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, BCLxL, 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 BCLxL 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 hematologic-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 BCLxL 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 proteins BCL2, BCLxL 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 BCLxL 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 BCLxL 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 BCLxL 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 BCLxL 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 BCLxL 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 BCLxL inhibition alone. Furthermore, combined in vitro BCL2 and BCLxL inhibition, with venetoclax and A-1155463 respectively, demonstrated marked synergy amongst all ALL sub-types tested. This suggests that combined BCL2 and BCLxL 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 BCLxL specific inhibitors) showed strong in vitro efficacy, although the combination of BCLxL and BCL2 inhibition continued to demonstrate synergy amongst this sub-group. In addition, in MLLr-ALL xenografts navitoclax 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 BCLxL 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 BCLxL 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 BCLxL 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 BCLxL 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 novel approach to improving outcome in high-risk pediatric ALL.

Acknowledgements

None.

Footnote

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.

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