Am J Transl Res 2013;5(1):36-46
www.ajtr.org /ISSN:1943-8141/AJTR1212001

Original Article

Cladribine and bendamustine exhibit inhibitory activity in dexamethasone-sensitive and -resistant multiple myeloma cells

Bo Cai[1,2], Shuiliang Wang[2], Jingcao Huang[2,4], Choon-Kee Lee[3*], Chunji Gao[1], Bolin Liu[2]

*[1]Department of Hematology, Chinese PLA General Hospital, Beijing, China; [2]Department of Pathology, [3]Department of Medicine, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA; [4]State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China. *Current address: San Juan Cancer Center, Montrose Memorial Hospital, Montrose, CO, USA.*

Received December 10, 2012; Accepted January 2, 2013; Epub January 21, 2013; Published January 30, 2013

Abstract: Cladribine (2-CDA) is a well-known purine nucleoside analog with activities against lymphoproliferative disorders such as hairy cell leukemia (HCL). Bendamustine, a hybrid molecule of purine analog and alkylator, induces apoptosis via DNA damage response and inhibition of mitotic checkpoint. Their therapeutic potential in patients with multiple myeloma (MM), particularly those become resistant to traditional chemotherapeutic agents, remains unclear. Here we study the effects of cladribine or bendamustine on dexamethasone-sensitive (MM1.S) and -resistant (MM1.R) MM cells. MTS-based proliferation assays showed that cladribine and bendamustine exhibited similar anti-proliferation/anti-survival effects on MM1.S and MM1.R cells in a dose-dependent manner. The IC50s of cladribine were approximately 35.3 nmol/L and 58 nmol/L for MM1.S and MM1.R cells, respectively. The IC50s of bendamustine were approximately 119.8 μmol/L (MM1.S) and 138 μmol/L (MM1.R). An apoptotic-ELISA and western blot assays of PARP cleavage and activation of caspase-8 and caspase-3 indicated that cladribine or bendamustine induced apoptosis in both cell lines. Similar results were obtained with flow cytometric analysis showing that cladribine or bendamustine increased the sub-G1 population. Treatment with bendamustine but not cladribine also resulted in cell cycle S-phase arrest. Either cladribine or bendamustine led to a remarkable increase of the phosphorylated H2A.X, CHK1 and CHK2 in both MM1.S and MM1.R cells, suggesting an induction of DNA damage response. Collectively, we demonstrate that cladribine and bendamustine exert potent inhibitory effects on dexamethasone-sensitive and -resistant MM cells *in vitro*. Our data suggest that MM patients, including those with dexamethasone resistance, may particularly benefit from cladribine or bendamustine.

Keywords: Cladribine, bendamustine, dexamethasone resistance, DNA damage response, apoptosis, multiple myeloma

Introduction

Multiple myeloma (MM), a clonal bone marrow disorder characterized by the neoplastic transformation of B cells, is the second most common hematological malignancy, and is responsible for approximately 2% of cancer deaths [1]. Despite progress in recent years, MM is still incurable and therapeutic challenges remain, especially for patients at high risk for early relapse and for those with multi-drug resistant disease and refractoriness to traditional combination regimens. Identification of novel and more effective therapeutic agents is critical to improve the survival of MM patients.

Cladribine (also known as 2-chlorodeoxyadenosine, 2-CDA) is classified as one of the therapeutic purine nucleoside analogs. In cells, it requires phosphorylation by deoxycytidine kinase (DCK) to convert to its lymphocytotoxic form, 2-chlorodeoxyadenosine triphosphate, inhibiting DNA and RNA synthesis and subsequently enhancing DNA strand break [2, 3]. Since lymphocytes have high levels of DCK as compared with other cell types, cladribine

shows potent cytotoxicity in lymphoid tissues [4]. Cladribine exerts remarkable activity, produces long-lasting complete remissions, and has been standard treatment in hairy cell leukemia (HCL), a chronic B-cell lymphoproliferative disorder [5]. Although cladribine has not become first-line therapy as another purine nucleoside analog fludarabine, it has been shown to have similar and even more impressive clinical results with single agent or combination with conventional agents in treating chronic lymphocytic leukemia (CLL) [6-9]. In addition, cladribine has also been proved to be active in other hematologic malignancies such as indolent non-Hodgkin lymphoma and acute myeloid leukemia [10, 11]. However, its therapeutic potential in MM is still controversial. Clinical studies found that cladribine was less effective in MM patients; however the data were resulting from insufficient samples and low dose of the agent [12, 13]. While some investigators observed negative results in *in vitro* studies [14, 15], others showed that cladribine displayed a marked heterogeneous effect on MM cell lines [4], and clearly inhibited proliferation of RPMI8226 MM cells at high concentrations [16]. Our previous studies also confirmed that cladribine exhibited inhibitory effects on U266, RPMI8226 and MM1.S cells *in vitro*, and MM1.S was the only cell line showing significant response to the clinically achievable concentrations [17].

Bendamustine was synthesized firstly in 1960s in German. Although classified as one of alkylating agents, bendamustine combines the alkylating activity of the mustard group with the antimetabolite activity of the purine analog structure, which makes it possess different pharmacological profile. It has been shown that bendamustine induces DNA damage response, apoptosis, mitotic catastrophe, and inhibition of mitotic checkpoint, however, it does not show cross-resistance with other cytotoxic agents [18]. Preclinical studies have demonstrated that bendamustine has potential to overcome resistance to other alkylating agents [19], and also shows synergistic toxicity with cladribine or rituximab in lymphoma cell lines or xenograft models [20, 21]. In MM treatment, several clinical studies have shown promising results of bendamustine for relapsed/refractory MM patients using single agent or combination with conventional chemotherapeutics such as thalidomide, lenalidomide, and dexamethasone [22-24]. Nonetheless, the precise molecular mechanisms of how bendamustine overcomes chemotherapeutic resistance in MM remain unclear.

Dexamethasone is widely used in combination with other classical or novel chemotherapeutics for MM treatment. However, development of resistance to dexamethasone has limited the use of this agent. Here, based upon our previous studies evaluating the efficacy of cladribine in MM treatment [17], we seek to explore whether cladribine or bendamustine may have therapeutic potential against MM sensitive or resistant to dexamethasone.

Materials and methods

Reagents and antibodies

Dexamethasone (D1756) and cladribine (C4438) were purchased from Sigma-Aldrich (St. Louis, MO). Dexamethasone was dissolved in ethanol to make a stock solution at 1 mg/ml. Cladribine and bendamustine (Cephalon, Inc., Frazer, PA) were dissolved in dimethyl sulfoxide (DMSO) to make a stock solution at 10 mg/ml and 526 mmol/L, respectively. All the stock solutions were stored at -20 °C.

