Effects of the aurora kinase inhibitors AZD1152-HQPA and ZM447439 on growth arrest and polyploidy in acute myeloid leukemia cell lines and primary blasts

Elisabeth Walsby,[1] Val Walsh,[1] Chris Pepper,[1] Alan Burnett,[1] and Ken Mills[1,2]

[1]Department of Haematology, School of Medicine, Cardiff University, Cardiff, South Glamorgan, UK, and [2]Center for Cancer Research and Cell Biology, Queens University Belfast, Lisburn Belfast, UK

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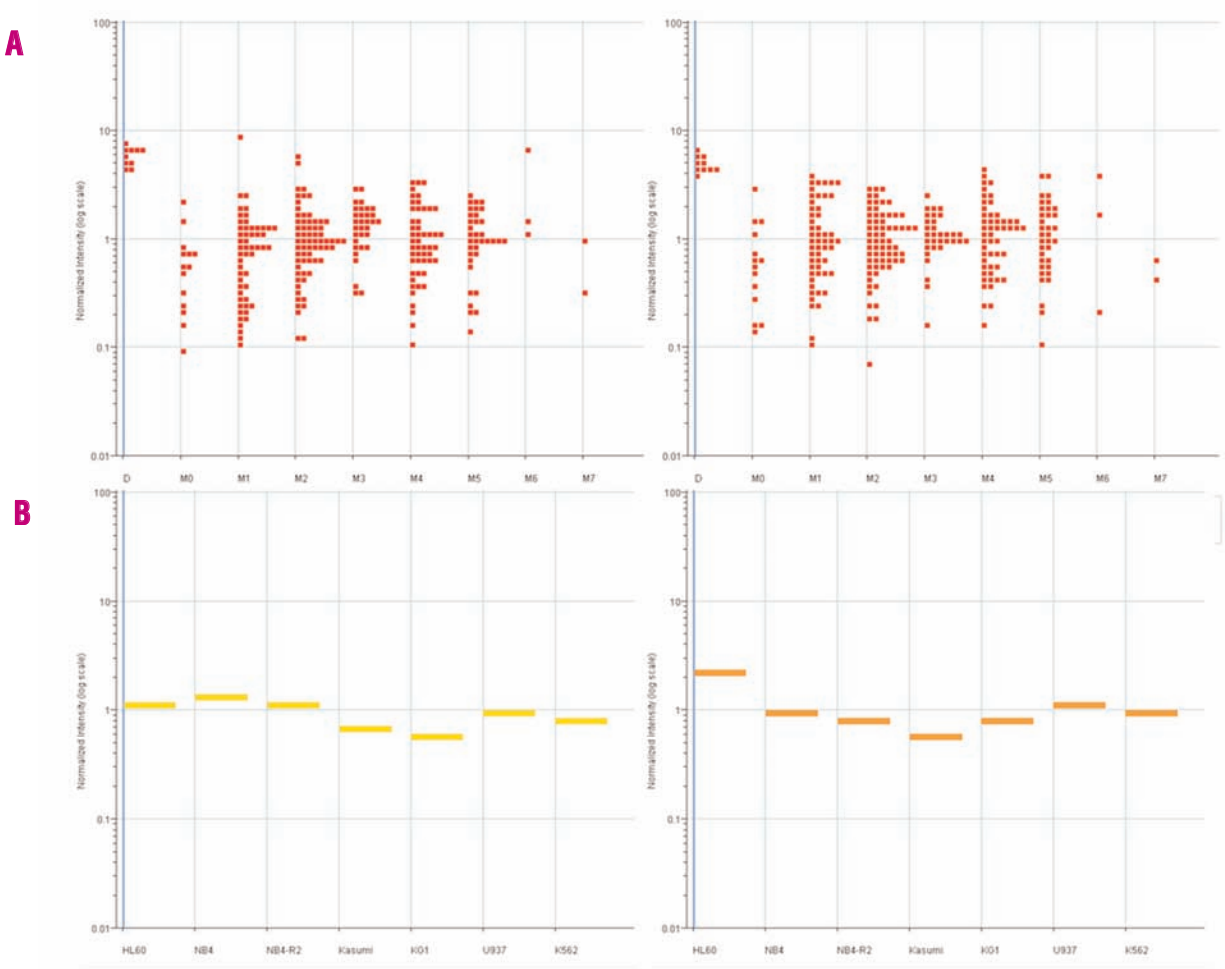
Supplementary Table S1. Cell cycle results for cell lines treated with AZD1152-HQPA and ZM447439 and analyzed using Cylchred.

