Mitochondrial Priming: “Lowering” the Bar for Chemotherapeutic Success

Song Zhao MD PhD
December 14, 2012
Recent results have highlighted the importance of negative-feedback mechanisms that attenuate proliferative signaling. Disruptions of negative-feedback mechanisms that usually trigger growth factor receptors by ligand-mediated receptor transduction are being detected in an array of tumor types, which serve to hyperactivate the PI3-kinase isoforms.

Disruptions of negative-feedback mechanisms are capable of enhancing proliferative signaling. The functional impact of crosstalk between the multiple pathways radiating from growth factor receptors, for example, the one responding to the Ras signal transducer, serves to hyperactivate the PI3-kinase isoforms of signaling and thereby ensure homeostatic regulation of the feedback loops that normally operate to dampen various types of signaling powers; instead, the oncogenic mutations in the catalytic subunit of phosphoinositide 3-kinase (PI3-kinase) isoforms are being detected in an array of tumor types, which serve to hyperactivate the PI3-kinase isoforms.

Given that a number of distinct downstream signaling pathways radiate from a ligand-stimulated receptor, the activating mutations affecting the structure of the B-Raf protein, constitutive activation of signaling circuits facilitates ligand-independent firing.

Remarkably, the precise identities and sources of the proliferative signals operating within normal tissues were poorly understood a decade ago and in general remain so. Moreover, we still know relatively little about the mechanisms controlling the release of these mitogenic signals. In part, the understanding of these mechanisms is complicated by the fact that the growth properties, such as cell survival and energy metabolism, are thought to be transmitted in a temporally and spatially regulated fashion from one cell to its neighbors; such paracrine stimulation is difficult to access experimentally. In addition, the ability of cancer cells to acquire the capability to sustain proliferative signaling in a highly specific and localized fashion.

Conventional growth factor signaling is affected by elevating the bioavailability of growth factors, frequently by the actions of other enzymes that liberate and activate them, apparently in a highly specific and localized fashion. The pericellular space and extracellular matrix, and by the actions of a number of alternative ways: They may produce growth factor ligands themselves, to which they can respond via the expression of alternative receptors, typically containing intracellular tyrosine kinase activity. Cancer cells, by deregulating these signals, become masters of their own destinies. The enabling signals are transmitted in a temporally and spatially regulated fashion from one cell to its neighbors; such paracrine stimulation is difficult to access experimentally. In addition, the ability of cancer cells to acquire the capability to sustain proliferative signaling in a highly specific and localized fashion.

Growth factor independence may also result from structural alterations in the receptor molecules that facilitate ligand-independent firing. The outcome can result from structural alterations in the receptor molecules that facilitate ligand-independent firing. The consequence of growth factor independence is that the cell cycle as well as cell growth (that is, increases in cell number and thus maintenance of normal tissue architecture and number of alternative ways: They may produce growth factor ligands themselves, to which they can respond via the expression of alternative receptors, typically containing intracellular tyrosine kinase activity. Cancer cells, by deregulating these signals, become masters of their own destinies. The enabling signals are transmitted in a temporally and spatially regulated fashion from one cell to its neighbors; such paracrine stimulation is difficult to access experimentally. In addition, the ability of cancer cells to acquire the capability to sustain proliferative signaling in a highly specific and localized fashion.

High-throughput DNA sequencing analyses of cancer cell genomes have revealed somatic mutations in certain human genes that compromise Ras GTPase activity, which obviating the need to stimulate these components of signaling pathways operatively, such as cell survival and energy metabolism. The enabling signals are transmitted in a temporally and spatially regulated fashion from one cell to its neighbors; such paracrine stimulation is difficult to access experimentally. In addition, the ability of cancer cells to acquire the capability to sustain proliferative signaling in a highly specific and localized fashion.