The sources of antibodies for western blots were as follows: caspase-8 mouse mAb (1C12), caspase-3 rabbit mAb (8G10), PARP rabbit mAb, P-Histone H2A.X (Ser139) rabbit antibody, Histone H2A rabbit polyclonal antibody II, P-CHK1 (Ser345) (133D3) rabbit mAb, CHK1 rabbit antibody, P-CHK2 (Thr68) rabbit polyclonal antibody, and CHK2 rabbit polyclonal antibody (Cell Signaling Technology, Inc., Beverly, MA); cyclin E (HE12), E2F1 (KH95), $p27^{kip1}$ (C-19) (Santa Cruz Biotechnology Inc., Santa Cruz, CA); β-actin mouse mAb (clone AC-75) (Sigma Chemical Co., St. Louis, MO). All other reagents were purchased from Sigma unless otherwise specified.

Cells and cell culture

Human MM cell line MM1.S and MM1.R [25] were kindly provided by Dr. Steven Rosen (Department of Medicine, Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL). The cells were maintained in RPMI1640 cell culture medium sup-

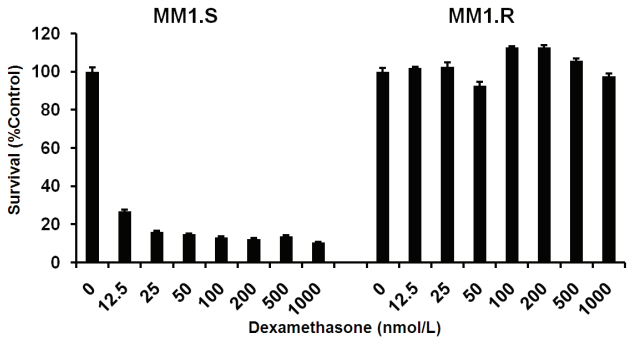

Figure 1. Dexamethasone inhibits proliferation of MM1.S, but not MM1.R cells. MM1.S or MM1.R cells were plated onto 96-well plates with fresh RPMI1640 medium (0.5% FBS) or same medium containing indicated concentrations of dexamethasone for 72 h. The percentages of surviving cells as compared to controls, defined as 100% survival, were determined by reduction of MTS. Data shows the representative of three independent experiments. Bars, SD.

plemented with 10% fetal bovine serum (FBS) at a 37 °C humidified atmosphere containing 95% air and 5% CO_2 and were split twice a week.

Cell proliferation assays

The CellTiter96™ AQ non-radioactive cell proliferation kit (Promega Corp., Madison, WI) was used to evaluate cell viability as we previously described [26]. In brief, cells were plated onto 96-well plates with 0.1 ml complete medium containing 0.5% FBS as control, or 0.1 ml of the same medium with a series doses of dexamethasone, cladribine or bendamustine, and incubated for 72 h in a 37 °C humidified atmosphere containing 95% air and 5% CO_2. After reading all wells at 490 nM with a 96-plate reader, the percentages of surviving cells from each group relative to control groups, defined as 100% survival, were determined by reduction of MTS.

Quantification of apoptosis

An apoptosis ELISA kit (Roche Diagnositics Corp., Indianapolis, IN) was used to quantitatively measure cytoplasmic histone-associated DNA fragments (mononucleosomes and oligonucleosomes) as previously reported [26].

Western blot analysis

Protein expression levels were measured as previously described [26]. In brief, cells were lysed in a buffer containing 50 mM Tris, pH 7.4, 50 mM NaCl, 0.5% NP-40, 50 mM NaF, 1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, 25 μg/ml leupeptin, and 25 μg/ml aprotinin. The protein concentrations of total cell lysates were measured by the Coomassie Plus protein assay reagent (Pierce Chemical Co., Rockford, IL). Equal amounts of cell lysates were boiled in Laemmli SDS-sample buffer, resolved by SDS-PAGE, and western blot analysis with specific antibodies as described in the figure legends.

Flow cytometric analysis of cell cycle and apoptosis

Flow cytometric analyses were performed as described previously [26] to define the cell cycle distribution and apoptosis for treated and untreated cells. In brief, cells grown in 100-mm culture dishes were harvested and fixed with 70% ethanol. Cells were then stained for total DNA content with a solution containing 50 μg/ml propidium iodide and 100 μg/ml RNase I in PBS for 30 min at 37 °C. Data was acquired using a Coulter FC500 instrument (Beckman-Coulter, Fullerton, CA), and analyzed using ModFit LT 3.2 software (Verity Software House, Topsham, ME).

Statistical analysis

Statistical analyses of the experimental data were performed using a two-sided Student's t test. Significance was set at a $P<0.05$. Calculation of IC50 was performed using the Calcusyn software (Biosoft, Ferguson, MO).

Results

Dexamethasone induces growth inhibition, cell cycle G1 arrest, and apoptosis in MM1.S, but not MM1.R cells.

MM1.S and MM1.R cell lines were established from the same MM patient that paralleled progression of the disease. While MM1.S cells are sensitive to steroid hormones such as dexamethasone, MM1.R cells have no response to the treatment of dexamethasone [25]. Thus, this pair of MM cell lines provides us an excellent system to identify novel and more effective therapeutics against MM, particularly those

