Mitochondrial Priming of New Targeted Agents in Acute Myeloid Leukemia

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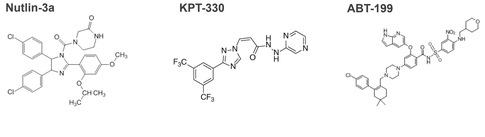
Abstract

As numerous molecularly targeted agents are entering clinical trials, predictive testing is highly desirable. We investigated if response to certain agents correlates with the recently reported method of BH3 profiling (Chonghaile TN. et al, Science, 2011), a functional assay developed by Letai's group that measures tumor cell mitochondrial priming by measuring mitochondrial outer membrane permeabilization (MOMP) following exposure to the peptide-mimicking BH3 domains of BH3-only proteins. Mitochondrial priming has been reported to be correlated with clinical responses to conventional chemotherapy in solid tumors and hematological malignancies (Vo TT, et al, Cell, 2012, Pierceall W et al, Mol Cancer Ther, 2013). Twenty-two AML lines were tested. Cells were permeabilized with digitonin and exposed to standardized doses of BH3 peptides (BIM, PUMA, NOXA, BAD, BMF, HRK, or PUMA2A). JC-1 was used for detection of MOMP and served as a measure of sensitivity to each peptide (reported as %[BH3 peptide]). Also, BCL-2, BCL-XL and MCL-1 expression levels were determined by Western blot. In addition to studies of untreated cells, treatment effects of different anti-leukemia drugs (AraC, Nutlin-3a, KPT-330 [Selinexor] and ABT-199) were determined over a wide dose range and denoted as ([specific apoptosis] = [(%Annexin V+ cells at each dose) - (%Annexin V+ cells at 0 μM)]/[100- (%Annexin V+ cells at 0 μM)]). Mixed linear models were used for analysis. As expected, ABT-199 sensitivity positively correlated with % [BAD]-%[HRK] (|b| = 3.22, p < 0.001), which is compatible with BCL-2 dependency of ABT-199 (while BAD is binding to BCL-2 and BCL-XL, HRK is binding to BCL-XL only and the difference is therefore BCL-2-specific). This was supported by the observed correlation between %[BAD]-% [HRK] and BCL-2 protein expression levels (r=0.619; p = 0.018). AraC sensitivity showed a similar correlation with %[BAD]-%[HRK] (|b| = 1.61, P < 0.05). Unexpectedly, Nutlin-3a activity did not correlate with any of the BH3 peptides. Results indicate that ABT-199, KPT-330 and Nutlin-3a show different modes of action in terms of BH3 peptide dependency, supporting potential combination effects of these agents. For ABT-199, increased MCL1 levels were associated with diminished cytotoxicity (r= 0.720; p < 0.005), as expected. For Ara-C, a similar correlation with MCL-1 was noted (r = 0.623; p < 0.05), but no correlations were observed for Nutlin-3a and KPT-330.