The enabling signals are transmitted in a temporally and spatially regulated fashion from one cell to its neighbors; such paracrine stimulation is difficult to access experimentally. In addition, the ability of cancer cells to acquire the capability to sustain proliferative signaling in a highly specific and localized fashion.
Cell Death

Apoptosis:
- Shrinkage and chromatin condensation
- Budding
- Apoptotic bodies phagocytosed with no inflammation

Necrosis:
- Swelling
- Blebbing with disruption of cell membrane
- Release of proteolytic enzymes with important inflammatory reaction

Mitochondrion at Center of Cell Death

Activator BH3-only

Effector multidomain pro-apoptotic

Sensitizer BH3-only

Multidomain anti-apoptotic

Mitochondrion

Bax/Bak channel

Bcl-2

Cyt c release

ATP Depletion, ROS generation

Apoptosome formation

Mitochondrial dysfunction

Caspase Inhibitors

Caspase activation

NECROSISS

APOPTOSIS

Modified from Martin et al. Molecular Cell 46, 2012
Mitochondrial outer membrane permeabilization (MOMP) is the central event signifying irreversible commitment to cell death.
HYPOTHESES

- Chemosensitivity is determined by the proximity of a cell to the threshold of apoptosis.
- Some cancer cells are closer to the threshold than others, i.e. more *primed* to cell death than others.
- *Mitochondrial priming* can be used to predict response to chemotherapy and can be modulated to enhance chemosensitivity.
- Therapeutic index for chemotherapy is determined by differences in mitochondrial priming in cancer cells versus normal cells.
Proapoptotic BH3-only Peptides

<table>
<thead>
<tr>
<th></th>
<th>BID</th>
<th>BIM</th>
<th>BIDmut</th>
<th>BAD</th>
<th>BIK</th>
<th>NOXA A</th>
<th>NOXA B</th>
<th>HRK</th>
<th>BNIP</th>
<th>PUMA</th>
<th>BMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL-2</td>
<td>66 (6)</td>
<td>&lt;10</td>
<td>-</td>
<td>11 (3)</td>
<td>151 (2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>18 (1)</td>
<td>24 (1)</td>
</tr>
<tr>
<td>BCL-XL</td>
<td>12 (9)</td>
<td>&lt;10</td>
<td>-</td>
<td>&lt;10</td>
<td>10 (2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>92 (11)</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>BCL-w</td>
<td>&lt;10</td>
<td>38 (7)</td>
<td>-</td>
<td>60 (19)</td>
<td>17 (12)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25 (12)</td>
<td>11 (3)</td>
</tr>
<tr>
<td>MCL-1</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
<td>109 (33)</td>
<td>19 (2)</td>
<td>28 (3)</td>
<td>-</td>
<td>-</td>
<td>&lt;10</td>
<td>23 (2)</td>
</tr>
<tr>
<td>BFL-1</td>
<td>53 (3)</td>
<td>73 (3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>59 (11)</td>
<td>-</td>
</tr>
</tbody>
</table>

Certo et al. Cancer Cell 2006, 9

Martin et al. Molecular Cell 46, 2012
BH3 Profiling: Measuring Mitochondrial Priming

Tissue sample → Single cell Suspension → JC-1 stain & Permeabilize → Peptide treatment

Kinetic trace → BH3 profile → Heat map

Davids and Letai, JCO 2012, 30:25
Mitochondrial Priming Among Primary Human Cancers and Normal Tissues

Further, it may be that loss of cell-cycle checkpoint control governs some aspects of chemosensitivity. Once again, however, these principles are rarely examined in patient samples.

Most conventional chemotherapeutic agents kill via the mitochondrial pathway of apoptosis regulated by the BCL-2 family of proteins. Commitment to the mitochondrial pathway of apoptosis

**Fig 4.** BCL-2 homology 3 (BH3) profiling. (A) Single-cell suspensions derived from peripheral blood, bone marrow, lymph nodes, solid tumors, or normal tissue are permeabilized via digitonin and stained with JC-1. Permeabilized cells are then exposed to BH3-only peptides in a 384-well format, and the decay of /H9004/H9023m is measured as fluorescence at 590 nm, via the JC dye, by plate reader. Note that for heterogeneous clinical samples, this process can be performed with fluorescence activated cell sorting and a BH3 profile derived for a cellular subpopulation of interest. Data adapted.

(B) Comparison of mitochondrial priming among a variety of primary human cancers and normal tissues. Cancers with clinical follow-up were classified as known chemosensitive or known chemoresistant. Cancers classified as typically chemoresistant or typically chemosensitive lacked individual clinical follow-up data. Data shown are mean ± standard deviation across all specimens tested. Analysis of variance was used to demonstrate statistical significance between the different categories, with a Tukey’s multiple comparison post-test. NS, not significant (ie, \( P \geq 0.05 \)). (*) \( P \leq 0.01 \). (†) \( P \leq 0.001 \). Data adapted.