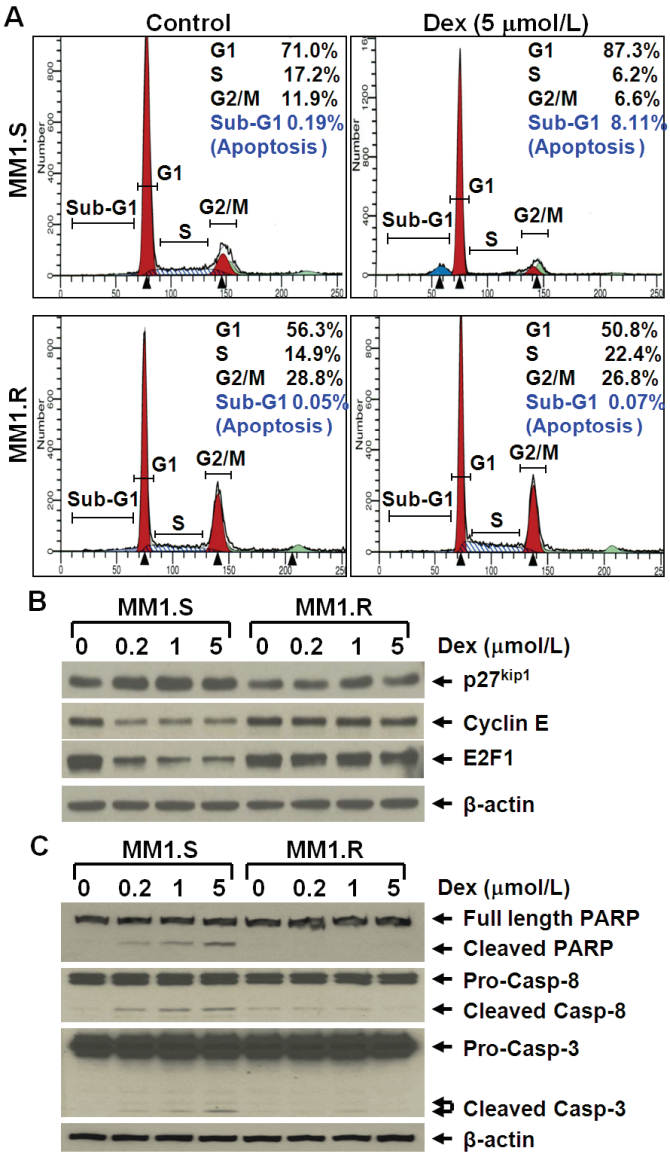

Figure 2. Dexamethasone induces cell cycle G1 arrest and apoptosis in MM1.S cells. A, MM1.S or MM1.R cells cells were cultured with RPMI1640 (0.5% FBS) in the absence or presence of dexamethasone (Dex, 5 μmol/L) for 24 h. Cells were collected and subjected to flow cytometric analysis of cell cycle distribution. Data shows the representative of three independent experiments. B & C, MM1.S or MM1.R cells were cultured with RPMI1640 (0.5% FBS) in the absence or presence of indicated concentrations of dexamethasone (Dex) for 24 h. Cells were collected and subjected to western blot analyses with specific antibody directed against $p27^{kip1}$, Cyclin E, E2F1, or β-actin (B), or PARP, caspase-8 (Casp-8), caspase-3 (Casp-3), or β-actin (C).

become resistant to dexamethasone. We first performed cell proliferation (MTS) assays to confirm the responsiveness of MM1.S and MM1.R cells to dexamethasone. MM1.S cells were much more sensitive to dexamethasone than MM1.R cells with the survival rate less than 30% at the concentration of 12.5 nmol/L. In contrast, MM1.R cells were highly resistant to dexamethasone treatment with the survival rate almost 100% at the concentration of 1000 nmol/L (**Figure 1**). Then, flow cytometric analysis of cell cycle progression revealed that treatment with dexamethasone (5 μmol/L) increased the percentage of cells in G1 phase and reduced the percentage of cells in S phase in MM1.S cells but not MM1.R cells (**Figure 2A**). Consistently, western blot analysis on the critical molecules controlling the G1-S transition showed that dexamethasone enhanced the expression of the CDK inhibitor $p27^{kip1}$ and reduced the positive regulators Cyclin E and E2F1 only in MM1.S cells (**Figure 2B**). Furthermore, dexamethasone induced cleavages of PARP and caspase-8 and -3, the hallmarks of apoptosis, in MM1.S cells in a dose-dependent manner (**Figure 2C**). In addition, the enhanced apoptosis was also detected with the presence of a sub-G1 population in MM1.S rather than MM1.R cells upon dexamethasone treatment (**Figure 2A**). Collectively, our data indicate that dexamethasone induces growth inhibition, cell cycle G1 arrest, and apoptosis in the sensitive, but not resistant MM cells.

Cladribine and bendamustine inhibit cell proliferation and induce apoptosis in both MM1.S and MM1.R cells

Next, we sought to determine whether cladribine and bendamustine may overcome dexamethasone resistance in MM cells. After treated with different concentration of cladribine or bendamustine for 72 h, both MM1.S and MM1.R cells' proliferation was significantly inhibited by either cladribine or bendamustine (**Figure 3A** & **3B**). The response of MM1.S cells to cladribine was in accordance with our previous report [17]. The IC50s of cladribine were approximately 35.3 nmol/L and 58 nmol/L for MM1.S and MM1.R cells, respectively. The IC50s of bendamustine were approximately

