Analysis of Aurora B kinase in non-Hodgkin lymphoma

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This study explored the levels of Aurora B, a key regulator of mitosis, in 71 lymph nodes and tumor specimens excised operatively from individuals with various types of non-Hodgkin lymphoma (NHLs). Immunohistochemical examination found that diffuse large B-cell lymphoma (10/21, 48%) and Burkitt lymphoma (BL) (6/7, 86%) cells highly (percentage of positive cells, >20%) expressed Aurora B in their nuclei. On the other hand, none of the low-grade B-cell lymphoma ($n = 20$), except for one case of follicular lymphoma, highly expressed this protein kinase, suggesting that levels of Aurora B correlated with histological grade in B-cell NHLs ($P < 0.01$). Exposure of BL/leukemia cells to AZD1152-HQPA *in vitro*, a selective inhibitor of Aurora B kinase, potently induced growth arrest and apoptosis in a caspase-dependent, as well as -independent manner. Moreover, AZD1152 synergistically enhanced the effects of vincristine (VCR) to induce growth arrest of these cells. Further experiments found that VCR increased levels of the p-Aurora B through the activation of c-Jun N-terminal kinase, which was blocked in the presence of AZD1152-HQPA.

The Aurora family of serine/threonine kinases has an important role in chromosome alignment, segregation and cytokinesis during mitosis. The Aurora family consists of three members: Aurora A, B and C, which share 67–76% amino acid sequence identity in their catalytic domains, and few similarities in their N-terminus.[1,2] Aurora A localizes on centrosomes and has a crucial role in each step of mitosis including bipolar spindle formation.[2] Aurora B is a chromosomal passenger protein and localizes at centromeres during prometaphase and subsequently relocates to midzone microtubules and midbodies during anaphase and telophase.[1,3] Aurora B has a role in chromosome alignment, kinetochore–microtubule biorientation, activation of the spindle assembly checkpoint and cytokinesis in association with phosphorylation of histone H3 on Ser 10.[1,3] Aurora C is specifically expressed in the testis and has a role in spermatogenesis.[4] Recent studies found that Aurora C also acted as a chromosomal passenger protein and might compensate for Aurora B function.[5]

Several studies found that Aurora A and B were overexpressed in a variety of solid tumors, including colon,[6,7] breast,[7–10] prostate,[11] pancreas,[12] thyroid,[13] and head and neck cancers.[14] We have shown recently that hematological malignant cells including those from acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), as well as chronic myeloid leukemia aberrantly expressed Aurora A and B kinases.[15] In addition, we have shown that ZM447439, a novel and selective inhibitor of Aurora A and B kinases, effectively induced growth arrest and apoptosis of a variety of types of leukemia cells.[15] Studies conducted by other groups noted that the phenotype of HeLa cells after their exposure to ZM447439 was similar to that induced by knock down of the Aurora B kinase gene.[16] We, therefore, hypothesized that Aurora B might be a more favorable molecular target for cancer therapy than Aurora A.

AZD1152 is a novel acetanilide-substituted pyrazole-aminoquinazoline prodrug that is converted rapidly to the active drug, AZD1152 hydroxy-QPA (AZD1152-HQPA), in human plasma.[17] AZD1152-HQPA is a specific inhibitor of the enzymatic activity of Aurora kinase, with selectivity for Aurora B (IC50 of 0.36 nM *versus* 1369 nM for Aurora B and A kinases, respectively); the inhibitor had less activity against a panel of more than 50-fold against other serine–threonine and tyrosine kinases including FLT3, JAK2 and Abl.[17,18] AZD1152 was active against a variety of solid tumors as well as hematological malignant cells.[18,19]

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c-Jun NH2-terminal kinase (JNK; also known as stress-activated protein kinase) is one of the mitogen-activated protein kinase superfamily.[20] JNK is known to be activated in response to certain growth factors, as well as environmental stresses such as ultraviolet radiation. Activated JNK mediates both apoptotic and prosurvival signal pathways. For example, 12-*O*-tetradecanoyl phorbol 13-acetate-induced phosphorylation of JNK, resulting in apoptosis of androgen-dependent human prostate cancer cells.[21] On the other hand, activation of JNK was required for interleukin-3-dependent proliferation of Ba/F3 murine pre-B cells.[22] In addition, JNK was shown to be required for survival and proliferation of B-lymphoma cells.[23]

This study analyzed the expression of Aurora B in various types of non-Hodgkin lymphoma (NHLs) by immunohistochemistry and found that this kinase was highly expressed in histologically aggressive types of lymphoma such as diffuse large B cell (DLBC) and Burkitt lymphoma (BL). Inhibition of Aurora B by AZD1152 caused apoptosis of these cells in parallel with activation of the caspase pathway. In addition, AZD1152 synergistically enhanced the ability of vincristine (VCR), a tubulin-depolymerizing agent, to induce apoptosis of BL cells. Moreover, we found that VCR induced activation of Aurora kinases through stimulation of JNK, which was blocked by AZD1152 in BL cells.

MATERIALS AND METHODS
Immunohistochemistry
A total of 71 paraffin-embedded malignant lymphoma samples were obtained by the Laboratory of Diagnostic Pathology of Kochi University Hospital during the period from 1992 to 2008. All specimens were obtained at the initial presentation of the patients. Patients' ages ranged from 23 to 95 years with a mean age of 65 years. Histology of malignant lymphoma was diagnosed according to the WHO classification by at least two experienced pathologists (TT and MF). This study included DLBC lymphoma (DLBCL, 21 cases), BL (7 cases), follicular lymphoma (FL, 13 cases), mantle cell lymphoma (MCL, 4 cases), mucosa-associated lymphoid tissue lymphoma (MALT, 3 cases), adult T-cell leukemia/lymphoma (11 cases), peripheral T-cell lymphoma (9 cases), and Hodgkin lymphoma (3 cases). In total, 3 (two DLBCL and one MCL) out of 48 patients with B-cell lymphoma were diagnosed before 2001, and were treated with CHOP (cyclophosphamide, adriamycin, VCR and prednisone). Rest of the patients were treated with retuximab in combination with anti-cancer agents. The immunohistochemical procedure was performed with a Ventana DISCOVERY autostainer system according to the protocol provided by the manufacturer (Ventana Japan, Osaka, Japan). The anti-Aurora B antibody (EP1009Y, Epitomics, Burlingame, CA, USA) was used. The tissue sections were counterstained with hematoxylin and semiquantitatively scored as weakly positive (+ ; >5 to 20%), moderately positive (+ + ; >20 to 80%) or markedly positive (+ + + ; >80%).