Davids and Letai, JCO 2012, 30:25
Relative Mitochondrial Priming of Myeloblasts and Normal HSCs Determines Chemotherapeutic Success in AML

Thanh-Trang Vo, Jeremy Ryan, Ruben Carrasco, Donna Neuberg, Derrick J. Rossi, Richard M. Stone, Daniel J. DeAngelo, Mark G. Frattini, and Anthony Letai

1Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA
2Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA 02215, USA
3Department of Stem Cell and Regenerative Biology, Harvard Medical School, Boston, MA 02115, USA
4Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY 10065
Mitochondrial Priming Predicts Response to Topoisomerase II Inhibitors

The increase in priming was associated with increased killing.

Depolarization = MOMP

Annexin-PI- = viable cells
We combined a patient AML sample with normal leukocytes to identify cells of interest by FACS analysis (blasts. For this reason we adapted our BH3 profiling method to a minority of nucleated cells in the bone marrow are AML myeloid patient blood and bone marrow samples. Often, only

**Figure 2.** Specifically Increasing Mitochondrial Priming Increases Sensitivity to Chemotherapy

**A.** BCL-2 protein levels after lentiviral introduction of shRNAs into the MOLM13 cell line. (A) BCL-2 protein levels after lentiviral introduction of shRNAs into the MOLM13 cell line.

**B.** JC-1 dye was used to detect mitochondrial charge loss. B. JC-1 dye was used to detect mitochondrial charge loss.

**C.** Daunorubicin. C. Most patient

**D.** Mitoxantrone. E. Mitoxantrone.

**E.** Mitoxantrone. E. Mitoxantrone.

Depolarization = MOMP     Annexin-PI- = viable cells     Vo et al. Cell 2012, 151(2)
BCL-2 antagonist ABT-737 Increases Mitochondrial Priming & Sensitivity to Chemotherapy

We combined a patient AML sample with normal leukocytes to a minority of nucleated cells in the bone marrow are AML myelogenous patient blood and bone marrow samples. Often, only method of BH3 profiling normal and malignant cells in heterogeneous in the clinical setting. We first needed to develop a reliable

We next wanted to test whether priming was similarly determin-

Figure 2. Specifically Increasing Mitochondrial Priming Increases Sensitivity to Chemotherapy

(B) Priming as measured by depolarization induced by BIM BH3 at two concentrations.

(A) BCL-2 protein levels after lentiviral introduction of shRNAs into the MOLM13 cell line.

Figure 3: Increased priming by ABT-737 also increased these cell lines' sensitivity to etoposide, mitoxantrone, and daunorubicin. All data represent mean ± SD. Depolarization = MOMP. Annexin-PI- = viable cells.

(C–F and H) BCL-2 knockdown increased cellular sensitivity to (C) daunorubicin, (D) etoposide, and (E) mitoxantrone. Alternatively, priming was increased by

(G) THP-1

(D) Moreover, the digitonin used to permit peptide access

(H) OCI-AML3

(Vo et al. Cell 2012, 151(2))
Relative Priming of AML Determines Clinical Outcome

Bim % Response = Depolarization = MOMP

** **P < 0.005, *** **P < 0.0005
Relative Priming of AML Determines Clinical Outcome

Restricting our analysis to those patients from 4A for whom we had cytogenetic data, we segregated patients as favorable, intermediate (combining intermediate I and II), or poor risk according to this system. As expected, clinical outcome was best in the favorable group, worse in the intermediate, and worst in the poor risk group. However, even within each of these groups, there was a mixture of clinical responses: CR (cured), CR (relapse), and no CR. We asked whether BH3 profiling could better refine the prognostic information provided by the ELN criteria, even within these genetically defined groups. In each case it could. In the favorable group, BH3 profiling could distinguish between CR (cured) and CR (relapse) (Figure 4D). In the intermediate group, BH3 profiling could distinguish between those destined for cure, relapse, or no CR (Figure 4E). Finally, BH3 profiling could distinguish between those destined to relapse and those who would be refractory to initial therapy in the poor category (Figure 4F).