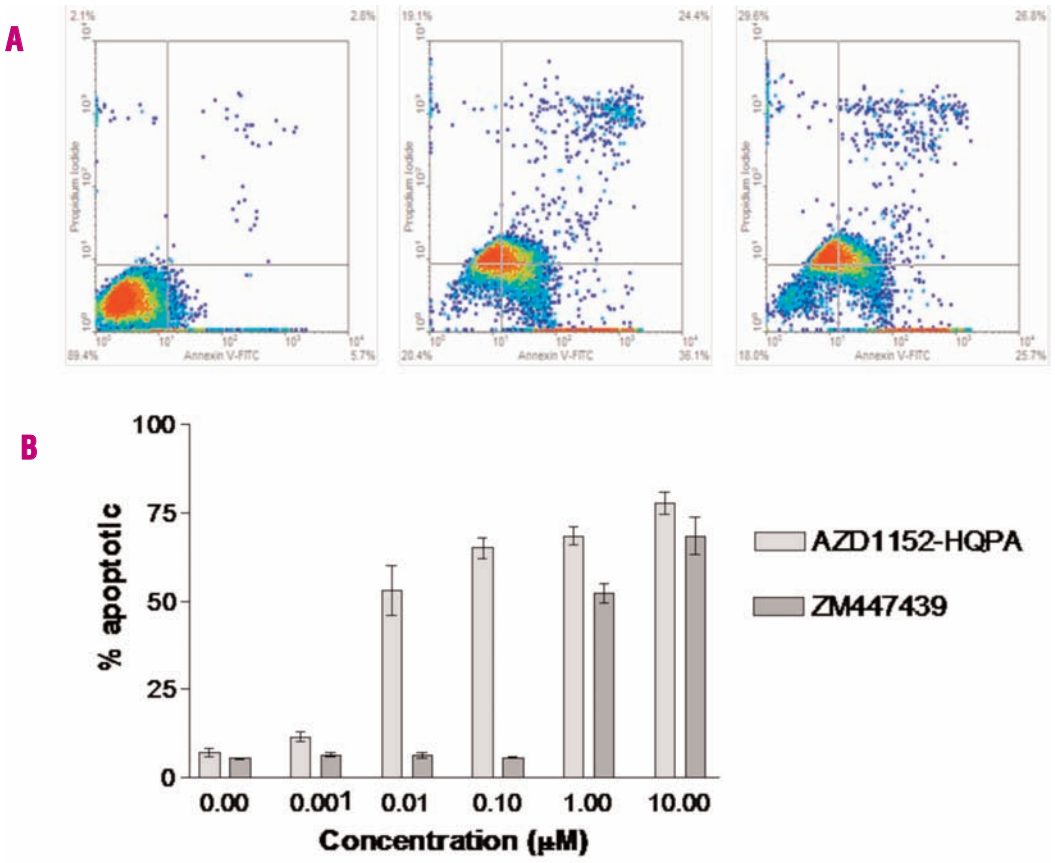
Region	*AZD1152-HQPA (μM)*	*%*	*p value*	*ZM447439 (μM)*		*p value*
2N	1.0	26.37	0.034	1.0	24.52	0.0008
	0.1	26.42	0.022	0.1	36.73	0.395
	0.01	27.16	0.043	0.01	32.96	0.278
	untreated	39.94	–	untreated	40.98	–
S	1.0	34.83	0.121	1.0	37.56	0.597
	0.1	30.47	0.009	0.1	41.36	0.470
	0.01	37.07	0.113	0.01	38.94	0.776
	untreated	44.01	–	untreated	39.99	–
4N	1.0	15.47	0.044	1.0	11.43	0.503
	0.1	17.93	0.012	0.1	8.34	0.514
	0.01	14.98	0.071	0.01	12.18	0.374
	untreated	7.55	–	untreated	10.11	–
>4N	1.0	17.86	0.001	1.0	18.18	0.003
	0.1	18.23	0.001	0.1	3.79	0.026
	0.01	10.69	0.007	0.01	5.65	0.066
	untreated	2.67	–	untreated	1.80	–

Supplementary Table S2. Cell cycle distribution results for primary AML samples treated with AZD1152-HQPA and ZM447439 for 48 hours and analyzed using Cylchred software.