The study was approved by the Ethics Committee of Kochi University (approved #20-54), and complied with the Helsinki Declaration.

Cells
Burkitt lymphoma/leukemia cell lines, BALM14, −18 and −27 were kind gifts from Dr A Hrashima (Hayashibara Biochemical Laboratories, Okayama, Japan). Daudi and Ramos Burkitt lymphoma cell lines were obtained from RIKEN BRC Cell Bank (Tsukuba, Japan). Acute promyelocytic leukemia NB4 and AML MOLM-13 cell lines were a kind gift from Dr M Lanotte (St Louis Hospital, Paris, France) and Dr Y Matsuo (Fujisaki Cell Center, Hayashibara Biochemical Laboratories), respectively. Bone marrow mononuclear cells and peripheral blood lymphocytes were isolated from healthy volunteers after obtaining informed consent, as previously described.[24]

Reagents
AZD1152-HQPA was provided by AstraZeneca (Macclesfield, UK). AZD1152-HQPA was dissolved in 100% dimethyl sulfoxide (DMSO; Burdick & Jackson, Muskegon, MI, USA) to a stock concentration of 10 mM and stored at −80°C. The pan-caspase inhibitor z-Val-Ala-Asp (OMe)-Fluoromethylketone (z-VAD-FMK) and the JNK inhibitor SP600125 were purchased from BIOMOL International (Plymouth Meeting, PA, USA) and Calbiochem (San Diego, CA, USA), respectively, and were dissolved in 100% DMSO to a stock concentration of 20 mM.

3-(4,5–dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) Assays
Cells (5×10^5 per ml) were cultured with various concentrations of AZD1152-HQPA for 2 days in 96-well plates. After culture, MTT assay was performed as previously described.[25] All experiments were performed in triplicate and repeated at least thrice.

Measurement of p-Histone H3 at Ser10 and P-JNK by Flow Cytometry
The Alexa Fluor 488-conjugated anti-p-histone H3 (Ser10, Cell Signaling Technology, Beverly, MA, USA) and anti-p-JNK (SC-6254, Santa Cruz, Santa Cruz, CA, USA) antibodies were used to quantify the population of leukemia/lymphoma cells expressing the phosphorylated forms of histone H3 and JNK, respectively. These experiments were performed using flow cytometry.

Immunoblotting
Immunoblotting was performed as previously described.[26] The antibodies used were anti-PARP (Cell Signaling Technology), anti-caspase 3 (Cell Signaling Technology), anti-GAPDH (Abcam Japan, Tokyo, Japan), anti-Aurora B kinase (Cell Signaling Technology) and anti-p-Aurora A/B/C kinases (Cell Signaling Technology). Band intensities were measured by densitometry.

Small interfering RNA

Control siRNA and a siRNA against JNK were purchased from Sigma (St Louis, MO, USA). Control siRNA targets green fluorescence proteins.

Transfections

NB4 cells were transiently transfected with either control or JNK siRNA by Amaxa electroporator Nucleofector II (Wako Pure Chemical Industries, Osaka, Japan), using the Nucleofector Kit V (program X-001). The preliminary experiments using the green fluorescence protein-expressing vector found that efficacy of transfection with this program was approximately 70%, with approximately 80% cell viability, as measured by FACS and annexin V staining (data not shown). We used NB4 cells for these studies, because none of BL/leukemia cell lines were suitable for transfection (data not shown). We previously showed that NB4 cells expressed Aurora kinases and the inhibitor of these kinases effectively inhibited their proliferation.[15]

Data analysis

The χ^2-test was used to examine the relation between levels of Aurora B and clinical factors. The combination index (CI) for growth inhibition elicited by AZD1152-HPQA and VCR in leukemia cells was calculated using the median effect method of Chou and Talalay[27] (Calcusyn Software available from Biosoft, Cambridge, United Kingdom). CI values <1 indicate synergy, a $CI = 1$ shows an additive effect and a CI >1 shows antagonism between the two agents.