Cytogenetic abnormalities have long been demonstrated to provide prognostic information about both short- and long-term clinical outcomes in AML (Mroz et al., 2004). However, the important physiological effects of major chromosomal alterations that confer altered prognosis and chemotherapy response are obscure. We hypothesize that poor-risk cytogenetic abnormalities are related to low mitochondrial priming, resulting in relative chemoresistance. Monosomy 7 is the one cytogenetic abnormality present in sufficient abundance in our sample for us to test this hypothesis. We therefore examined the mitochondrial priming of myeloblasts containing monosomy 7 and found that they were significantly less primed than responsive AML samples lacking monosomy 7 (Figure S3B). Low mitochondrial priming may provide for the first time a physiological mechanism to connect this poor-risk cytogenetic finding to poor chemotherapy response in AML.

Priming of Myeloblast Relative to Hematopoietic Stem Cell Determines Therapeutic Index

If poorly primed AML is less sensitive to chemotherapy, what is limiting oncologists from just giving higher doses of chemotherapy to the poorly primed population? In the clinic, drug
Primed mice received a single dose of 5-fluorouracil (5-FU) or 6-thioguanine (6-TG). We then measured the priming of normal human HSCs with the method for Long-Term Survival.

**Figure 5. Priming of AML Relative to HSC Priming Determines Clinical Outcome**

- A. Bim % Response = Depolarization = MOMP
- B. Kaplan-Meier survival curves based on pretreatment priming. Using the priming of normal HSCs as a cut-off, patients were categorized as high primed or low primed. **p < 0.005, ***p < 0.0005. See also Figure S4.

- We indeed found that the priming of HSCs represents the index of conventional induction chemotherapy in AML. The biological significance of this finding is that it suggests that apoptosis is irrelevant to the mechanism of graft-versus-host disease. Therefore, the priming of HSCs is an index of efficacy of induction therapy.

- The survival of the low-primed AML patients not receiving allogeneic transplant was not significantly different from that of the high-priming patients. Thus, although there are subpopulations that are of dominant importance in determining clinical outcome.

- We proceeded to test the hypothesis that the therapeutic index of induction therapy was dependent on the difference in priming between patients and HSCs. We noted that some AML patients that were cured despite presenting with very poorly primed myeloblasts.

- Poorly Primed AML Requires Allogeneic Transplantation

- The biological significance of this finding is that it suggests that given the otherwise poor prognosis of AML, killing of myeloblasts in vivo in humans. Furthermore, that apoptosis is irrelevant to the mechanism of graft-versus-host disease. Therefore, the priming of HSCs is an index of efficacy of induction therapy.

- The distribution of mitochondrial priming among population was the least primed, often much less primed than more mature populations. That for all of these cases, the least mature CD34+ CD38 subpopulation is thought to most commonly harbor AML stem cells. Although it would take a much greater sample to test this thoroughly, this raises the question of whether there are subpopulations that are of dominant importance in determining clinical outcome.
Most AML mitochondria from both refractory and sensitive patients are more sensitive to the BAD BH3 peptide. HSCs are selective for MCL-1, whereas most AML cells are selective for BCL-2. We found that the mitochondrial response to the BAD BH3 peptide correlated well with cellular killing by ABT-737, which antagonizes function of BCL-2, BCL-XL, and BCL-w.

When we compared the response of mitochondria of primary AML cells, whether sensitive or refractory, we found that mitochondrial killing correlated with mitochondrial response to ABT-737. We also found that the majority of AML cells were sensitive to the BAD BH3 peptide, whereas a subset of AML cells were sensitive to the NOXA BH3 peptide. We found that normal HSCs are more sensitive to the BAD BH3 peptide than AML myeloblasts. HSCs are selectively more dependent on MCL-1 for survival than are most AML cells. These results are congruent with the increased mitochondrial sensitivity to BAD BH3 in normal human hematopoietic progenitor cells.

In contrast, mitochondria from HSCs were significantly more sensitive to treatment with ABT-737 than were the majority of AML samples we tested. We found that the mitochondrial response to the HRK BH3 peptide correlated with cellular sensitivity to ABT-737. We also found that cellular response to ABT-737 correlated with mitochondrial response to the BAD BH3 peptide.

To further validate our findings, we compared the response of mitochondria of primary AML cells to the BH3 profiling results. We found that primary AML cells, whether sensitive or refractory, had a cellular dependence on the antiapoptotic protein with which they inhibit. Dysfunction induced by such peptides is therefore a measure of specific dependence on the antiapoptotic protein with which they inhibit. Priming measures based on the promiscuously interactive BIM BH3 peptide, other BH3 peptides used in the BH3 profile inhibit cellular killing by ABT-737.

