



Published in final edited form as:

Cell Stem Cell. 2013 March 7; 12(3): 269–270. doi:10.1016/j.stem.2013.02.006.

BCL-2 inhibition – stemming the tide of myeloid malignancies

Leah J. Hogdal and Anthony Letai

Department of Medical Oncology, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215

Abstract

Leukemia stem cells (LSC) are likely to be the source for both therapeutic resistance and relapse in myeloid malignancies. (Lagadinou et al., 2013) and (Goff et al., 2013) find that LSC can be selectively targeted by small molecule antagonists of anti-apoptotic BCL-2 family proteins.

Cancer stem cells possess the capacity to self-renew as well as generate the heterogeneous cancer cells that comprise a tumor. Although the theory of cancer stem cells was proposed nearly 50 years ago, prospectively identifying cancer stem cells remains a challenge, as does killing these quiescent, chemoresistant cancer stem cells. Myeloid cancers, particularly chronic myeloid leukemia (CML) and acute myeloid leukemia (AML) have been useful model systems in the establishment of cancer stem cells (Wang & Dick, 2005). In this week's *Cell Stem Cell*, Lagadinou et al., 2013 and Goff et al., 2013 find that small molecule inhibition of anti-apoptotic Bcl-2 family members selectively harms leukemia cells that possess stem cell properties.

Instead of using canonical cell surface markers to identify leukemia stem cells (LSCs), Lagadinou et al. apply a functional approach. They hypothesize that LSCs, being quiescent in nature, have lower metabolic activity and thus lower reactive oxygen species (ROS) production. The authors profile primary acute myeloid leukemia cells (AML) for their ROS levels and find that ROS-low cells not only exhibit less proliferation and a higher fraction of cells in G₀, but also exhibit an increased ability to engraft in immunodeficient mice compared to ROS-high cells. In order to identify a mechanism underlying this phenomenon, they perform RNA-seq on ROS-low and ROS-high cells and find that the anti-apoptotic BH3-only family member BCL-2 is up-regulated in ROS-low cells. This finding is validated by qRT-PCR and immunoblot analysis. Importantly, Lagadinou et al. demonstrate that inhibiting BCL-2 with the battle-tested BCL-2 family inhibitor ABT-263 (Roberts et al., 2012) selectively kills ROS-low cells *in vivo*. In addition, *ex vivo* treatment of AML cells with ABT-263 inhibits engraftment of AML cells into NSG mice.

The authors' exploitation of ROS measurements to functionally identify LSCs and BCL-2 dependent subsets is both novel and potentially of great utility. The perturbation of metabolism and oxygen consumption by BCL-2 inhibition may require further clarification, however. It is known that in sensitive cells, BCL-2 inhibition can cause mitochondrial outer membrane permeabilization (MOMP) very rapidly, even within an hour (Del Gaizo Moore et al., 2008; Vogler et al., 2008). This permeabilization by itself could cause significant perturbations in oxidative phosphorylation and oxygen consumption. Thus, it needs to be clarified whether the metabolic effects of BCL-2 inhibition observed by the authors occur before or after MOMP.

Similar to Lagadinou et al, Goff et al., find that inhibition of BCL-2 (and its family members) selectively target LSCs in primary human CML cells. Instead of taking a functional approach, the authors more traditionally define CML LSCs as CD34⁺CD⁺38⁻Lin⁻. They find that isolated human BC LSCs transplanted into RAG^{-/-}γ^{-/-} which engraft

into the bone marrow are more quiescent and chemoresistant compared to those that engraft at the tumor site, spleen or liver. Based on a fairly modest relative increase in expression of anti-apoptotic proteins like BCL-2 in blast crisis (BC) LSCs compared to normal or chronic phase (CP) CD34⁺CD⁺38Lin⁻ cells, the authors treat the cells with sabutoclax, developed as a pan-BCL-2 inhibitor. They observe that BC progenitor cells are more sensitive to sabutoclax compared to normal progenitor cells. Further, combination therapy of dasatinab and sabutoclax of CML cells serially transplanted into mice cause a modest increase in life span compared to untreated or single agent-treated animals (median survival increased from ~75 days to ~85 days).

To clarify that the observations are truly a leukemia stem cell based effect of direct inhibition of BCL-2 family proteins, a couple of issues may require future clarification. First, it is not completely clear that the mechanism of *in vivo* toxicity of sabutoclax is solely due to inhibition of BCL-2 family proteins. This is pertinent because gossypol, from which sabutoclax was derived, exhibits mechanisms of killing that are distinct from direct inhibition of BCL-2 family proteins (Van Delft et al., 2006). In this respect, it will be interesting to see whether at effective doses sabutoclax induces thrombocytopenia in mammals *in vivo*. While thrombocytopenia can be a problem clinically, it is a useful *in vivo* biomarker of BCL-XL inhibition, as circulating platelets have been found to be dependent on BCL-XL for survival (Mason et al., 2007). In addition, it is not clear whether these results from BC CML cells can be extended to stem cells from de novo AML. In the former case, the authors identified their stem cells as bearing a CD34⁺/CD38⁺ phenotype, while in the latter case, the CD34⁺/CD38⁻ population is more commonly thought to bear the stem cells (Wang & Dick, 2005).

