Commentary

A new approach to high risk pediatric acute lymphoblastic leukemia?

Diane Hanna[1,2], **Mary Ann Anderson**[1,3]

[1]Department of Cancer and Hematology, Walter and Eliza Hall Institute, Parkville, Australia; [2]Children's Cancer Centre, Royal Children's Hospital, Parkville, Australia; [3]Department of Clinical Hematology and Bone Marrow Transplantation, Royal Melbourne Hospital, Parkville, Australia

Correspondence to: Mary Ann Anderson. Department of Cancer and Hematology, Walter and Eliza Hall Institute, Parkville, Australia; Department of Clinical Hematology and Bone Marrow Transplantation, Royal Melbourne Hospital, Parkville, Australia. Email: manderson@wehi.edu.au.

Comment on: Khaw SL, Suryani S, Evans K, *et al*. Venetoclax responses of pediatric ALL xenografts reveal sensitivity of MLL-rearranged leukemia. Blood 2016;128:1382-95.

Submitted Nov 03, 2016. Accepted for publication Nov 17, 2016.
doi: 10.21037/tcr.2016.12.68
View this article at: http://dx.doi.org/10.21037/tcr.2016.12.68

Introduction

Advances in our understanding of acute lymphoblastic leukemia (ALL) disease biology and optimized chemotherapeutic regimens, as well as bone marrow transplantation, have resulted in pediatric ALL changing from a disease with an almost universally fatal outcome 50 years ago, to one in which the expectation is cure in 2016. Unfortunately, there remain a sub-group of children who still do poorly due to the refractory nature of the disease from identifiable high-risk genetic features or from relapsed disease. For these children, identifying novel therapeutic approaches is a priority.

BH3 mimetics, which trigger apoptotic death of cells, have shown promise both *in vitro* and *in vivo* in a number of human lymphoid malignancies (1). Like many targeted agents BH3 mimetics have a relatively favorable toxicity profile, without the majority of systemic side effects associated with conventional cytotoxic chemotherapy. Furthermore, acting via a truly novel mechanism BH3 mimetics also offer therapeutic hope for cancers that are predicted to respond poorly to conventional cytotoxic treatments. Thus, understanding the anti-leukemic activity of BH3 mimetics in poor prognosis pediatric ALL offers a potential avenue toward improving outcomes for this subgroup of patients.

Background

Failure of programed cell death or apoptosis has long been recognized as one of the key hallmarks of cancer (2) contributing both to the development of malignancy and chemotherapy resistance (3-8). There are two major signal cascades by which apoptotic death can occur: the extrinsic and intrinsic pathways. It is the intrinsic pathway of apoptosis that is most commonly dysregulated in human B cell malignancies (9). Central to the intrinsic pathway of apoptosis are the BCL2 family of proteins comprising of three subgroups: (I) pro-survival BCL2 proteins (including BCL2, $BCLx_L$, BCLw, MCL1 and A1); (II) pro-apoptotic mediators (BAX and BAK) and; (III) the pro-apoptotic BH3 only proteins (including BIM, BID, PUMA, BAD, and NOXA). In normal cells, BH3 only proteins are activated by cellular stress signals and function by binding to and inhibiting the pro-survival BCL2 proteins. This abrogates the capacity of the pro-survival BCL2 proteins to restrain the pro-apoptotic mediators BAX and BAK, allowing them to trigger mitochondrial outer membrane permeabilization (MOMP). MOMP culminates in the release of cytochrome C, which is an essential cofactor for caspase activation, and irreversible commitment to cell death ensues.

Selective binding between different BCL2 family proteins (10), as well as differential expression of various family members in diverse tissue compartments (11,12), allows for an exquisite control of cell survival at an individual cell level. This balance ultimately determines cellular life death decisions. For example, we know that BCL2 is critical for the prevention of apoptosis in normal lymphoid cells (11), whereas $BCLx_L$ is a key component of

platelet survival (12). In malignant lymphoid cells, however, BCL2 over-expression overwhelms the capacity of BH3 only proteins to trigger the intrinsic pathway of apoptosis, resulting in inappropriate cell survival.