Figure 7

**Certo et al., 2006**

**Oltersdorf et al., 2005**

Allogeneic Transplantation Benefits Patients with Low Priming

![Graph A](image1.png)

![Graph B](image2.png)

**Figure 7**

A). This suggests a specific dependence on BCL-2 or BCL-w for these cells' survival. Prior work has indicated that MOMP induced by the BAD BH3 peptide indicates an increased mitochondrial sensitivity to BAD BH3. This result indicates that cellular response to ABT-737 correlated with mitochondrial response to the BAD BH3 peptide.

B). As predicted, these results suggest that normal human hematopoietic progenitor cells are more sensitive to the BAD BH3 peptide than are AML myeloblasts. These results are congruent with the increased mitochondrial sensitivity to BAD BH3 in normal human hematopoietic progenitor cells.

Recall that all of the refractory AML patient cells were poorly primed. Because most of these refractory cells are sensitive to treatment with ABT-737, it is possible that they are sensitive to treatment with ABT-737. This illustrates a strength of the BH3 profiling tool, that it can provide important information about genetic dependencies and the apoptotic pathway without requiring the priming and postremission therapy. Low-primed patients who received a transplant had a better overall survival than no-transplanted, low-primed patients.

In contrast, mitochondria from HSCs were significantly more sensitive to treatment with ABT-737 than were the majority of AML samples we tested. We found that normal HSCs are more sensitive to the BAD BH3 peptide than AML myeloblasts. HSCs are selectively more dependent on MCL-1 for survival than are most AML cells. These results are congruent with the increased mitochondrial sensitivity to BAD BH3 in normal human hematopoietic progenitor cells.

![Graph A](image1.png)

![Graph B](image2.png)

**Figure 7**

A). This suggests a specific dependence on BCL-2 or BCL-w for these cells' survival. Prior work has indicated that MOMP induced by the BAD BH3 peptide indicates an increased mitochondrial sensitivity to BAD BH3. This result indicates that cellular response to ABT-737 correlated with mitochondrial response to the BAD BH3 peptide.

B). As predicted, these results suggest that normal human hematopoietic progenitor cells are more sensitive to the BAD BH3 peptide than are AML myeloblasts. These results are congruent with the increased mitochondrial sensitivity to BAD BH3 in normal human hematopoietic progenitor cells.

Recall that all of the refractory AML patient cells were poorly primed. Because most of these refractory cells are sensitive to treatment with ABT-737, it is possible that they are sensitive to treatment with ABT-737. This illustrates a strength of the BH3 profiling tool, that it can provide important information about genetic dependencies and the apoptotic pathway without requiring the priming and postremission therapy. Low-primed patients who received a transplant had a better overall survival than no-transplanted, low-primed patients.
Sensitivity of AML cells to BCL-2 Antagonizing Therapy

MCL-1 can promote resistance to ABT-737 (Konopleva et al., 2006; van Delft et al., 2006). Thus, BH3 profiling can detect resistance as well as sensitivity to ABT-737. In summary, these results suggest that there is a useful therapeutic index for BCL-2 inhibition between malignant myeloblasts and normal HSCs that can be exploited. Significantly, BCL-2 inhibition offers a therapeutic index even in those patients who are poorly primed and respond poorly to conventional chemotherapy. These results suggest a promising therapeutic intervention for AML patients with poor conventional options, and furthermore, BH3 profiling could provide a predictive biomarker of potential utility in guiding BCL-2 directed therapy.

Figure 7. BCL-2 Independence of HSCs Provides a Therapeutic Window for ABT-737

(A) BH3 profiling responses to BH3 peptides show BCL-2 dependency in all AML lines.

(B) Comparison of IC₅₀ killing by ABT-737 with BAD peptide response for each line after 24 hr of treatment. Correlation determined by a one-tailed Spearman correlation.

(C) Most AML cells from both sensitive and refractory patients are responsive to the BAD peptide, whereas HSCs are not.

(D) BAD peptide response of primary CD34⁺CD38⁻/CO AML population is more pronounced than HSC response.

(E) HSCs and most primary AML are not responsive to the HRK peptide.

(F) HSCs are responsive to the NOXA peptide but most primary AML are not.