RESULTS

High-Grade B-cell Lymphoma Cells Aberrantly Expressed Aurora B

Representative results of immunohistochemistry exploring the expression of Aurora B in BL and FL were shown in Figure 1. DLBCL (10/21, 48%) and BL (6/7, 86%) samples highly expressed Aurora B in their nuclei (percentage of positive cells, $>20\%$) (Table 1). On the other hand, FL (12/13, 92%), MCL (1/4, 25%) and MALT (1/3, 33%) samples slightly expressed Aurora B (percentage of positive cells,
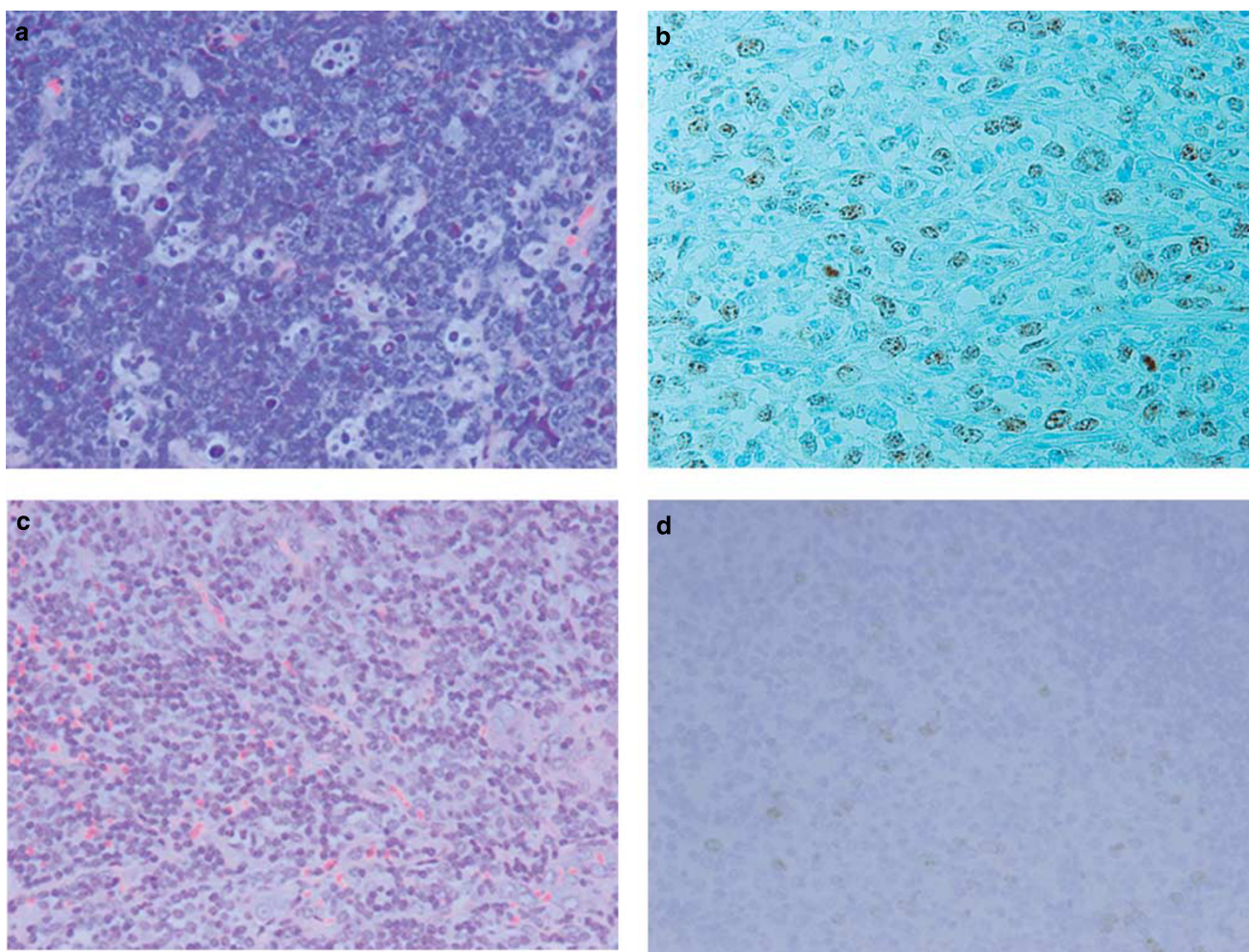
Figure 1 Representative histology and immunohistochemistry of lymph nodes from individuals with Burkitt lymphoma (BL) and follicular lymphoma (FL). Hematoxylin and eosin staining of BL (**a**) and FL (**c**). Lymph nodes from BL (**b**) and FL (**d**) were stained with anti-Aurora B antibody. Original magnifications were $\times 400$.

Table 1 Expression of Aurora B in malignant lymphomas

Levels of Aurora B	− No. (%)	+ No. (%)	++ No. (%)	+++ No. (%)	Total	
Age at diagnosis						$P = 0.018$
<65 years	9 (28)	17 (53)	6 (19)	0 (0)	32	
>65 years	11 (28)	12 (31)	11 (28)	5 (13)	39	
Gender						$P > 0.05$
Male	8 (24)	16 (48)	7 (21)	2 (6)	33	
Female	11 (29)	14 (37)	10 (26)	3 (8)	38	
Histology						$P < 0.01$
DLBCL	2 (10)	9 (43)	9 (43)	1 (5)	21	
BL	0 (0)	1 (14)	3 (43)	3 (43)	7	
FL	0 (0)	12 (92)	1 (8)	0 (0)	13	
MCL	3 (75)	1 (25)	0 (0)	0 (0)	4	
MALT	2 (67)	1 (33)	0 (0)	0 (0)	3	
HL	1 (33)	2 (67)	0 (0)	0 (0)	3	
ATL	8 (73)	0 (0)	3 (27)	0 (0)	11	
PTCL	3 (33)	5 (56)	0 (0)	1 (11)	9	
Clinical stage						$P = 0.024$
I/II	13 (38)	13 (38)	8 (24)	0 (0)	34	
III/IV	6 (16)	18 (49)	8 (22)	5 (14)	37	
IPI						$P < 0.01$
Low/low-int	16 (31)	25 (49)	10 (20)	0 (0)	51	
High/high-int	4 (20)	4 (20)	7 (35)	5 (25)	20	

ATL, adult T-cell lymphoma; BL, Burkitt lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HL, Hodgkin lymphoma; IPI, International Prognostic Index; int, intermediate; MCL, mantle cell lymphoma; MALT, mucosa-associated lymphoid tissue lymphoma; PTCL, peripheral T-cell lymphoma. Statistical significance of difference between age <65 years and >65 years, male and female, DLBCL+BL and FL+MCL+MALT, clinical stage I/II and III/IV, and IPI low/low-int and high/high-int was determined by χ^2-test.

5–20%) (Figure 1b) and none of them, except for one case of FL, highly expressed this protein kinase, suggesting that expression of Aurora B correlated with histological grade in B-cell NHLs ($P < 0.01$). In addition, levels of Aurora B correlated with advanced clinical stage ($P = 0.024$) and high International Prognostic Index (IPI) score ($P < 0.01$) (Table 1).