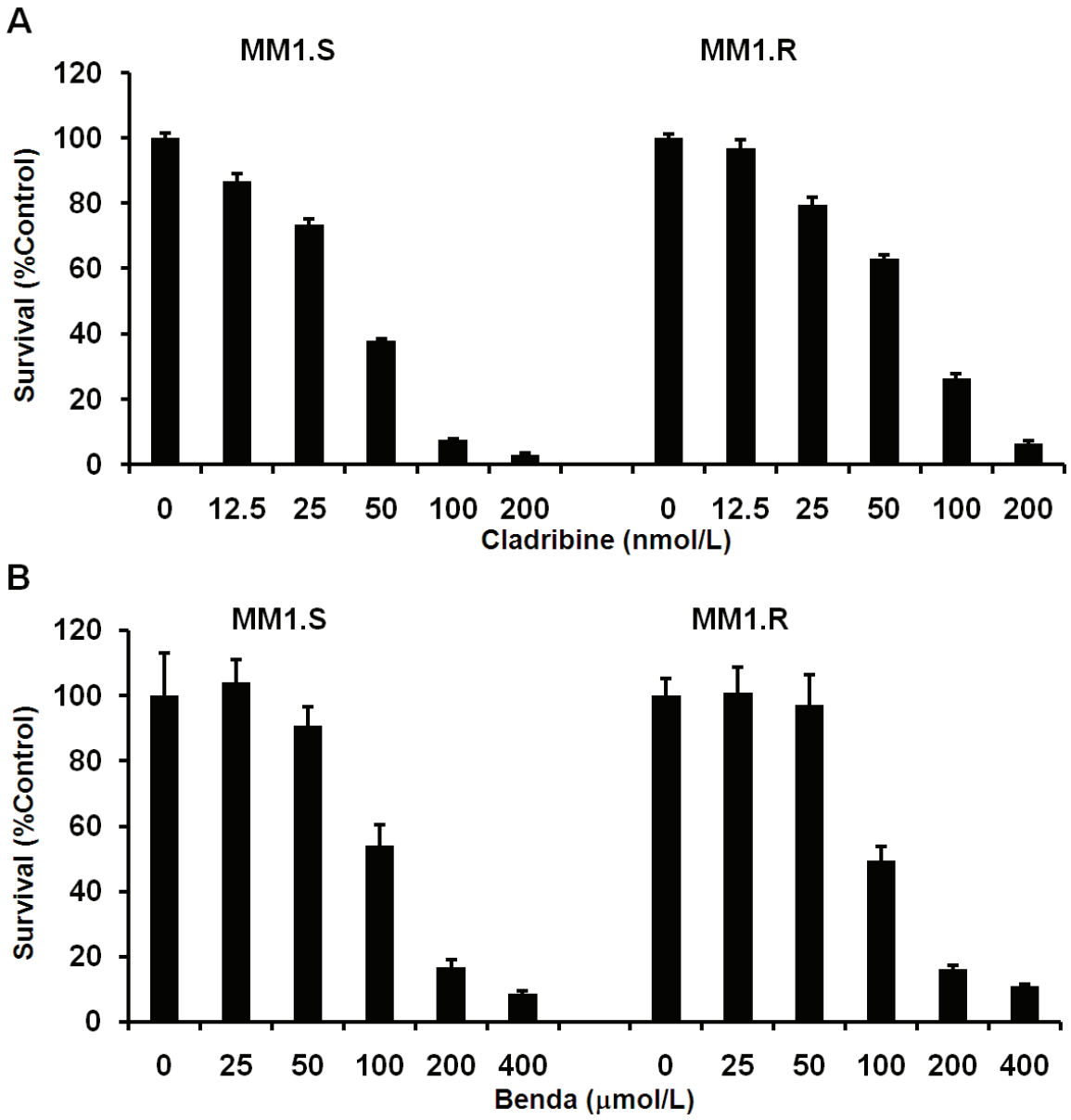

Figure 3. Cladribine and bendamustine inhibit cell growth in both MM1.S and MM1.R cells. MM1.S or MM1.R cells were plated onto 96-well plates with fresh RPMI1640 medium (0.5% FBS) or same medium containing indicated concentrations of cladribine or bendamustine (Benda) for 72 h. The percentages of surviving cells as compared to controls, defined as 100% survival, were determined by reduction of MTS. Data shows the representative of three independent experiments. Bars, SD. A. cladribine; B. bendamustine.

119.8 μmol/L (MM1.S) and 138 μmol/L (MM1.R). To elucidate the molecular mechanisms by which cladribine and bendamustine induced growth inhibition, we performed apoptosis-related analysis after treating MM cells with either agent for 24 h. An apoptotic-ELISA showed that dexamethasone (5 μmol/L), cladribine (0.5 μmol/L) or bendamustine (100 μmol/L) induced potent apoptotic effects on MM1.S cells. Less potent but still significant apoptosis was observed in MM1.R cells upon treatment with cladribine or bendamustine, but not dexamethasone (**Figure 4A**). Consistent data were also obtained from western blot assays on the apoptosis-related proteins and flow cytometric analysis. Either cladribine or bendamustine strongly induced PARP cleavage and activation of caspase-8 and -3 evidenced by the increases of cleaved caspase-8 and -3 in both cell lines, while dexamethasone showed

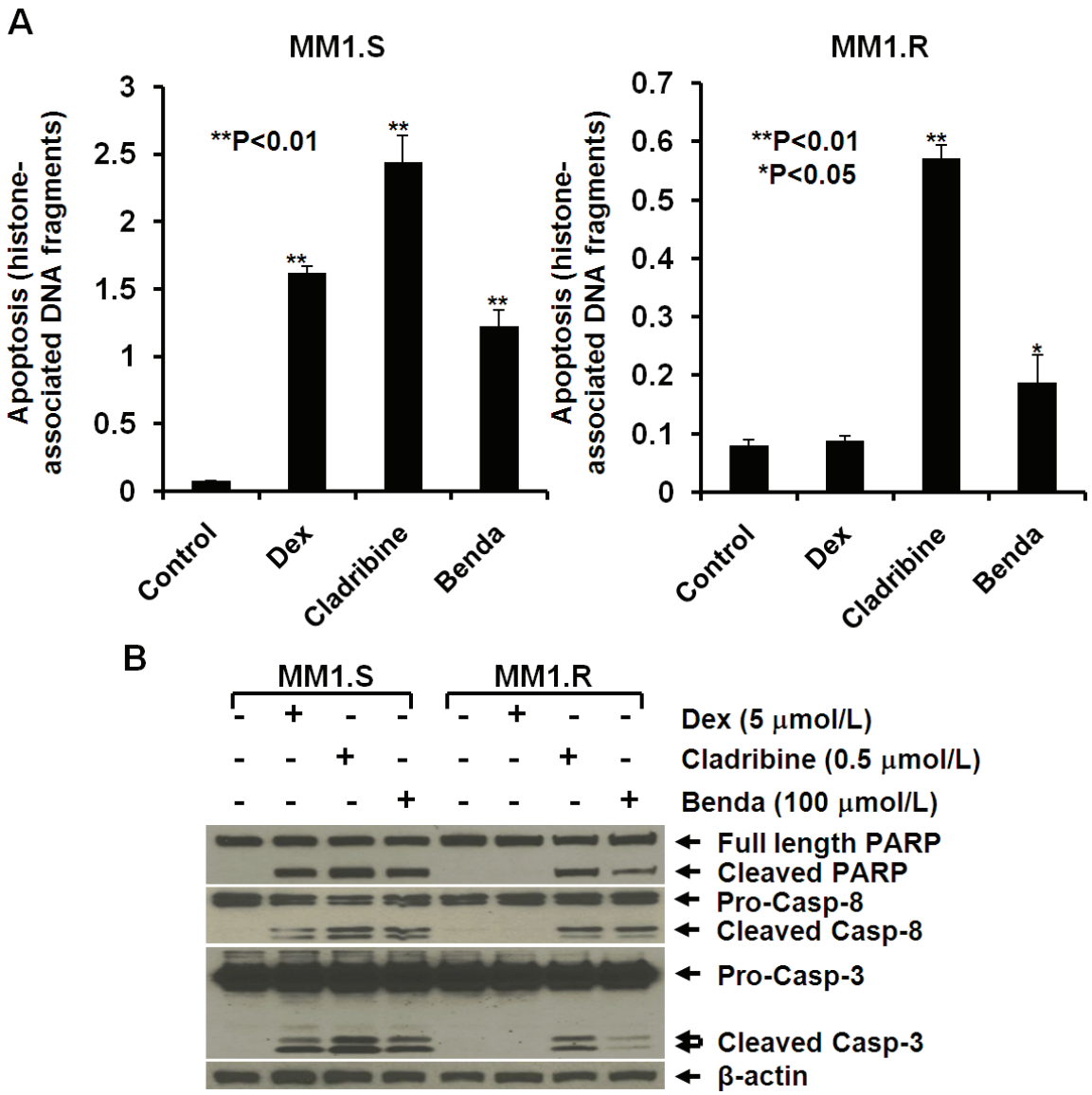

Figure 4. Cladribine and bendamustine induce apoptosis in both MM1.S and MM1.R cells. MM1.S or MM1.R cells were cultured with RPMI1640 (0.5% FBS) in the absence or presence of dexamethasone (Dex, 5 μmol/L), cladribine (0.5 μmol/L) or bendamustine (Benda, 100 μmol/L) for 24 h. Cells were collected and subjected to a specific apoptotic ELISA (A), or western blot analyses with specific antibody directed against PARP, caspase-8 (Casp-8), caspase-3 (Casp-3), or β-actin (B). Bars, SD. P values vs control.