An exciting, translational message that emerges from both papers is that inhibition of BCL-2 is a promising approach to the treatment of myeloid malignancies. These results are consistent with prior studies that found that bulk and stem-like AML cells are more dependent on BCL-2 than normal HSC, and are therefore more sensitive to BCL-2 inhibition by small molecules (Konopleva et al., 2006; Vo et al., 2012). This evidence comes at a particularly propitious time with the recent introduction of ABT-199 into clinical trials (Souers et al., 2013). As discussed briefly above, the dose-limiting toxicity of ABT-263 thrombocytopenia, caused by the inhibition of BCL-XL, is a particular problem in the treatment of AML patients that usually already suffer from thrombocytopenia. ABT-199, by selectively targeting BCL-2 but not BCL-XL, avoids this toxicity, likely making its use in AML better tolerated. With the identification of a therapeutic index and elimination of a major toxicity, it would seem that conditions are ripe for clinical testing of BCL-2 inhibition in myeloid cancers.

References

- Del Gaizo Moore V, Schlis KD, Sallan SE, Armstrong Sa, Letai A. BCL-2 dependence and ABT-737 sensitivity in acute lymphoblastic leukemia. *Blood*. 2008; 111:2300–2309. [PubMed: 18056841]
- Goff DJ, Recart AC, Sadarangani A, Chun HJ, Barrett CL, Krajewska M, Leu H, Low-Marchelli J, Ma W, Shih AY, et al. A Pan-BCL2 Inhibitor Renders Bone-Marrow-Resident Human Leukemia Stem Cells Sensitive to Tyrosine Kinase Inhibition. *Cell Stem Cell*. 2013
- Konopleva M, Contractor R, Tsao T, Samudio I, Ruvolo PP, Kitada S, Deng X, Zhai D, Shi YX, Sneed T, et al. Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. *Cancer Cell*. 2006; 10:375–388. [PubMed: 17097560]
- Lagadinou ED, Sach A, Callahan K, Rossi RM, Neering SJ, Minhajuddin M, Ashton JM, Pei S, Grose V, O'Dwyer KM, et al. BCL-2 Inhibition Targets Oxidative Phosphorylation and Selectively Eradicates Quiescent Human Leukemia Stem Cells. *Cell Stem Cell*. 2013

- Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Coretes J, Minden M, Paterson B, Caligiuri M, Dick J. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature*. 1994; 367:345–648.
- Mason KD, Carpinelli MR, Fletcher JI, Collinge JE, Hilton Aa, Ellis S, Kelly PN, et al. Programmed anuclear cell death delimits platelet life span. *Cell*. 2007; 128(6):1173–86. [PubMed: 17382885]
- Roberts AW, Seymour JF, Brown JR, Wierda WG, Kipps TJ, Khaw SL, Carney Da, He SZ, Huang DCS, Xiong H, et al. Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. *Journal of Clinical Oncology*. 2012; 30:488–496. [PubMed: 22184378]
- Souers AJ, Levenson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, Dayton BD, Ding H, Enschede SH, Fairbrother WJ, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nature Medicine*. 2013; 19:202–208.
- Van Delft MF, Wei AH, Mason KD, Vandenberg CJ, Chen L, Czabotar PE, Willis SN, Scott CL, Day CL, Cory S, et al. The BH3 mimetic ABT-737 targets selective Bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if Mcl-1 is neutralized. *Cancer Cell*. 2006; 10:389–399. [PubMed: 17097561]
- Vo TT, Ryan J, Carrasco R, Neuberg D, Rossi DJ, Stone RM, Deangelo DJ, Frattini MG, Letai A. Relative mitochondrial priming of myeloblasts and normal HSCs determines chemotherapeutic success in AML. *Cell*. 2012; 151:344–355. [PubMed: 23063124]
- Vogler M, Dinsdale D, Sun XM, Young KW, Butterworth M, Nicotera P, Dyer MJS, Cohen GM. A novel paradigm for rapid ABT-737-induced apoptosis involving outer mitochondrial membrane rupture in primary leukemia and lymphoma cells. *Cell Death and Differentiation*. 2008; 15:820–830. [PubMed: 18309326]
- Wang J, Dick J. Cancer Stem Cells: Lessons from Leukemia. *Trends in Cell Biology*. 2005; 15:594–501.