novel and evolving therapies for chronic lymphocytic leukemia, Pharmacotherapy 34 (2014) 1298–1316.
[6] K. Alimoghaddam, A review of arsenic trioxide and acute promyelocytic leukemia, Int. J. Hematol. Oncol. Stem Cell. Res. 8 (2014) 44–54.
[7] L. Zhou, Jian Hou, G. Chi-Fung Chan, D. Man-Yuen Sze, Arsenic trioxide for non acute promyelocytic leukemia hematological malignancies: a new Frontier, J. Blood Dis. 1 (2014) 1018.
[8] P.R. Subbarayan, B. Ardalan, In the war against solid tumors arsenic trioxide needs partners, J. Gastrointest. Cancer 45 (2014) 363–371.
[9] O. Merkel, C. Heyder, D. Asslaber, et al., Arsenic trioxide induces apoptosis preferentially in B-CLL cells of patients with unfavourable prognostic factors including del17p13, J. Mol. Med. 86 (2008) 541–552.
[10] J. Redondo-Muñoz, E. Escobar-Díaz, M. Hernández del Cerro, et al., Induction of B-chronic lymphocytic leukemia cell apoptosis by arsenic trioxide involves suppression of the PI3K/Akt pathway via JNK activation and PTEN upregulation, Clin. Cancer Res. 16 (2010) 4382–4391.
[11] I. Amigo-Jiménez, E. Bailón, E. Ugarte-Berzal, et al., Matrix metalloproteinase-9 is involved in chronic lymphocytic leukemia cell response to fludarabine and arsenic trioxide, PLoS One 9 (2014) e99993.
[12] A.B. Roecklein, B. Torok-Storb, Functionally distinct human marrow stromal cell lines immortalized by transduction with the human papilloma virus E6/E7 genes, Blood 85 (1995) 997–1005.
[13] T.C. Chou, P. Talalay, Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors, Adv. Enzyme Regul. 22 (1984) 27–55.
[14] D. Rossi, M. Fangazio, S. Rasi, et al., Disruption of BIRC3 associates with fludarabine chemorefractoriness in TP53 wild-type chronic lymphocytic leukemia, Blood 119 (2012) 2854–2862.
[15] 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.
[16] A.D. Ramsay, M. Rodriguez-Justo, Chronic lymphocytic leukaemia–the role of the microenvironment pathogenesis and therapy, Br. J. Haematol. 162 (2013) 15–24.
[17] J.A. Burger, J.G. Gribben, The microenvironment in chronic lymphocytic leukemia (CLL) and other B cell malignancies: insight into disease biology and new targeted therapies, Semin. Cancer Biol. 24 (2014) 71–81.
[18] S. Chiaretti, M. Marinelli, I. Del Giudice, et al., NOTCH1, SF3B1, BIRC3 and TP53 mutations in patients with chronic lymphocytic leukemia undergoing first-line treatment: correlation with biological parameters and response to treatment, Leuk. Lymphoma 55 (2014) 2785–2792.
[19] K. Ishitsuka, J. Suzumiya, M. Aoki, et al., Therapeutic potential of arsenic trioxide with or without interferon-alpha for relapsed/refractory adult T-cell leukemia/lymphoma, Haematologica 92 (2007) 719–720.
[20] M.T. Park, Y.H. Kang, I.C. Park, et al., Combination treatment with arsenic trioxide and phytosphingosine enhances apoptotic cell death in arsenic trioxide-resistant cancer cells, Mol. Cancer Ther. 6 (2007) 82–92.
[21] H. Yan, Y.C. Wang, D. Li, Y. Wang, et al., Arsenic trioxide and proteasome inhibitor bortezomib synergistically induce apoptosis in leukemic cells: the role of protein kinase Cδ, Leukemia 21 (2007) 1488–1495.
[22] S. Takahashi, Combination therapy with arsenic trioxide for hematological malignancies, Anticancer Agents Med. Chem. 10 (2010) 504–510.
[23] F. Efficace, F. Mandelli, G. Avvisati, et al., Randomized phase III trial of retinoic acid and arsenic trioxide versus retinoic acid and chemotherapy in patients with acute promyelocytic leukemia: health-related quality-of-life outcomes, J. Clin. Oncol. 32 (2014) 3406–3412.
[24] M.F. Arteaga, J.H. Mikesch, T.K. Fung, C.S. So, Epigenetics in acute promyelocytic leukaemia pathogenesis and treatment response: a transition to targeted therapies, Br. J. Cancer 112 (2015) 413–418.
[25] A.M. Ramos, C. Fernández, D. Amrán, et al., Pharmacologic inhibitors of PI3K/Akt potentiate the apoptotic action of the antileukemic drug arsenic trioxide via glutathione depletion and increased peroxide accumulation in myeloid leukemia cells, Blood 105 (2005) 4013–4020.
[26] G. Tabellini, A. Cappellini, P.L. Tazzari, et al., Phosphoinositide 3-kinase/Akt involvement in arsenic trioxide resistance of human leukemia cells, J. Cell. Physiol. 202 (2005) 623–634.
[27] B.C. Bornhauser, L. Bonapace, D. Lindholm, et al., Low-dose arsenic trioxide sensitizes glucocorticoid-resistant acute lymphoblastic leukemia cells to dexamethasone via an Akt-dependent pathway, Blood 110 (2007) 2084–2091.
[28] M.C. Estaño, E. Calviño, E. de Blas, et al., 2-Deoxy-D-glucose cooperates with arsenic trioxide to induce apoptosis in leukemia cells: involvement of IGF-1R-regulated Akt/mTOR, MEK/ERK and LKB-1/AMPK signaling pathways, Biochem. Pharmacol. 84 (2012) 1604–1616.
[29] A.G. Buggins, C.J. Pepper, The role of Bcl-2 family proteins in chronic lymphocytic leukaemia, Leuk. Res. 34 (2010) 837–842.
[30] K. Balakrishnan, J.A. Burger, M. Fu, et al., Regulation of Mcl-1 expression in context to bone marrow stromal microenvironment in chronic lymphocytic leukemia, Neoplasia 16 (2014) 1036–1046.