ALL is the commonest childhood cancer. It encompasses several distinct entities that are characterized by chromosomal rearrangements, structural variations and sequence mutations that perturb lymphoid maturation, proliferation, growth suppression and epigenetic regulation (13-16). For most children and adolescents enrolled on clinical trials internationally, survival comes close to or exceeds 90%. This has been achieved through risk-adapted therapy based on the biological features of the disease as well as early treatment response, defined by minimal residual disease (MRD). Despite excellent survival outcomes for most children, ALL remains the leading cause of disease related death in children and young adults due to significant treatment related toxicity as well as refractory or relapsed disease associated with lower rates of disease response (17,18). New approaches directed against rational therapeutic targets are urgently required to improve outcomes for children with poor prognosis ALL. One such group is patients with mixed lineage leukemia rearrangements (MLLr) (approximately 6% of all childhood ALL) (19). MLLr-ALL is one of the most vexing clinical problems in pediatric hematology-oncology due to its aggressive clinical presentation, often in the uniquely vulnerable infant age group. Perhaps most importantly, the outcomes remain poor despite the use of maximally intensified standard chemotherapy with or without HSCT (20). BCL2 and $BCLx_L$ are up-regulated in ALL (21-23) and BCL2 over-expression has been associated with slow response to initial therapy (24). This information lead naturally to the hypothesis that inhibition of BCL2 and related family members may have a role in improving outcomes in poor prognosis ALL.

In the early to mid-2000's, utilization of nuclear magnetic resonance spectroscopy led to the improved understanding of the structural binding of BH3, only proteins to the pro-survival BCL2 proteins (25) and in 2005 the first true BH3 mimetic, ABT-737, were described (26). ABT-737, and its orally available analogue navitoclax (ABT-263) (27), bind to the pro-survival proteins BCL2, $BCLx_L$ and BCLw with high affinity, inhibiting their function. Both agents have demonstrated efficacy in pediatric ALL xenografts both alone and in combination with standard cytotoxic therapy (28,29). For instance, navitoclax demonstrated a 61% objective response rate in pediatric xenograft models of high risk ALL (28,30).

In the clinic, navitoclax has been tested in adults with chronic lymphocytic leukemia (CLL) (31) and non-Hodgkin lymphoma (NHL) (32) where it demonstrated overall response rates of 35% and 22% respectively, with no complete remissions. Unfortunately, navitoclax exposure was limited by predictable dose related thrombocytopenia (31,32), due to on-target inhibition of $BCLx_L$ which is critical to survival of circulating platelets (12,33). As a result, a BCL2 selective inhibitor, venetoclax, was developed (1). The phase I clinical trial results of venetoclax in relapsed and refractory CLL have recently been published with venetoclax demonstrating an almost 80% overall response rate with a 20% complete response rate, without dose limiting thrombocytopenia (34). The possible role of venetoclax in other hematological malignancies such as ALL, however, remains unresolved.

Key steps forward

In their recent paper Khaw *et al.* (35), address a number of unanswered questions relating to the use of BH3 mimetics in pediatric ALL, specifically: (I) Is the superior efficacy of venetoclax compared with navitoclax in CLL recapitulated in ALL? (II) What is the relative importance of pro-survival family members, namely $BCLx_L$ and BCL2, in ALL survival? (III) What biomarkers for pediatric ALL response to venetoclax? and (IV) Which subgroups of ALL may respond better than others to venetoclax?

Disappointingly, the work by Khaw *et al.* showed that venetoclax demonstrated an inferior objective response rate [26% compared with 61% (28,30)] to navitoclax in high-risk pediatric ALL xenografts, suggesting that $BCLx_L$ or BCLw may be critical to the response of this disease to BH3 inhibition. Somewhat predictably Khaw *et al.* also showed that the ALL xenografts that responded to venetoclax had significantly lower $BCLx_L$ protein expression and significantly higher BCL2 protein expression, compared with the others. Taken together, Khaw *et al.* concluded that for pediatric ALL xenografts, higher levels of pro-survival proteins such as $BCLx_L$ that are not targeted by venetoclax undermine the efficacy of this agent. While protein expression of BCL2 family members may be a useful biomarker for venetoclax efficacy, Khaw *et al.* further demonstrated that *in vitro* sensitivity of ALL cells to venetoclax also accurately predicted *in vivo* responsiveness of the xenografts.

In keeping with the above findings, Khaw *et al.* show

that across most ALL subtypes navitoclax demonstrates greater *in vitro* efficacy than either venetoclax or selective $BCLx_L$ inhibition alone. Furthermore, combined *in vitro* BCL2 and $BCLx_L$ inhibition, with venetoclax and A-1155463 respectively, demonstrated marked synergy amongst all ALL sub-types tested. This suggests that combined BCL2 and $BCLx_L$ inhibition is likely to be critical to the efficacy of BH3 mimetics in ALL. The notable exception to this, however, was seen in MLLr-ALL where venetoclax (but not the $BCLx_L$ specific inhibitors) showed strong *in vitro* efficacy, although the combination of $BCLx_L$ and BCL2 inhibition continued to demonstrate synergy amongst this sub-group. In addition, in MLLr-ALL xenografts venetoclax was associated with a higher objective response rate of 50% compared with 26% in non-MLLr-ALL xenografts.