the same impact only on MM1.S cells (**Figure 4B**). In addition, increased percentage of the cells in sub-G1 population further confirmed that treatment with cladribine or bendamustine promoted both MM1.S and MM1.R cells undergoing apoptosis (**Figure 5A** & **5B**). Interestingly, we did not detect significant alterations of cell cycle progression upon treatment with cladribine in both MM1.S and MM1.R cells (**Figure 5A**), consistent with our previous findings [17]. In contrast, a remarkable S phase arrest was observed in both MM1.S and MM1.R cells after treatment with bendamustine (**Figure 5B**), suggesting that induction of cell cycle S phase arrest by bendamustine may contribute to its inhibitory effects on MM cells. Taken together, our data demonstrate that cladribine and bendamustine exhibit potent anti-proliferative/anti-survival activity in both dexamethasone-sensitive and -resistant MM cells, and both agents accelerate apoptosis via caspase-dependent signaling pathways.

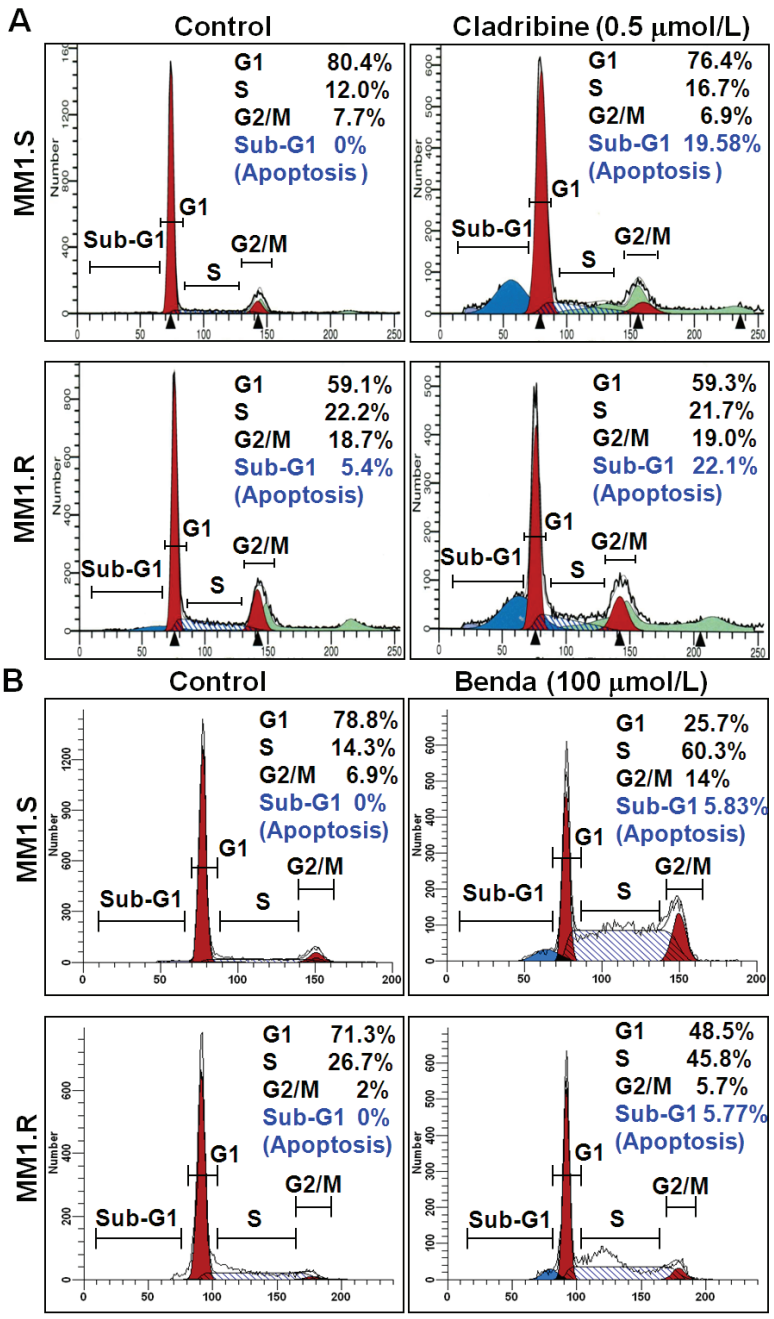

Figure 5. Cladribine and bendamustine show distinct effects on cell cycle progression in MM1.S and MM1.R cells. MM1.S or MM1.R cells were cultured with RPMI1640 (0.5% FBS) in the absence or presence of cladribine or bendamustine (Benda) for 24 h. Cells were collected and subjected to flow cytometric analysis of cell cycle distribution. Data shows the representative of three independent experiments. A. cladribine; B. bendamustine.

Cladribine and bendamustine enhance DNA damage response in both MM1.S and MM1.R cells

It has been reported that both cladribine and bendamustine have capability to induce DNA double strand break in other cells [3, 18], we wondered whether both agents could trigger DNA damage response to result in growth inhibition in both MM1.S and MM1.R cells. We examined the expression of DNA damage checkpoint proteins after treating the cells with dexamethasone, cladribine or bendamustine for 24 h. Without surprise, treatment with dexamethasone (5 μmol/L) increased phosphorylated H2A.X (P-H2A.X) and CHK2 (P-CHK2) in MM1.S but not MM1.R cells. P-CHK1 remained unchanged (**Figure 6A**). In contrast, the levels of P-H2A.X, P-CHK1, and P-CHK2 were dramatically induced by cladribine (0.5 μmol/L) or bendamustine (100 μmol/L) in both cell lines (**Figure 6A**). Nonetheless, unlike the effects of dexamethasone on cell cycle G1-S transition in MM1.S cells, neither cladribine nor bendamustine significantly altered the expression levels of $p27^{kip1}$, Cyclin E, and E2F1 in both cell lines (**Figure 6B**), which was consistent with the cell cycle analysis showing that neither agent induced G1 arrest (**Figure 5**). Our studies suggest that both cladribine and bendamustine enhance DNA damage response mainly through induction of P-H2A.X, P-CHK1, and P-CHK2 in MM cells.

