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Hematol Oncol (2011)
Published online in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/hon.981

Letter to the Editor

In vitro efficacy of tyrosine kinase inhibitors: SYK and BCR-ABL inhibitors in lymphomas

To the Editor

The B cell receptor (BCR) signalling pathway is believed to play a major role in B cell lymphoma growth and survival [1,2]. SYK is a critical non-receptor tyrosine kinase involved in the BCR signalling, that, when activated, leads to intracellular calcium mobilization, activation of AKT, mitogen-activated protein kinases and NFκB. A relatively strong expression of SYK can be demonstrated in the vast majority of non-Hodgkin lymphoma subtypes [3], and inhibition of SYK has been shown as a new promising therapeutic approach for lymphoid neoplasms [4–8]. Here, we report new *in vitro* data on the activity of different SYK inhibitors in lymphoma cell lines and lymphoma primary cells.

Based upon our previous data on mantle cell lymphoma (MCL) cell lines [4], we first treated six diffuse large B cell lymphomas (DLBCL) cell lines (Karpas 422, VAL, SUDHL-6, OCI Ly19, U2392 and DOHH2) with increasing doses of the SYK inhibitor piceatannol (3,4,3′,5′-tetrahydroxy-*trans*-stilbene; Sigma, Fluka Chemie GmbH, Buchs, Switzerland) for 72 h (Figure 1A). Cell growth