In summary, using *ex vivo* human pediatric ALL cells, as well as ALL pediatric xenografts, Khaw *et al.* have shown that: (I) venetoclax has inferior efficacy to navitoclax across many *in vitro* and *in vivo* preclinical models of pediatric ALL; (II) both $BCLx_L$ and BCL2 appear to be instrumental to survival of pediatric ALL; (III) BCL2 family member protein expression as well as *in vitro* sensitivity assays may be useful biomarkers to predict response of pediatric ALL to BH3 inhibition; and (IV) in contrast to other pediatric ALL subtypes, MLLr-ALL appears to be sensitive *in vitro* and *in vivo* to BCL2 selective inhibition with venetoclax.

A number of caveats should be considered in the interpretation of these conclusions, however. Chief among these are the limitations inherent in xenograft models for predicting the clinical behavior and response of cancers in patients. Additionally, the reasons for the unique sensitivity of MLLr-ALL to venetoclax inhibition remain unresolved but possibly relate to up-regulation of BCL2 by DOT1L-mediated H3K79 methylation at the BCL-2 locus (36). Further, the clinical utility of BH3 mimetics in the treatment of ALL requires validation especially since $BCLx_L$ inhibition (with attendant dose limiting thrombocytopenia) is likely to be required for most subtypes. The role for potential MCL1 inhibitors in the therapeutic landscape and their combination with standard cytotoxic agents and novel therapies may also impact on the clinical development of agents such as venetoclax for the treatment of ALL. Nevertheless, these results add further impetus for investigation of both the safety and efficacy of these agents in high-risk pediatric ALL where clinical outcomes with standard therapeutic approaches remain sub-optimal.

Conclusions

Navitoclax has shown pre-clinical promise in the treatment of pediatric ALL. Unfortunately, however, the safety of this agent at higher doses is limited by the development of thrombocytopenia. While venetoclax avoids the dose limiting thrombocytopenia associated with navitoclax, the work of Khaw *et al*, suggest that loss of $BCLx_L$ inhibition is likely to significantly undermine the efficacy of this agent in most subgroups of pediatric ALL. Still, Khaw *et al*. were able to identify predictors of ALL response to venetoclax therapy including *in vitro* sensitivity, higher BCL2 protein expression and the presence of rearranged MLL. This implies that while venetoclax may not be as efficacious as navitoclax as a single agent in many pediatric ALL subtypes, it may have a useful role in highly selected poor prognosis sub-groups such as MLLr-ALL or in combination with established anti-leukemic drugs and/or immune- and cellular therapies. Further work will need to focus on understanding why $BCLx_L$ appears to be less critical in the survival of MLLr ALL and also investigating the safety of both these BH3 mimetics alone and in rational combinations, especially in children. Nonetheless this work provides the basis for a promising a novel approach to improving outcome in high-risk pediatric ALL.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by Section Editor Peipei Xu (Department of Hematology, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China).