Discussion

Recent advances in identifying novel agents for MM treatment have provided promising results. Among them purine nucleoside analogs are rationally designed as anti-cancer drugs that

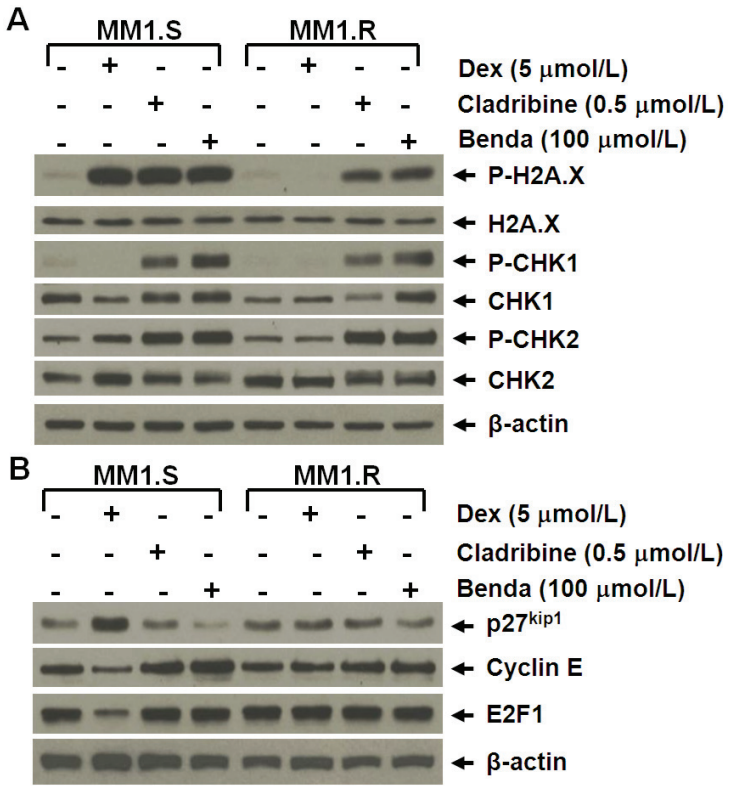

Figure 6. Cladribine and bendamustine enhance DNA damage response in both cell lines. MM1.S or MM1.R cells were cultured with RPMI1640 (0.5% FBS) in the absence or presence of dexamethasone (Dex), cladribine or bendamustine (Benda) for 24 h. Cells were collected and subjected to western blot analyses with specific antibody directed against P-H2AX, H2AX, P-CHK1, CHK1, P-CHK2, CHK2, or β-actin (A) , or $p27^{kip1}$, Cyclin E, E2F1, or β-actin (B).

exert cytotoxicity via inhibition of DNA and RNA synthesis, and are frequently used in treating hematologic malignancies [27, 28]. Despite successful application of cladribine in B-cell originated hematologic malignancies such as HCL and CLL [5, 6], its efficacy in MM remains controversial. Bendamustine is an old alkylating agent possessing purine analog structure, and has been currently used to treat B-cell originated cancer [29]. Although recent clinical trials showed promising results of bendamustine in treating relapse/refractory MM patients, its mechanisms of action in overcoming resistance are not fully understood. Here we provide strong evidence indicating that both cladribine and bendamustine potently inhibit cell proliferation and induce apoptosis in MM cells, and have potential to overcome dexamethasone resistance. We previously reported that the MM1.S cells showed significant growth inhibition and apoptosis induced by cladribine with the dose under the peak plasma concentration [17]. Here our data not only further confirmed this finding, they also revealed that another cell line MM1.R, which was derived from the same MM patient and highly resistant to dexamethasone, presented a similar sensitivity to cladribine treatment as compared with MM1.S (**Figure 3A**). In addition, the doses of bendamustine we evaluated for growth inhibition and apoptosis were also within clinically tolerated concentrations [30]. Therefore, these facts facilitate us to propose that cladribine and bendamustine may be valuable options to treat MM patients, especially those become resistant to dexametha -sone.

Dexamethasone is frequently used for the treatment of MM patients, but resistance to this agent has limited its application. MM1.S and MM1.R cells were established as a good model to explore mechanisms of resistance to dexamethasone [25]. We utilized this pair of cell lines not only to confirm their clearly distinct sensitivities to dexamethasone, but also to clarify the reasons why dexamethasone acts so differently in these two cell lines. Our data indicate that dexamethasone fails to induce cell cycle G1 arrest and activation of caspase-dependent apoptosis signaling in MM1.R cells, which leads to dexamethasone resistance. It has been reported that dexamethasone-induced apoptosis is associated with a decrease in the activities of MAPK and $p70^{S6K}$ and the induction of PARP cleavage and caspase activation in the sensitive MM cells. In contrast, these effects can be blocked by interleukin-6 (IL-6) via inhibiting MAPK and $p70^{S6K}$ kinases and PARP cleavage, or triggering activation of PI3-K/Akt signaling [31, 32]. In addition, activated Stat3 signaling induced by IL-6 confers resistance to apoptosis in human U266 myeloma cells [33]. Therefore, it will be very interesting to study if IL-6 mediated survival signaling contributes to the resistance to dexamethasone in MM1.R cells.

Like other purine nucleoside analogs, cladribine needs to be phosphorylated by deoxycytidine kinase (DCK) to convert to its active form [2]. Thus, the expression levels of DCK in MM cells may be critical for cladribine to become effective. Considering our previous findings that MM1.S cells are more sensitive to cladribine than the other cells [17], we are currently trying to figure out whether MM1.S and MM1.R cells have similar DCK activity, and whether or not this pair of cell lines express higher levels of DCK than other MM cell lines. Distinct from cladribine, bendamustine does not require any kinase to activate, and directly induces DNA double strand breaks. Furthermore, it has also been reported that bendamustine activates genes involved in DNA damage responses, apoptosis, inhibition of mitotic checkpoint, and induction of mitotic catastrophe [18]. Previous studies also demonstrated that expression of the glucocorticoid receptor (GR) is required for glucocorticoid response in hematologic malignancies. Thus, GR and its regulation are crucial factors of glucocorticoid effectiveness [34]. Compared to wild-type expression of GR, as observed in MM1.S cells, GR expression is reduced or absent in MM1.R cells [25]. Both cell lines have similar responses to either cladribine or bendamustine, which may indicate less involvement of GR. It is still worth exploring whether the expression levels or function of GR are altered upon treatment with cladribine or bendamustine, and whether either agent may re-sensitize MM1.R cells to the treatment of dexamethasone.