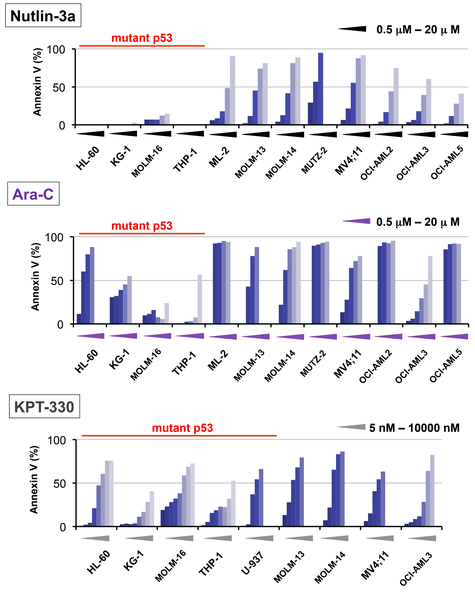


Methods

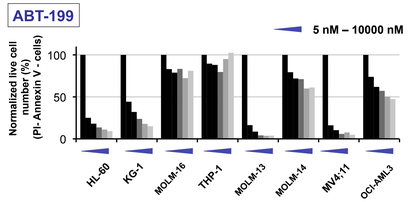
1. Reagents and cells: KPT-330 was synthesized at Karyopharm Therapeutics. (Natick, MA). MDM2 antagonist Nutlin-3a was purchased from Cayman Chemical Company (Ann Arbor, MI). BCL-2 inhibitor ABT-199 was purchased from Selleckchem. Total 22 AML cell lines, including OCI-AML3, MOLM-13 and MV4;11 cells were transduced with lentiviruses encoding either p53- or MCL-1- specific shRNA or scrambled shRNA, were used. BH3 profiling was conducted for all the lines. Independently, cells were treated with KPT-330, Nutlin3a, ABT-199 or Ara-C and apoptosis was assessed by Annexin V and PI staining.
2. Protein expression was assessed by Western blotting.
3. BH3 profilings: Cells were permeabilized with digitonin and exposed to BH3 peptides (BIM, PUMA, NOXA, BAD, BMF, HRK, or PUMA2A). JC-1 was used for detection of MOMP.
4. Statistical analysis: Student t test or Mann-Whitney (two-sided) results were considered significant for $P < 0.05$. For analysis of the association between BH3 profiling and the sensitivity to each agent, Mixed linear models were used.

Results

Induced apoptosis in each cell line by each agent

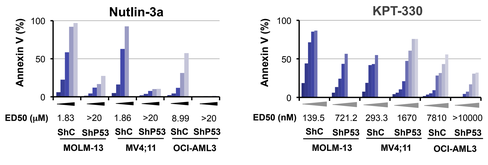


ABT-199

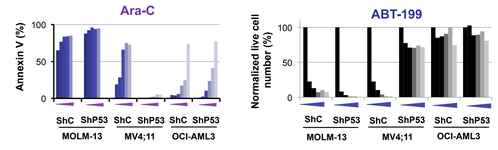


p53-dependency in induced apoptosis by each agent

Apoptosis induced by Nutlin-3a and KPT-330 (partially) is p53-dependent.

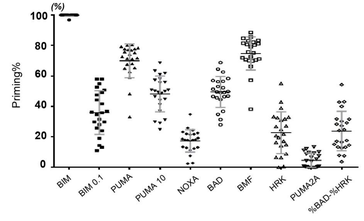


Apoptosis induced by Ara-C or ABT-199 is p53-independent.



BH3 profiling and correlation with drug sensitivity

BH3 profiling in 22 AML cell lines.



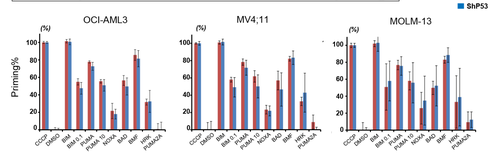
[%BAD - %HRK] can predict BCL-2 dependency (the sensitivity to ABT-199), and Ara-C-induced apoptosis also revealed BCL-2 dependency.

Table 1. Multivariate Mixed linear models

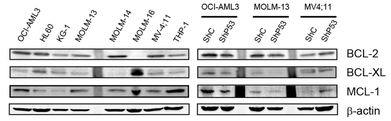
	Nutlin-3a		Ara-C		KPT-330		ABT-199*	
	β	p-value	β	p-value	β	p-value	β	p-value
%BIM 0.1	0.1	0.671	0.13	0.83	-0.04	0.921	-2.75	0.056
% PUMA 10	0.13	0.661	-0.34	0.642	0.78	0.088	0.38	0.734
% NOXA	0.34	0.555	0.95	0.506	1.04	0.124	2.39	0.249
% BAD	0.27	0.412	-0.26	0.756	0.08	0.875	-1.06	0.31
% BMF	0.34	0.199	-1	0.126	-0.54	0.453	1.98	0.271
% HRK	0.24	0.293	-1.05	0.056	0.49	0.22	0.76	0.366
% BAD - % HRK	-0.18	0.56	1.61	0.022	-0.88	0.109	3.22	<0.001

* normalized dead cell number

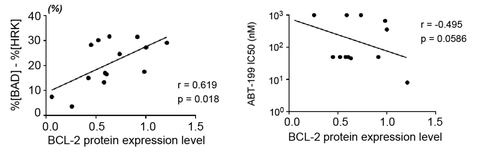
Baseline p53 levels do not affect mitochondrial priming.