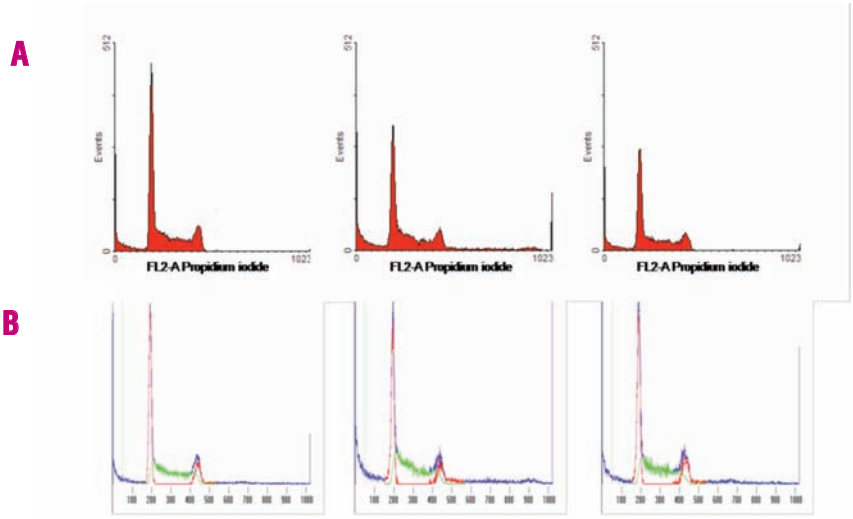
Region	*AZD1152-HQPA (μM)*	*%*	*p value*	*ZM447439 (μM)*	*%*	*p value*
2N	0.1	41.08	0.089	0.1	46.15	0.691
	0.01	52.40	0.039	0.01	49.80	0.010
	untreated	55.69	–	untreated	55.69	–
S	0.1	33.45	0.282	0.1	31.34	0.784
	0.01	28.51	0.033	0.01	30.74	0.012
	untreated	11.70	–	untreated	11.70	–
4N	0.1	15.22	0.447	0.1	13.93	0.868
	0.01	10.93	0.986	0.01	10.76	0.876
	untreated	6.00	–	untreated	6.00	–
>4N	0.1	3.55	0.308	0.1	3.33	0.288
	0.01	2.57	0.681	0.01	3.39	0.156
	untreated	2.06	–	untreated	2.06	–



Supplementary Figure S1. (A) Expression levels of AK-A and AK-B in donor (D) and AML samples sorted by FAB group and (B) Expression of AK-A and AK–B in AML cell lines. The expression levels shown were determined from analysis of normalized signal intensities from Affymetrix U133A gene chips.



Supplementary Figure S2. (A) Apoptosis induced in NB4 cells that were untreated (left) or treated with 0.1 µM AZD1152-HPQA (middle) or 0.1 µM ZM447439 (right) as assessed by annexin V and propidium iodide positivity measured by flow cytometry. (B) Concentration-dependent increases in apoptosis induction in NB4 cells by AZD1152-HPQA and ZM447439 after 48 hours of treatment.