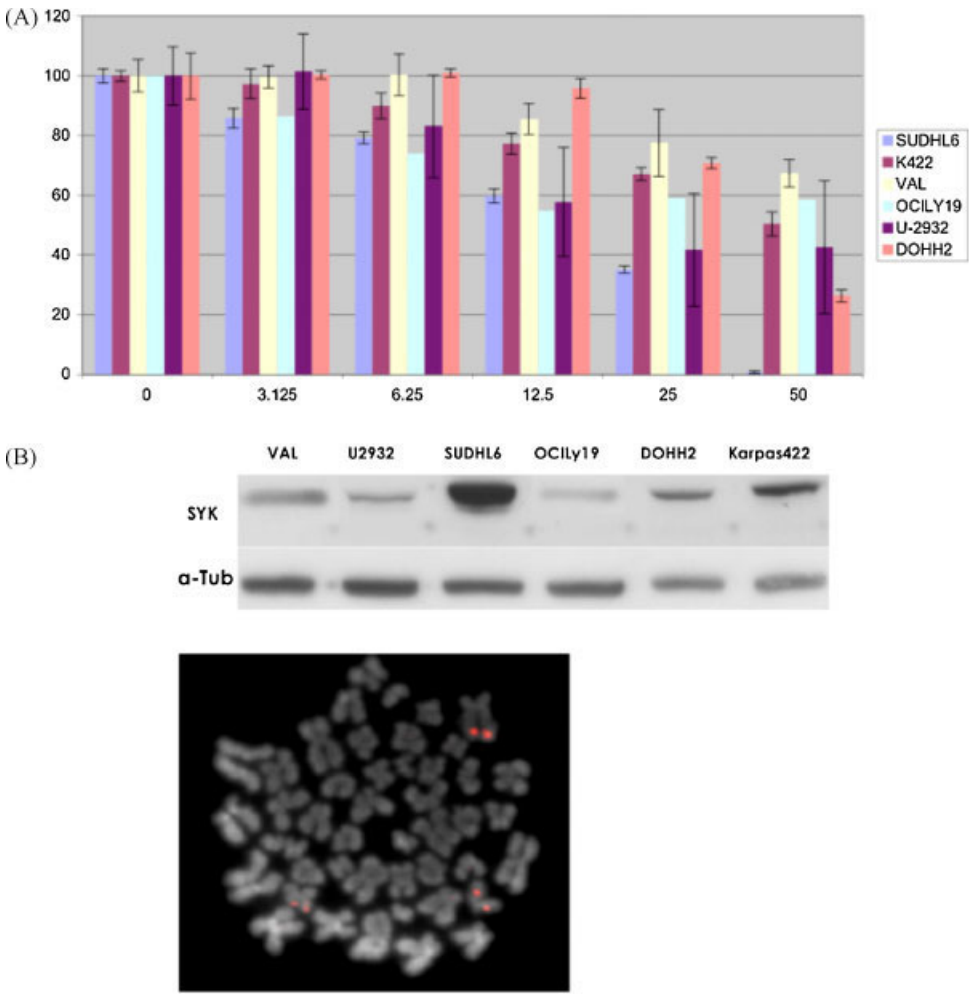

Figure 1. (A) Cytotoxic effect of piceatannol on DLBCL cell lines. Cells were exposed for 72 h to the indicated concentration of the drug. Y-axis, percentage of viable cells. X-axis, doses of piceatannol in μM. Error-bars represent the standard deviation calculated for at least 12 replicates derived from three different experiments. Viable cell number was determined by measuring 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide (MTT) dye absorbance as previously described [4,17]. (B) Basal levels of SYK in DLBCL cell lines and demonstration of the presence of an extra-copy of the gene by FISH. Upper panel: Immunoblotting showing SYK protein expression. Lower panel: FISH analysis using BAC targeting *SYK* region (red) metaphases of SUD-HL-6 DLBCL cell line. FISH was performed as previously described [4]

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inhibition was observed: the 50% inhibitory concentrations (IC_{50}) were 18 μM in SUDHL-6, 25 μM in U2392, 37 μM in DOHH2, 48 μM in Karpas 422 and higher than 50 μM in OCI-Ly19 and in VAL. SUDHL-6, which was the most sensitive and that showed a clear dose–response, was the cell line with the highest level of SYK at protein level and it presented an extra-copy of the locus containing *SYK*, as shown by fluorescence *in situ* hybridization (FISH) (Figure 1B). In SUDHL6, the cell growth inhibition was due to apoptosis induction, as shown by Western blotting with antibodies specific for the cleaved form of poly (ADP-ribose) polymerase (PARP) (Figure 2A). Treatment of the MCL cell line Jeko1 and of DLBCL cell line SUDHL-6 with the corresponding IC_{50} doses of piceatannol determined a reduction of the levels of total cellular phospho-tyrosine residues at 24, 48 and 72 h (Figure 2B), showing that indeed the activity of piceatannol might be mediated by a reduction in tyrosines phosphorylation, as described in chronic myeloid leukaemia (CML) after treatment with imatinib [9].