Inhibition of Aurora B by AZD1152-HQPA Induced Growth Arrest and Apoptosis of BL/Leukemia Cells

We examined whether Aurora B could be a molecular target for the treatment of NHLs using BL/leukemia cells. Ramos, Daudi, BALM-14, −18 and −27 BL/leukemia cells expressed Aurora B protein as measured by western blot analysis (Figure 2a). On the other hand, bone marrow mononuclear cells as well as peripheral blood lymphocytes from healthy volunteers did not express this kinase protein (Figure 2a). Ph-positive B-ALL PALL-2 and AML MOLM-13 cells were used as a positive control. Exposure of BL/leukemia cells to various concentrations of AZD1152-HQPA (12.5–100 nM) for 2 days effectively inhibited their proliferation with IC50s between 50 and 100 nM, as measured by MTT assay (Figure 2b). As expected, AZD1152-HQPA (12.5–100 nM) did not affect the viability of peripheral blood lymphocytes from healthy volunteers (Figure 2b).

Western blot analysis found that exposure of Ramos, BALM-14 and -27 cells to AZD1152-HQPA (12.5–100 nM) for 2 days increased levels of the cleaved forms of PARP and caspase 3, suggesting that AZD1152-HQPA induced apoptosis of these cells (Figure 2c). Cleavage of PARP in Daudi cells was not associated with activation of caspase 3 (Figure 2c). Other caspases such as caspase 7 or 2 could cleave PARP, leading to the apotosis of Daudi cells. Pre-incubation of Ramos and BALM-27 cells with the pan-caspase inhibitor Z-VAD-FMS (50 μM, 1 h) blocked AZD1152-HQPA-induced cleavage of PARP and caspase 3; and AZD1152-HQPA-mediated growth inhibition of these cells was blunted (Figure 2d). These observations suggested that AZD1152-HQPA-induced growth inhibition of BL/leukemia cells was, at least in part, caspase dependent.

AZD1152-HQPA Potentiated the Antiproliferative Activity of VCR Directed Against BALM-27 cells

We examined the ability of AZD1152-HQPA to enhance the antiproliferative effects of the conventional anti-lymphoma/leukemia agent, VCR. BALM-27 cells were cultured with various concentrations of AZD1152-HQPA (15–100 nM) and/or VCR (0.625–5 nM) for 48 h; and proliferation was measured by MTT assay (Figure 3a). AZD1152-HQPA (30 nM) or VCR (2.5 nM) alone inhibited MTT activity by approximately 25 and 30%, respectively (Figure 3a). When cells were exposed to both compounds in combination, proliferation was inhibited by approximately 80% (Figure 3a), resulting in a CI value of <1 (synergistic antiproliferative effect, figure not shown).