Three major signaling pathways play critical roles in MM growth. Jak pathway activates Stat3 transcription factors to regulate cell survival [35]. The MAPK pathway is involved in MM cell growth and proliferation [36]. In addition, the PI-3K/Akt pathway leads to NF-κB and FKHR activation and p53 degradation [37]. We have observed that cladribine treatment reduces phosphorylated Stat3 in U266, RPMI8226 and MM1.S cells, and addition of the Stat3 inhibitor enhances cladribine-induced apoptosis [17]. Thus, it is interesting to explore whether cladribine and bendamustine inhibit MM growth via Jak/Stat3, MAPK, or PI-3K/Akt pathways, and have potential to enhance the efficacy via specifically inhibiting the key proteins in these pathways. Besides, since most of MM patients are treated with multi-drug regimens, uncovering the therapeutic potential of cladribine and bendamustine in a combinational context may further extend their clinical application. While bendamustine and cladribine exhibit *in vitro* synergy in non-Hodgkin's lymphoma cells [20], we did not observe synergistic effects on inhibiting MM growth (data not shown), and their anti-MM activities when combined with conventional therapeutics remain to be elucidated.

In summary, cladribine and bendamustine exhibit similar anti-proliferative activities, and induce DNA damage response and caspase-dependent apoptosis in both dexamethasone-sensitive and -resistant MM cells. While cladribine has no effects on cell cycle progression, bendamustine potently arrested the cells at S phase. Our studies suggest that cladribine and bendamustine hold therapeutic potential against MM, and may be developed as novel therapeutics to treat MM patients, particularly those resistant to dexamethasone.

Competing interests

The authors declare no conflict of interests.

Acknowledgements

The authors are grateful to Dr. Stephen P Trusko of Cephalon, Inc. (Frazer, PA) for providing bendamustine. This work was supported in part by a Research Fund from Cephalon, Inc. (to CKL & BL). BC was sponsored by China Scholarship Council (2011006001).

Abbreviations

MM, multiple myeloma; HCL, hairy cell leukemia; CLL, chronic lymphocytic leukemia; DMSO, dimethyl sulfoxide; PARP, poly(ADP-ribose) polymerase; FBS, fetal bovine serum; ELISA, enzyme-linked immunosorbent assay; IC50, inhibitory concentration 50; MAPK, mitogen-activated protein kinase; PI-3K, phosphoinositide 3- kinase; IL-6, interleukin-6; JAK, c-Jun N-terminal kinase; STAT, signal transducers and activators of transcription; DCK, deoxycytidine kinase; GR, glucocorticoid receptor; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt.

Address correspondence to: Dr. Bolin Liu, Department of Pathology, Mail Stop-8104, School of

Medicine, University of Colorado Anschutz Medical Campus, 12801 E. 17th Ave., Aurora, CO 80045, USA. E-mail: bolin.liu@ucdenver.edu; Dr. Chunji Gao, Department of Hematology, Chinese PLA General Hospital, 28 Fuxing Road, Beijing 100853, China. E-mail: gaochunji@medmail.com.cn

References

[1] Jemal A, Siegel R, Xu J and Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.

[2] Liliemark J. The clinical pharmacokinetics of cladribine. Clin Pharmacokinet 1997; 32: 120-131.

[3] Seto S, Carrera CJ, Kubota M, Wasson DB and Carson DA. Mechanism of deoxyadenosine and 2-chlorodeoxyadenosine toxicity to nondividing human lymphocytes. J Clin Invest 1985; 75: 377-383.

[4] Hjertner O, Borset M and Waage A. Comparison of the effects of 2-chlorodeoxyadenosine and melphalan myeloma cell lines. Leuk Res 1996; 20: 155-160.

[5] Goodman GR, Burian C, Koziol JA and Saven A. Extended follow-up of patients with hairy cell leukemia after treatment with cladribine. J Clin Oncol 2003; 21: 891-896.

[6] Karlsson KA, Stromberg M, Jonsson V, Mulligan SP, Liliemark J and Juliusson G. Cladribine (CdA) gives longer response duration than fludarabine (F) and high-dose intermittent chlorambucil (Chl) as first-line treatment of symptomatic chronic lymphocytic leukemia (CLL). First results from the international randomized phase III trial. Blood 2007; 110: 194a-194a.

[7] Robak T, Jamroziak K, Gora-Tybor J, Stella-Holowiecka B, Konopka L, Ceglarek B, Warzocha K, Seferynska I, Piszcz J, Calbecka M, Kostyra A, Dwilewicz-Trojaczek J, Dmoszynska A, Zawilska K, Hellmann A, Zdunczyk A, Potoczek S, Piotrowska M, Lewandowski K and Blonski JZ. Comparison of cladribine plus cyclophosphamide with fludarabine plus cyclophosphamide as first-line therapy for chronic lymphocytic leukemia: a phase III randomized study by the Polish Adult Leukemia Group (PALG-CLL3 Study). J Clin Oncol 2010; 28: 1863-1869.

[8] Robak T, Blonski JZ, Wawrzyniak E, Gora-Tybor J, Palacz A, Dmoszynska A, Konopka L, Warzocha K and Jamroziak K. Activity of cladribine combined with cyclophosphamide in frontline therapy for chronic lymphocytic leukemia with 17p13.1/TP53 deletion: report from the Polish Adult Leukemia Group. Cancer 2009; 115: 94-100.

[9] Bertazzoni P, Rabascio C, Gigli F, Calabrese L, Radice D, Calleri A, Gregato G, Negri M, Liptrott SJ, Bassi S, Nassi L, Sammassimo S, Laszlo D, Preda L, Pruneri G, Orlando L and Martinelli G. Rituximab and subcutaneous cladribine in chronic lymphocytic leukemia for newly diagnosed and relapsed patients. Leuk Lymphoma 2010; 51: 1485-1493.

[10] Sigal DS, Miller HJ, Schram ED and Saven A. Beyond hairy cell: the activity of cladribine in other hematologic malignancies. Blood 2010; 116: 2884-2896.

[11] Holowiecki J, Grosicki S, Giebel S, Robak T, Kyrcz-Krzemien S, Kuliczkowski K, Skotnicki AB, Hellmann A, Sulek K, Dmoszynska A, Kloczko J, Jedrzejczak WW, Zdziarska B, Warzocha K, Zawilska K, Komarnicki M, Kielbinski M, Piatkowska-Jakubas B, Wierzbowska A, Wach M and Haus O. Cladribine, But Not Fludarabine, Added to Daunorubicin and Cytarabine During Induction Prolongs Survival of Patients With Acute Myeloid Leukemia: A Multicenter, Randomized Phase III Study. J Clin Oncol 2012; 30: 2441-2448.