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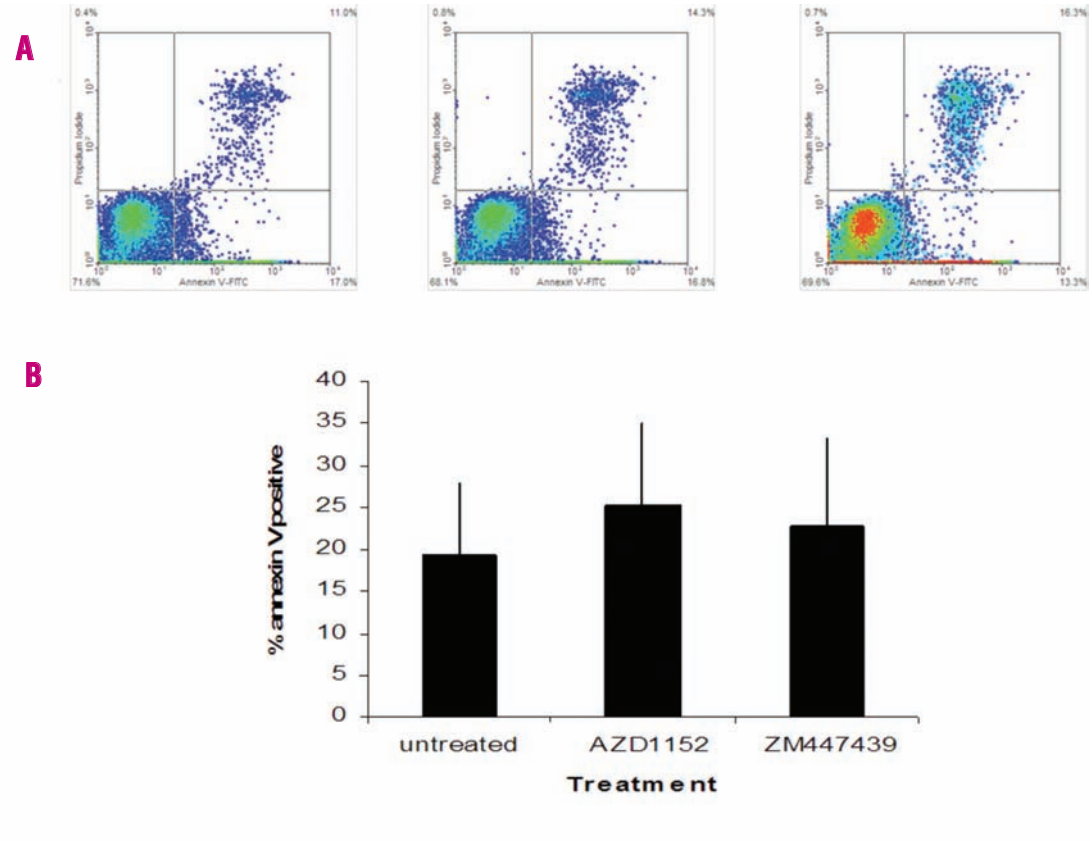
Untreated		AZD1152 0.01µM		ZM447439 0.01 µ M	
4N	>4N	4N	>4N	4N	>4N
10.3%	5.6%	10.3%	12.7%	12.8%	8.6%

Supplementary Figure S3. (A) Histograms showing the cell cycle measured by propidium iodide staining in untreated NB4 cells (left), in NB4 (left) cells treated with 0.01µM AZD1152 (center) and in NB4 cells treated with 0.01µM ZM447439 (right) following 48 hours of exposure to the compounds. In the cells treated with AZD1152 the emergence of polyploid cells is visible. (B) Cell cycle histograms produced using Cylchred software. The layout of the histograms is as described in part A. (C) Table showing the percentages of cells in the populations having 4N and >4N DNA contents. The effect of AZD1152-HPQA in particular is shown here, resulting in an increase from 5.6% to 12.7% of cells having >4N DNA contents.



Supplementary Figure S4. (A) Plots showing annexin V and propidium iodide staining in primary AML cells from one sample, (untreated cells (left), cells treated with 0.1 μM AZD1152 (centre) and cell treated with 0.1 μM ZM447439 (right) following 48 hours of exposure). Cells staining positive for both annexin V and propidium iodide (shown in the upper right section of the quadrant) were deemed to be apoptotic. An increase in apoptotic cells is visible for cells treated with both AZD1152 and ZM447439 is visible. (B) Bar chart showing the percentage of cells that stained annexin V positive. These results are the average of all those from the primary samples used and show the increase in apoptosis generated by exposure of these cells to AZD1152 and ZM447439 at 0.1 μM for 48 hours.