VCR Activated Aurora Kinases

We next attempted to verify the molecular mechanisms by which inhibition of Aurora B potentiated the effects of VCR in BALM-27 cells. Exposure of BALM-27 cells to various concentrations of VCR (1.25–5 nM, 24 h) induced phosphorylation of Aurora kinase and its substrate p-Histone H3 in a dose-dependent manner, which were effectively blocked in the presence of AZD1152-HQPA (25 nM), as measured by FACS (Figures 3b,c). Western blot analysis confirmed that VCR (1.25–5 nM, 24 h) stimulated the phosphorylation of Aurora kinases A and B in a dose-dependent manner, and
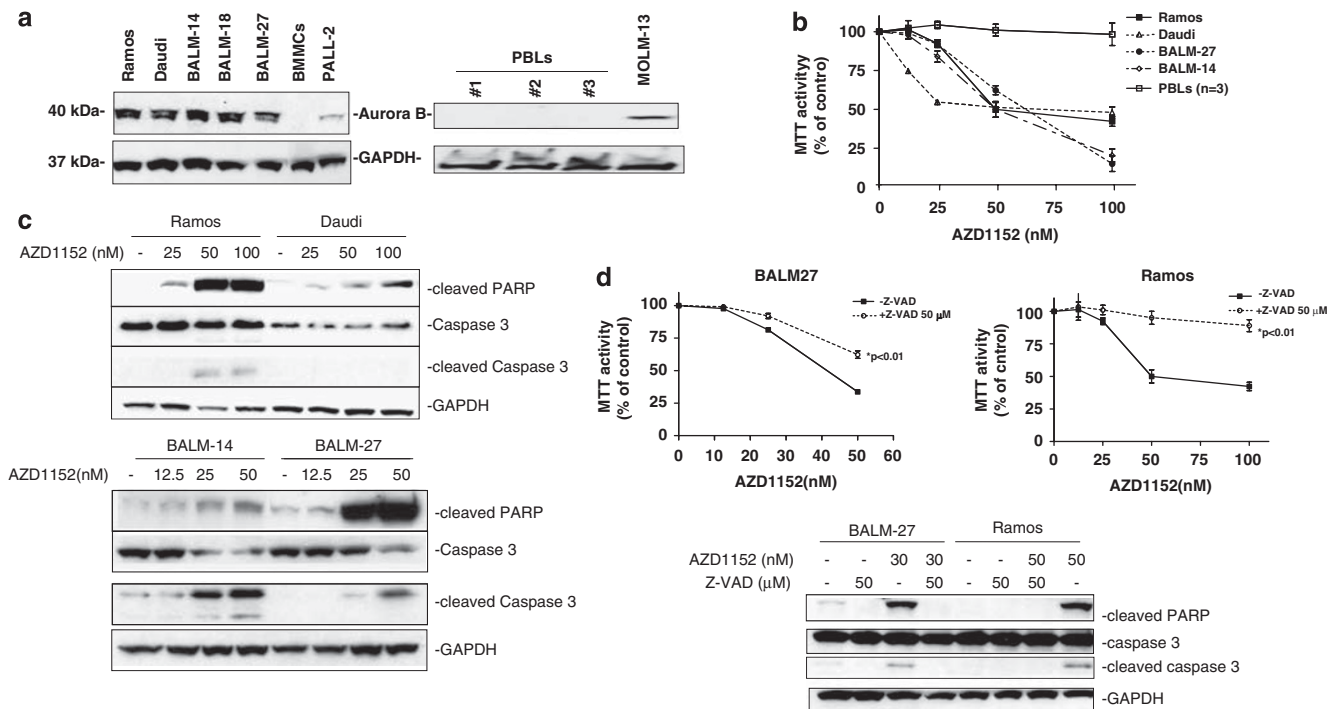
Figure 2 Burkitt lymphoma/leukemia cells express Aurora B. (**a**) Western blot analysis. Cell lysates from Burkitt lymphoma/leukemia cells and bone marrow mononuclear cells and peripheral blood lymphocytes from healthy volunteers were prepared and subjected to western blot analysis. The membrane was sequentially probed with anti-Aurora B and anti-GAPDH antibodies. PALL-2 and MOLM-13 cells were used as a positive control. (**b**) MTT assay. Burkitt lymphoma/leukemia and PBLs cells from healthy volunteers ($n = 3$) were exposed to various concentrations of AZD1152 (12.5–100 nM). After 48 h, cell viability was measured by MTT assay. Results represent the mean ± s.d. of three experiments performed in triplicate. (**c**) AZD1152-HQPA induces apoptosis of Burkitt lymphoma/leukemia cells. Western blot analysis. Cells were exposed to various concentrations of AZD1152 (12.5–100 nM). After 48 h, cells were harvested, and proteins were extracted and subjected to western blot analysis. The membranes were sequentially probed with antibodies to cleaved PARP, cleaved caspase 3, caspase 3 and GAPDH. Results represent one of three experiments performed independently. (**d**) Pre-incubation of BALM-27 and Ramos cells with Z-VAD-FMS rescues cells from AZD1152-mediated growth inhibition and apoptosis. BALM-27 and Ramos cells were pre-incubated with either Z-VAD-FMS (50 μM) (+ ZAD) or control diluent (−ZAD) for 1 h followed by the addition of AZD1152-HQPA (10–100 nM). After 48 h, cell viability was measured by MTT assay. At the same time, cell lysates were prepared and subjected to western blot analysis. The membranes were sequentially probed with anti-cleaved PARP, -cleaved caspase 3, -caspase 3 and GAPDH antibodies. BMMCs, bone marrow mononuclear cells; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PARP, poly (ADP-ribose) polymerase; PBMCs, peripheral blood mononuclear cells; PBLs, peripheral blood lymphocytes.
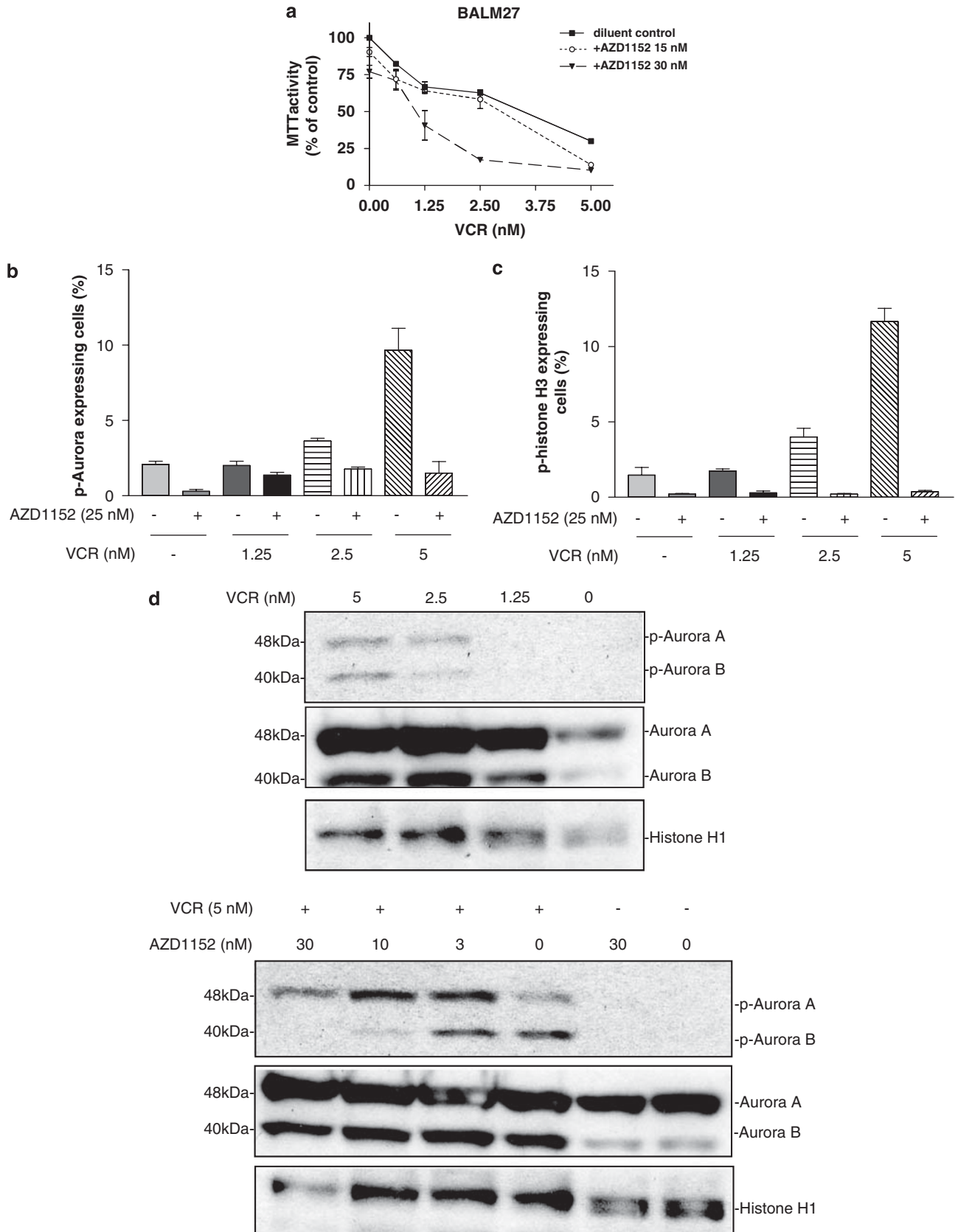
AZD1152-HQPA (25 nM) effectively blocked p-Aurora B but not Aurora A (Figure 3d).