[12] Dimopoulos MA, Kantarjian HM, Estey EH and Alexanian R. 2-Chlorodeoxyadenosine in the treatment of multiple myeloma. Blood 1992; 80: 1626.

[13] Niesvizky R, Siegel D and Michaeli J. 2-Chlorodeoxyadenosine for multiple myeloma. Blood 1993; 81: 868-869.

[14] Guchelaar HJ, Richel DJ and Schaafsma MR. Clinical and toxicological aspects of the antineoplastic drug cladribine: a review. Ann Hematol 1994; 69: 223-230.

[15] Nagourney RA, Evans SS, Messenger JC, Su YZ and Weisenthal LM. 2 chlorodeoxyadenosine activity and cross resistance patterns in primary cultures of human hematologic neoplasms. Br J Cancer 1993; 67: 10-14.

[16] Bagley RG, Roth S, Kurtzberg LS, Rouleau C, Yao M, Crawford J, Krumbholz R, Lovett D, Schmid S and Teicher BA. Bone marrow CFU-GM and human tumor xenograft efficacy of three antitumor nucleoside analogs. Int J Oncol 2009; 34: 1329-1340.

[17] Ma J, Wang S, Zhao M, Deng XS, Lee CK, Yu XD and Liu B. Therapeutic potential of cladribine in combination with STAT3 inhibitor against multiple myeloma. BMC Cancer 2011; 11: 255.

[18] Leoni LM, Bailey B, Reifert J, Bendall HH, Zeller RW, Corbeil J, Elliott G and Niemeyer CC. Bendamustine (Treanda) displays a distinct pattern of cytotoxicity and unique mechanistic features compared with other alkylating agents. Clin Cancer Res 2008; 14: 309-317.

[19] Kalaycio M. Bendamustine: a new look at an old drug. Cancer 2009; 115: 473-479.

[20] Chow KU, Boehrer S, Geduldig K, Krapohl A, Hoelzer D, Mitrou PS and Weidmann E. In vitro

induction of apoptosis of neoplastic cells in low-grade non-Hodgkin's lymphomas using combinations of established cytotoxic drugs with bendamustine. Haematologica 2001; 86: 485-493.

[21] Kanekal S, Crain B, Elliott G and Multani PS. SDX-105 (Treanda (TM)) enhances the tumor growth inhibitory effect of rituximab in Daudi lymphoma xenografts. Blood 2004; 104: 229b-229b.

[22] Damaj G, Malard F, Hulin C, Caillot D, Garidi R, Royer B, Marit G, Stoppa AM, Banos A, Morineau N, Moreau P, Fitoussi O and Tiab M. Efficacy of bendamustine in relapsed/refractory myeloma patients: results from the French compassionate use program. Leuk Lymphoma 2012; 53: 632-634.

[23] Ramasamy K, Hazel B, Mahmood S, Corderoy S and Schey S. Bendamustine in combination with thalidomide and dexamethasone is an effective therapy for myeloma patients with end stage renal disease. Br J Haematol 2011; 155: 632-634.

[24] Lentzsch S, O'Sullivan A, Kennedy RC, Abbas M, Dai L, Pregja SL, Burt S, Boyiadzis M, Roodman GD, Mapara MY, Agha M, Waas J, Shuai Y, Normolle D and Zonder JA. Combination of bendamustine, lenalidomide, and dexamethasone (BLD) in patients with relapsed or refractory multiple myeloma is feasible and highly effective: results of phase 1/2 open-label, dose escalation study. Blood 2012; 119: 4608-4613.

[25] Greenstein S, Krett NL, Kurosawa Y, Ma C, Chauhan D, Hideshima T, Anderson KC and Rosen ST. Characterization of the MM.1 human multiple myeloma (MM) cell lines: a model system to elucidate the characteristics, behavior, and signaling of steroid-sensitive and -resistant MM cells. Exp Hematol 2003; 31: 271-282.

[26] Lee CK, Wang S, Huang X, Ryder J and Liu B. HDAC inhibition synergistically enhances alkylator-induced DNA damage responses and apoptosis in multiple myeloma cells. Cancer Lett 2010; 296: 233-240.

[27] Korycka A, Lech-Maranda E and Robak T. Novel purine nucleoside analogues for hematological malignancies. Recent Pat Anticancer Drug Discov 2008; 3: 123-136.

[28] Robak T, Korycka A, Kasznicki M, Wrzesien-Kus A and Smolewski P. Purine nucleoside analogues for the treatment of hematological malignancies: pharmacology and clinical applications. Curr Cancer Drug Targets 2005; 5: 421-444.

[29] Chang JE and Kahl BS. Bendamustine for treatment of chronic lymphocytic leukemia. Expert Opinion on Pharmacotherapy 2012; 13: 1495-1505.

[30] Rasschaert M, Schrijvers D, Van den Brande J, Dyck J, Bosmans J, Merkle K and Vermorken JB. A phase I study of bendamustine hydrochloride administered once every 3 weeks in patients with solid tumors. Anti-Cancer Drugs 2007; 18: 587-595.

[31] Chauhan D, Pandey P, Ogata A, Teoh G, Treon S, Urashima M, Kharbanda S and Anderson KC. Dexamethasone induces apoptosis of multiple myeloma cells in a JNK/SAP kinase independent mechanism. Oncogene 1997; 15: 837-843.

[32] Hideshima T, Nakamura N, Chauhan D and Anderson KC. Biologic sequelae of interleukin-6 induced PI3-K/Akt signaling in multiple myeloma. Oncogene 2001; 20: 5991-6000.

[33] Catlett-Falcone R, Landowski TH, Oshiro MM, Turkson J, Levitzki A, Savino R, Ciliberto G, Moscinski L, Fernandez-Luna JL, Nunez G, Dalton WS and Jove R. Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. Immunity 1999; 10: 105-115.

[34] Frankfurt O and Rosen ST. Mechanisms of glucocorticoid-induced apoptosis in hematologic malignancies: updates. Curr Opin Oncol 2004; 16: 553-563.

[35] Monaghan KA, Khong T, Burns CJ and Spencer A. The novel JAK inhibitor CYT387 suppresses multiple signalling pathways, prevents proliferation and induces apoptosis in phenotypically diverse myeloma cells. Leukemia 2011; 25: 1891-1899.

[36] Mahindra A, Cirstea D and Raje N. Novel therapeutic targets for multiple myeloma. Future Oncol 2010; 6: 407-418.

[37] Younes H, Leleu X, Hatjiharissi E, Moreau AS, Hideshima T, Richardson P, Anderson KC and Ghobrial IM. Targeting the phosphatidylinositol 3-kinase pathway in multiple myeloma. Clin Cancer Res 2007; 13: 3771-3775.