(G) Primary AML cells are significantly more sensitive to 1µM ABT-737 than HSCs after 9 hr of treatment. Blue dots represent two low-primed AML refractory to standard induction.

(H and I) Greater ABT-737 sensitivity correlates with greater BAD BH3 peptide sensitivity (H) and also correlates with less NOXA BH3 peptide sensitivity (I).

*p < 0.05, **p < 0.005, ***p < 0.0005; viability = annexin V-/PI- population. Data represent mean ± SD. See also Figure S5.
Sensitivity of HSCs to MCL-1 Antagonizing Therapy

MCL-1 can promote resistance to ABT-737 (Konopleva et al., 2006; van Delft et al., 2006). Thus, BH3 profiling can detect resistance as well as sensitivity to ABT-737. In summary, these results suggest that there is a useful therapeutic index for BCL-2 inhibition between malignant myeloblasts and normal HSCs that can be exploited. Significantly, BCL-2 inhibition offers a therapeutic index even in those patients who are poorly primed and respond poorly to conventional chemotherapy. These results suggest a promising therapeutic intervention for AML patients with poor conventional options, and furthermore, BH3 profiling could provide a predictive biomarker of potential utility in guiding BCL-2 directed therapy.

---

**Figure 7. BCL-2 Independence of HSCs Provides a Therapeutic Window for ABT-737**

(A) BH3 profiling responses to BH3 peptides show BCL-2 dependency in all AML lines.

(B) Comparison of IC_{50} killing by ABT-737 with BAD peptide response for each line after 24 hr of treatment. Correlation determined by a one-tailed Spearman correlation.

(C) Most AML cells from both sensitive and refractory patients are responsive to the BAD peptide, whereas HSCs are not.

(D) BAD peptide response of primary CD34+ CD38/C0 AML population is more pronounced than HSC response.

(E) HSCs and most primary AML are not responsive to the HRK peptide.

(F) HSCs are responsive to the NOXA peptide but most primary AML are not.

(G) Primary AML cells are significantly more sensitive to 1 mM ABT-737 than HSCs after 9 hr of treatment. Blue dots represent two low-primed AML refractory to standard induction.

(H and I) Greater ABT-737 sensitivity correlates with greater BAD BH3 peptide sensitivity (H) and also correlates with less NOXA BH3 peptide sensitivity (I).

*p < 0.05, **p < 0.005, ***p < 0.0005; viability = annexin V-/PI- population. Data represent mean ± SD. See also Figure S5.
MCL-1 can promote resistance to ABT-737 (Konopleva et al., 2006; van Delft et al., 2006). Thus, BH3 profiling can detect resistance as well as sensitivity to ABT-737. In summary, these results suggest that there is a useful therapeutic index for BCL-2 inhibition between malignant myeloblasts and normal HSCs that can be exploited. Significantly, BCL-2 inhibition offers a therapeutic index even in those patients who are poorly primed and respond poorly to conventional chemotherapy. These results suggest a promising therapeutic intervention for AML patients with poor conventional options, and furthermore, BH3 profiling could provide a predictive biomarker of potential utility in guiding BCL-2 directed therapy.

**Figure 7. BCL-2 Independence of HSCs Provides a Therapeutic Window for ABT-737**

(A) BH3 profiling responses to BH3 peptides show BCL-2 dependency in all AML lines.

(B) Comparison of IC50 killing by ABT-737 with BAD peptide response for each line after 24 hr of treatment. Correlation determined by a one-tailed Spearman correlation.

(C) Most AML cells from both sensitive and refractory patients are responsive to the BAD peptide, whereas HSCs are not.

(D) BAD peptide response of primary CD34+CD38−/CD0 AML population is more pronounced than HSC response.

(E) HSCs and most primary AML are not responsive to the HRK peptide.

(F) HSCs are responsive to the NOXA peptide but most primary AML are not.

(G) Primary AML cells are significantly more sensitive to 1μM ABT-737 than HSCs after 9 hr of treatment. Blue dots represent two low-primed AML refractory to standard induction.

(H and I) Greater ABT-737 sensitivity correlates with greater BAD BH3 peptide sensitivity (H) and also correlates with less NOXA BH3 peptide sensitivity (I).

*p < 0.05, **p < 0.005, ***p < 0.0005; viability = annexin V−/PI− population. Data represent mean ± SD. See also Figure S5.**
Summary

http://dx.doi.org/10.1016/j.cell.2012.08.038