JNK Mediated VCR-Stimulated Phosphorylation of Aurora Kinases

Further experiments explored the signal pathways, which mediated VCR-induced phosphorylation of Aurora kinases. JNK was constitutively activated in BALM-27 cells as judged by the detection of p-JNK by FACS (Figure 4a). Exposure of BALM-27 cells to VCR (5 nM, 1 h) further stimulated the phosphorylation of JNK, which was effectively inhibited by the specific JNK inhibitor SP600125 (5 μM) (Figure 4a). As expected, SP600125 (1.25–5 μM) downregulated VCR (5 nM, 24 h) induced expression of p-Aurora B in a dose-dependent manner (Figure 4b), suggesting that VCR induced p-Aurora B through JNK signaling. Surprisingly, SP600125 more profoundly blocked VCR-induced phosphorylation of Aurora A (Figure 4b).

Figure 3 AZD1152-HQPA potentiates effect of VCR. (**a**) MTT assay. BALM-27 cells were cultured with AZD1152-HQPA (15 or 30 nM) and/or vincristine (VCR) (0.625–5 nM). After 2 days, cell proliferation was measured by MTT assay. (**b**, **c**) VCR increases levels of p-Aurora A and B kinases and p-histone H3 (Ser10). FACS analysis. BALM-27 cells were exposed to VCR (1.25–5 nM) and/or AZD1152-HQPA (25 nM). After 3 h, p-Aurora A/B (**b**) and p-histone H3 (**c**) expressing populations were measured by FACScan. Results represent the mean ± s.d. of the three experiments performed in duplicate plate. (**d**) Western blot analysis. BALM-27 cells were exposed to VCR (1.25–5 nM) and/or AZD1152-HQPA (3–30 nM). After 3 h, cells were harvested, and proteins were extracted and subjected to western blot analysis. The membranes were sequentially probed with anti-p-Aurora A/B/C, anti-Aurora A and B, and histone H1 antibodies. Results represent one of the three experiments performed independently. FACS, fluorescence-activated cell sorting; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.
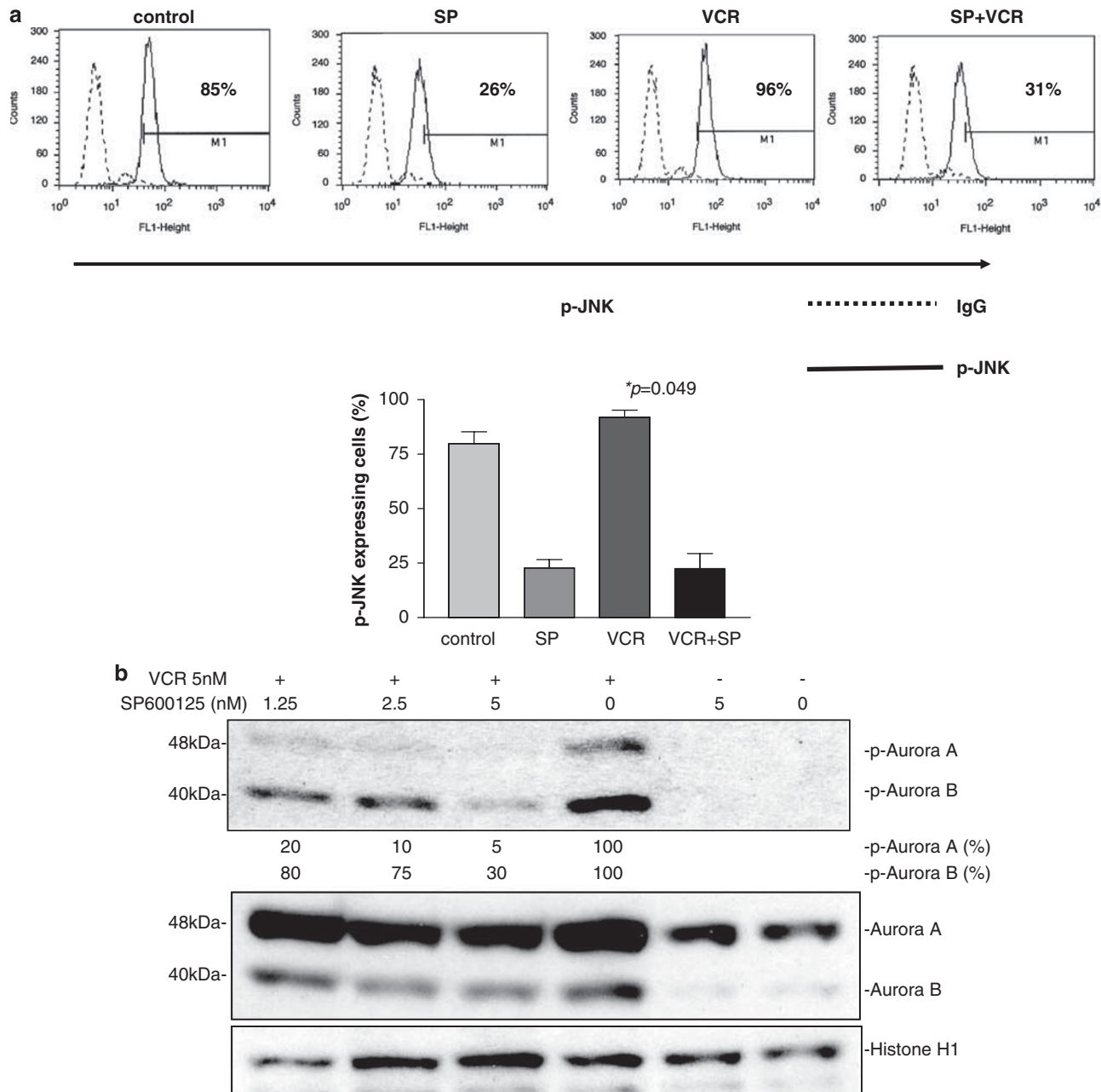
a BALM27