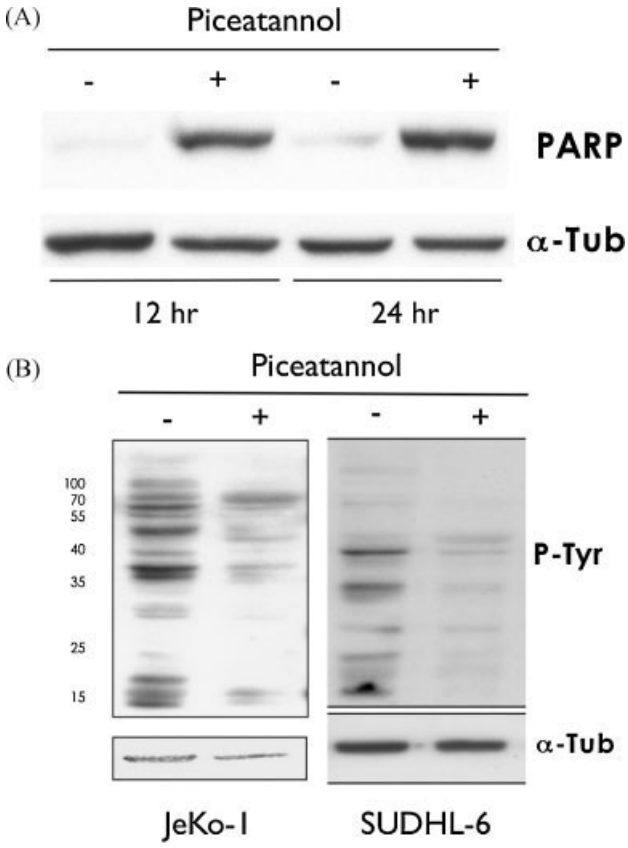

Figure 2. (A) Effect of piceatannol on the induction of apoptosis in SUDHL-6. Apoptosis was detected by immunoblotting with an antibody directed against the cleaved form of PARP in untreated control cells (-) and in cells treated (+) for 12 and 24 h with IC_{50} dose of piceatannol, as previously described [17]. Tubulin was used as a control for sample loading (lower panel). (B) Effect of piceatannol on total cellular level of phospho-tyrosine on MCL and DLBCL cell lines. Phospho-tyrosines were detected by immunoblotting with P-Tyr-100 antibody (Cell Signaling Technology, Danvers, MA, USA) directed against any phospho-Tyr residues in a manner largely independent of the surrounding amino acid sequence of the individual proteins. -, untreated control cells; +, cells treated for 48 h with IC_{50} dose of piceatannol. Tubulin was used as a control for sample loading

Three of the six DLBCL cell lines and the four MCL cell lines (NCEB-1, REC, Granta-519 and JeKo-1) [4] were then exposed to increasing doses of a potent SYK/ZAP7 inhibitor, NVP, for 72 h. Only the two cell lines expressing high levels of SYK, JeKo-1 and SUD-HL-6, showed an IC_{50} of 1 μM, a dose used to block mast cell degranulation without affecting cell viability [10], suggesting a SYK-mediated cytotoxic effect. The MCL cell line Granta-519 had a similar IC_{50}, but the inhibitory effect was not evident at higher doses, while Karpas 422 had an IC_{50} of 5 μM. VAL, Rec and NCEB1 were not sensitive to the compound at doses up to 25 μM.

Due to the suggested capacity of the BCR-ABL inhibitor imatinib to bind SYK [11], the MCL and DLBCL cell lines were exposed to increasing doses of the two BCR-ABL inhibitors imatinib and nilotinib already in the clinical setting. No cell line responded to doses of imatinib lower than 12.5 μM, thus beyond the concentration clinically achievable and used for CML and other sensitive neoplasms [12–15]. Still, JeKo-1 and SUD-HL-6 showed progressive inhibition of cell proliferation reaching the IC_{50} at a dose of 15–20 μM, indicating a possible dose-dependent cytotoxic effect in the two cell lines with constitutively high SYK levels.

To obtain further data on the relevance of SYK inhibition in lymphoma, we treated five lymphoma primary cells derived from DLBCL and three from leukaemic MALT lymphomas with increasing doses of NVP and piceatannol (0, 2, 5 and 10 μM) and with nilotinib at the clinically reachable [16] dose of 2 μM. A dose of 10 μM of piceatannol induced at least 50% reduction of cell proliferation in four DLBCL, but had no effect on the remaining primary cells. NVP induced more than 50% cytotoxicity in 2/3 MALT lymphoma samples at a dose of 2 μM, while DLBCL primary cells did not respond. Nilotinib induced more than 50% decrease of the viable cell number in 1/3 MALT lymphomas and in 1/5 DLBCL samples. These data obtained on lymphoma primary cells confirmed the cytotoxic activity of tyrosine kinase inhibitors in lymphoma primary cells, but the heterogenous pattern of response suggested that the effect was not necessarily mediated by SYK inhibition, but it could be due to inhibition of other molecules contributing to the constitutive BCR signalling.

In conclusion, our functional characterization of SYK in lymphoma cell lines and lymphoma primary cells support the design of prospective trials testing different molecules targeting SYK.

Acknowledgements

This work was supported by Oncosuisse grant KLS-01835-02-2006; Fondazione per la Ricerca e la Cura sui Linfomi (Lugano, Switzerland). The SYK inhibitor (NVP), imatinib mesylate (STI-571, Gleevec) and nilotinib (AMN107, Tasigna) were kindly provided by Novartis Pharma (Basel, Switzerland). We would like to thank Sara Pesenti and Anna Sasso for their technical assistance.

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DOI: 10.1002/hon

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DOI: 10.1002/hon