Conflicts of Interest: MA Anderson is an employee of Walter and Eliza Hall Institute which receives milestone payments in relation to venetoclax; the other author has no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Souers AJ, Leverson JD, Boghaert ER, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med 2013;19:202-8.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74.
3. Strasser A, Harris AW, Jacks T, et al. DNA damage can induce apoptosis in proliferating lymphoid cells via p53-independent mechanisms inhibitable by Bcl-2. Cell 1994;79:329-39.
4. Schmitt CA, Rosenthal CT, Lowe SW. Genetic analysis of chemoresistance in primary murine lymphomas. Nat Med 2000;6:1029-35.
5. Huang DC, O'Reilly LA, Strasser A, et al. The anti-apoptosis function of Bcl-2 can be genetically separated from its inhibitory effect on cell cycle entry. EMBO J 1997;16:4628-38.
6. Miyashita T, Reed JC. Bcl-2 oncoprotein blocks chemotherapy-induced apoptosis in a human leukemia cell line. Blood 1993;81:151-7.
7. Miyashita T, Reed JC. bcl-2 gene transfer increases relative resistance of S49.1 and WEHI7.2 lymphoid cells to cell death and DNA fragmentation induced by glucocorticoids and multiple chemotherapeutic drugs. Cancer Res 1992;52:5407-11.
8. Kamesaki S, Kamesaki H, Jorgensen TJ, et al. bcl-2 protein inhibits etoposide-induced apoptosis through its effects on events subsequent to topoisomerase II-induced DNA strand breaks and their repair. Cancer Res 1993;53:4251-6.
9. Anderson MA, Huang D, Roberts A. Targeting BCL2 for the treatment of lymphoid malignancies. Semin Hematol 2014;51:219-27.
10. Chen L, Willis SN, Wei A, et al. Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. Mol Cell 2005;17:393-403.
11. Veis DJ, Sorenson CM, Shutter JR, et al. Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. Cell 1993;75:229-40.
12. Mason KD, Carpinelli MR, Fletcher JI, et al. Programmed anuclear cell death delimits platelet life span. Cell 2007;128:1173-86.
13. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet 2013;381:1943-55.
14. Mullighan CG, Willman CL. Advances in the Biology of Acute Lymphoblastic Leukemia-From Genomics to the Clinic. J Adolesc Young Adult Oncol 2011;1:77-86.
15. Mullighan CG, Downing JR. Genome-wide profiling of genetic alterations in acute lymphoblastic leukemia: recent insights and future directions. Leukemia 2009;23:1209-18.
16. Mullighan CG. Genomic characterization of childhood acute lymphoblastic leukemia. Semin Hematol 2013;50:314-24.
17. Pui CH, Yang JJ, Hunger SP, et al. Childhood Acute Lymphoblastic Leukemia: Progress Through Collaboration. J Clin Oncol 2015;33:2938-48.
18. Pui CH, Pei D, Campana D, et al. Improved prognosis for older adolescents with acute lymphoblastic leukemia. J Clin Oncol 2011;29:386-91.
19. Pui CH, Evans WE. Acute lymphoblastic leukemia. N Engl J Med 1998;339:605-15.
20. Sanjuan-Pla A, Bueno C, Prieto C, et al. Revisiting the biology of infant t(4;11)/MLL-AF4+ B-cell acute lymphoblastic leukemia. Blood 2015;126:2676-85.
21. Hartung L, Bahler DW. Flow cytometric analysis of BCL-2 can distinguish small numbers of acute lymphoblastic leukaemia cells from B-cell precursors. Br J Haematol 2004;127:50-8.
22. Menendez P, Vargas A, Bueno C, et al. Quantitative analysis of bcl-2 expression in normal and leukemic human B-cell differentiation. Leukemia 2004;18:491-8.
23. Findley HW, Gu L, Yeager AM, et al. Expression and regulation of Bcl-2, Bcl-xl, and Bax correlate with p53 status and sensitivity to apoptosis in childhood acute lymphoblastic leukemia. Blood 1997;89:2986-93.
24. Bhojwani D, Kang H, Menezes RX, et al. Gene expression signatures predictive of early response and outcome in high-risk childhood acute lymphoblastic leukemia: A Children's Oncology Group Study [corrected]. J Clin Oncol 2008;26:4376-84.
25. Shuker SB, Hajduk PJ, Meadows RP, et al. Discovering high-affinity ligands for proteins: SAR by NMR. Science 1996;274:1531-4.
26. Oltersdorf T, Elmore SW, Shoemaker AR, et al. An inhibitor of Bcl-2 family proteins induces regression of

solid tumours. Nature 2005;435:677-81.
27. Tse C, Shoemaker AR, Adickes J, et al. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. Cancer Res 2008;68:3421-8.
28. Suryani S, Carol H, Chonghaile TN, et al. Cell and molecular determinants of in vivo efficacy of the BH3 mimetic ABT-263 against pediatric acute lymphoblastic leukemia xenografts. Clin Cancer Res 2014;20:4520-31.
29. High LM, Szymanska B, Wilczynska-Kalak U, et al. The Bcl-2 homology domain 3 mimetic ABT-737 targets the apoptotic machinery in acute lymphoblastic leukemia resulting in synergistic in vitro and in vivo interactions with established drugs. Mol Pharmacol 2010;77:483-94.
30. Lock R, Carol H, Houghton PJ, et al. Initial testing (stage 1) of the BH3 mimetic ABT-263 by the pediatric preclinical testing program. Pediatr Blood Cancer 2008;50:1181-9.
31. Roberts AW, Seymour JF, Brown JR, 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. J Clin Oncol 2012;30:488-96.
32. Wilson WH, O'Connor OA, Czuczman MS, et al. Navitoclax, a targeted high-affinity inhibitor of BCL-2, in lymphoid malignancies: a phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumour activity. Lancet Oncol 2010;11:1149-59.
33. Zhang H, Nimmer PM, Tahir SK, et al. Bcl-2 family proteins are essential for platelet survival. Cell Death Differ 2007;14:943-51.
34. Roberts AW, Davids MS, Pagel JM, et al. Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. N Engl J Med 2016;374:311-22.
35. Khaw SL, Suryani S, Evans K, et al. Venetoclax responses of pediatric ALL xenografts reveal sensitivity of MLL-rearranged leukemia. Blood 2016;128:1382-95.
36. Benito JM, Godfrey L, Kojima K, et al. MLL-Rearranged Acute Lymphoblastic Leukemias Activate BCL-2 through H3K79 Methylation and Are Sensitive to the BCL-2-Specific Antagonist ABT-199. Cell Rep 2015;13:2715-27.

Cite this article as: Hanna D, Anderson MA. A new approach to high risk pediatric acute lymphoblastic leukemia? Transl Cancer Res 2016;5(Suppl 7):S1428-S1432. doi: 10.21037/tcr.2016.12.68