MTT activity (% of control) vs VCR (nM)

- ■ diluent control
- ○ +AZD1152 15 nM
- ▼ +AZD1152 30 nM

b

p-Aurora expressing cells (%)

| AZD1152 (25 nM) | - | + | - | + | - | + | - | + |
| VCR (nM) | - | | 1.25 | | 2.5 | | 5 | |

c

p-histone H3 expressing cells (%)

| AZD1152 (25 nM) | - | + | - | + | - | + | - | + |
| VCR (nM) | - | | 1.25 | | 2.5 | | 5 | |

d

VCR (nM) 5 2.5 1.25 0

48kDa- -p-Aurora A
40kDa- -p-Aurora B

48kDa- -Aurora A
40kDa- -Aurora B

-Histone H1

VCR (5 nM) + + + + - -
AZD1152 (nM) 30 10 3 0 30 0

48kDa- -p-Aurora A
40kDa- -p-Aurora B

48kDa- -Aurora A
40kDa- -Aurora B

-Histone H1

Figure 4 VCR activates JNK. (**a**) FACS analysis. BALM-27 cells were exposed to VCR (5 nM) and/or SP600125 (5 μM). After 3 h, p-JNK-expressing population was quantified by FACScan. The statistical significance of difference between control and VCR-treated cells was determined by Mann–Whitney *U*-test. The bar graph represents a mean ± s.d. of three experiments performed in duplicate plate. (**b**) Western blot analysis. BALM-27 cells were exposed to VCR (5 nM) and/or SP600125 (1.25–5 μM). After 3 h, cells were harvested, and proteins were extracted and subjected to western blot analysis. The membranes were sequentially probed with anti-p-Aurora A/B/C, anti-Aurora A and B, and histone H1 antibodies. The band intensity was measured by densitometry. Results represent one of the three experiments performed independently. JNK, Jun N-terminal Kinase; SP, SP600125; VCR, vincristine.

Downregulation of JNK by a siRNA Blunted VCR-Stimulated Activation of Aurora Kinase

Moreover, we repressed JNK by a siRNA in NB4 leukemia cells. VCR (0.03 nM, 1 h) stimulated activation of p-Aurora kinase was effectively inhibited in the JNK siRNA-transfected NB4 cells (Figure 5a). Notably, synergistic growth inhibition of NB4 cells mediated by combination of VCR and AZD1152

was significantly ($P < 0.01$) attenuated when JNK was down-regulated in these cells (Figure 5b).

Discussion

This study, for the first time, showed that Aurora B was aberrantly expressed in histologically high-grade NHLs such as DLBCLs and BLs (Figure 1, Table 1). In addition, we found
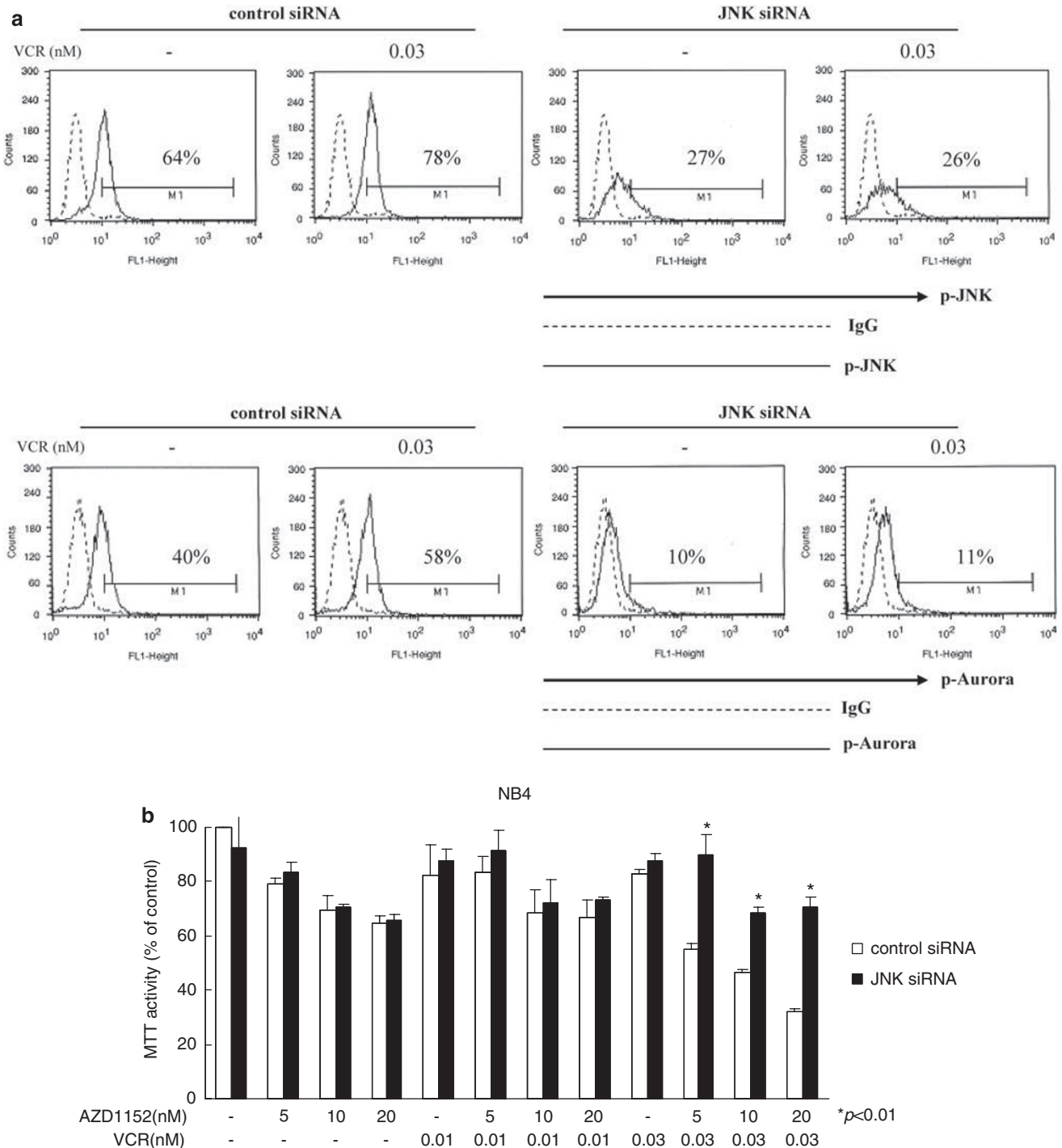
Figure 5 Downregulation of JNK by a siRNA blocked VCR-stimulated activation of Aurora kinase and attenuated synergistic growth inhibition mediated by combination of VCR and AZD1152. (**a**) FACS analysis. NB4 cells were transiently transfected with either control or JNK siRNA and then exposed to VCR (0.03 nM). After 3 h, p-JNK (upper panel) or p-Aurora kinase (lower panel) expressing population was quantified by FACScan. (**b**) MTT assay. NB4 cells were transiently transfected with either control or JNK siRNA, and then exposed to various concentrations of VCR and/or AZD1152. After 2 days, cell proliferation was measured by MTT assay. Results represent the mean ± s.d. of three experiments performed in triplicate. Statistical significance of difference between control and JNK siRNA-transfected cells was determined by Student's *t*-test. FACS, fluorescence-activated cell sorting; JNK, Jun N-terminal Kinase; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; VCR, vincristine.