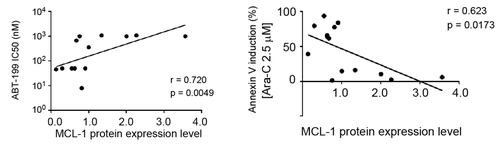
BCL-2 levels are predictive for mitochondrial priming but do not always correlate with cell death in AML lines



BCL-2 protein expression correlated with %[BAD] - %[HRK], but does not significantly correlate with cell death.

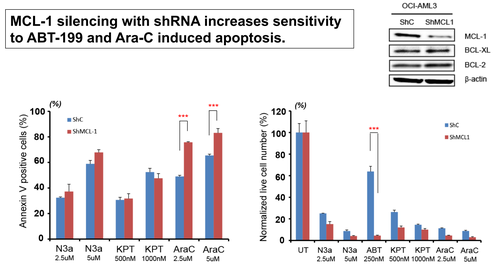


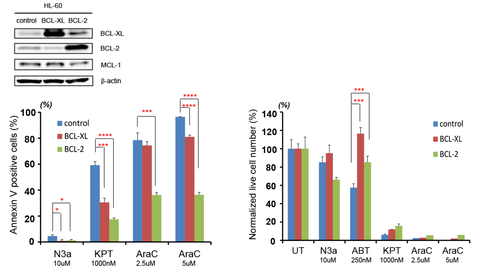
MCL-1 protein expression predicts resistance to ABT-199 and Ara-C.



Changes in BCL-2 family protein expression variably alter the senstivity to each agent.

MCL-1 silencing with shRNA increases sensitivity to ABT-199 and Ara-C induced apoptosis.





BCL-2 overexpression is associated with Ara-C resistance and BCL-2/XL with resistance to KPT-330.

BCL-XL overexpression is associated with ABT-199 resistance.

Conclusion

1. **BH3 profiling (%[Bad]-%[HRK]) is highly predictive for cell sensitivity to BCL-2 dependent apoptosis by ABT-199.**
2. **Ara-C-induced apoptosis unexpectedly revealed BCL-2 dependency, similar to ABT-199.**
3. **BH3 profiling does not predict for Nutlin-induced p53-mediated apoptosis or KPT-induced apoptosis.**
4. **BCL-2 protein levels correlate with BCL-2 - dependent mitochondrial priming.**
5. **BH3 profiling is superior to BCL-2 protein level in terms of predicting cell sensitivity to BCL-2 dependent apoptosis by ABT-199.**
6. **MCL-1 protein expression predicts resistance to ABT-199 and Ara-C.**
7. **BCL-2 overexpression is associated with Ara-C or KPT-330 resistance, and BCL-XL overexpression confers resistance to KPT-330 and ABT-199.**

BH3 profiling in conjunction with BCL-2 family proteins expression is a promising tool to predict the BH3 peptide dependency of chemotherapeutic agents.

Acknowledgements

This study was supported in part by grants from National Institutes of Health Lymphoma SPORE (CA136411), Leukemia SPORE (CA100632), P01 "The Therapy of AML" (CA55164), Cancer Center Support Grant (CA16672) and the Paul and Mary Haas Chair in Genetics (to MA).
It is also partially supported by Japan Society for the Promotion of Science, Postdoctoral Fellowship For Research Abroad (to JI).

Conflict of Interest

WP, MC, RL and CD are employees of Eutropics. SS and MK are employees of Karyopharm. has research support from Karyopharm and is a member of Eutropics.