that levels of Aurora B correlated with advanced clinical stage as well as high IPI scores (Table 1). Levels of Aurora B might be associated with a poor prognosis.

Inhibition of Aurora B kinase by the selective inhibitor AZD1152-HQPA produced profound growth arrest and apoptosis of BL/leukemia cells. The pan-caspase inhibitor

z-VAD-FMK partially restored the AZD1152-HQPA-mediated growth inhibition of BL cells (Figure 2d). These observations suggest that the AZD1152-HQPA-induced growth arrest of BL cells was mediated in both a caspase-dependent and -independent manner. Further studies are required to clarify the molecular mechanisms by which blockade of Aurora B kinase induces growth inhibition and apoptosis of BL cells.

This study found that AZD1152-HQPA potentiated the ability of VCR to arrest the growth of BL cells. This finding was consistent with our previous studies, showing that AZD1152-HQPA synergized with VCR to inhibit the growth of MOLM13 AML and PALL-2 Ph$^+$ ALL cells.[18] This study explored the molecular mechanisms, which produced synergistic growth inhibition of BALM-27 cells after exposure to combination of VCR and AZD1152. Exposure of these cells to VCR increased the levels of total, as well as the phosphorylated forms of Aurora B associated with the activation of JNK. Inhibition of JNK by the specific inhibitor SP600125 or the siRNA blocked VCR-stimulated phosphorylation of Aurora B, suggesting the involvement of JNK in VCR-induced activation of Aurora B kinase. Notably, when JNK was repressed by a siRNA, synergistic growth inhibition mediated by combination of VCR and AZD1152 was significantly attenuated (Figure 5). VCR-stimulated Aurora B through JNK signaling may have sensitized the cells to Aurora B kinase inhibition by AZD1152-HQPA. This may be the mechanism by which the combination of VCR and AZD1152-HQPA synergizes to inhibit the growth of BALM-27 cells.

Another group has shown Aurora A to be aberrantly expressed in high-grade lymphoma at the mRNA level.[28] In addition, these investigators found that the down-regulation of Aurora A by antisense oligonucleotides slowed the proliferation of lymphoma cells both *in vitro* and *in vivo*,[28] indicating that Aurora A may be a promising molecular target for treatment of NHLs.

Polo-like kinase 1 (PLK1) is also recognized as a mitotic kinase and has an important role in various events in mitosis, including chromosome alignment and segregation, and cytokinesis.[29] PLK1 was aberrantly expressed in DLBCLs in association with high levels of Ki-67, a marker of proliferation, and its levels were correlated with high IPI scores and poor prognosis.[30,31] We have recently found that BL/leukemia cells highly expressed PLK1 and inhibition of this kinae by a specific inhibitor or a siRNA induced growth arrest and apoptosis of these cells.[32] A variety of mitotic kinases may be disregulated in high-grade NHLs, and each of them can be a therapeutic target.

Both Aurora A and B kinases seem to be attractive molecular targets for the treatment of individuals with solid tumors as well as hematological malignancies. A number of small-molecule inhibitors with selectivity against Aurora A and/or Aurora B have been developed, including AZD1152 (a potent and selective inhibitor of Aurora B), MLN8054[33] (an orally available selective inhibitor of Aurora A), together with MK-0457,[34] and PHA-739358[35] (a pan-Aurora kinase inhibitor). These inhibitors are undergoing evaluation in clinical trials.

Taken together, our data indicate that Aurora B kinase may be a promising molecular target for individuals with high-grade lymphoma. Combined administration of AZD1152 and other chemotherapeutic agents such as VCR, should be further investigated in the clinical setting.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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