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616.ACUTE MYELOID LEUKEMIAS: INVESTIGATIONAL THERAPIES, EXCLUDING TRANSPLANTATION AND CELLULAR IMMUNOTHERAPIES

Mitochondrial Functional Profiling Links Apoptotic Sensitivity to Treatment Outcomes Based on Age and Cell Lineage in Acute Leukemia

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Background
Survival outcomes in paediatric T and B cell acute lymphoblastic leukemia (ALL) are far superior to acute myeloid leukemia (AML). In addition, children with ALL and AML have better response rates than adults. While the basis for these differences in therapy outcomes is multifactorial, it is poorly understood. The majority of anticancer therapies activate apoptosis signalling to target cancer cells. The balance between pro-survival (BCL-2, BCL-XL and MCL-1) and pro-apoptotic proteins as well as evasion of apoptosis, plays a key role in cancers. Dependencies to these anti-apoptotic pro-survival proteins is a hallmark of haematopoietic malignancies and can be exploited with novel agents especially by using Venetoclax (BCL-2 inhibitor) and Navitoclax (BCL-2 and BCL-XL inhibitor) that are now available in the clinic. We hypothesized that difference in apoptosis threshold (priming) based on cell lineage and patient age offers key functional insights into the differences in outcomes as well as gives us an in vitro window to assess inherent BCL-2 or BCL-XL dependencies that can be targeted.
Methods We prospectively and retrospectively examined functional regulation of apoptosis by performing a BH3 profiling assay on leukemia patient specimens from two tertiary cancer centres in Singapore. Since BCL-2 family consists of different anti-apoptotic proteins, we asked whether protection from specific pro-survival BCL-2 family proteins such as BCL-2, BCL-XL or MCL-1, predicted by sensitivity to BAD, HRK and MS-1 peptide, respectively, contribute to overall difference in apoptotic sensitivity between lymphoid and myeloid precursors. We also aimed to determine dominant factors that govern differences in clinical chemosensitivity to lymphoid compared to myeloid tumours. Blasts isolated via flow cytometry from bone-marrow aspirate (BMA) or peripheral blood were exposed to a variety of pro-apoptotic BH3 peptides to measure mitochondrial outer membrane permeabilization (cytochrome c release). The percentage of cytochrome c loss with each BH3 peptide at different concentrations was normalized to the cytochrome c release from negative control DMSO wells. Assays were performed in triplicate. Alamethacin was used as positive control. Flow cytometric data was acquired with a BD analyser, analysed with FlowJo and plotted in GraphPad Prism.
Results
Initial analysis of seventy one patient samples are presented here (40 AML, 17 T-ALL, 12 B-ALL and 2 MPAL). Lymphoblasts showed significantly increased apoptotic sensitivity as measured by Bim response compared to myeloblasts ($p<0.001$). We found lymphoblasts to be significantly more dependent on BCL-2 and BCL-XL compared to myeloblasts ($p<0.001$). Higher MCL-1 dependence was also noted in B-ALL cells as compared to AML ($p<0.05$). We next asked if apoptotic sensitivity measured by mitochondrial priming could explain higher chemosensitivity of pediatric over adult patients. Indeed we noted increase in overall priming in paediatric (N=26) as compared to adults (N=36) irrespective of cell of origin ($P<0.001$). Within the AML cohort, we found pediatric AML (N=8) to be more sensitive to Bim peptide compared to adults (N=32), as well as showed closer proximity to apoptosis ($p<0.05$). Of note there were no differences in overall priming or selective dependencies between diagnosis and relapse/refractory (R/R) patients in AML or ALL cohorts.

By using blasts of AML and +ALL patients (N=15), we found that increased mitochondrial priming response to HRK+MS1 peptides (which infer BCL-XL and MCL-1 dependence respectively) inversely correlated with achievement of remission with venetoclax based therapy ($p<0.001$). Mitochondrial sensitivity to MS-1 peptide, increased from pre-treatment to on treatment to relapse (N=3/3 patients) in sequential samples for three patients suggesting an opportunity for the use of MCL-1 inhibition in relapse setting.

Conclusion Our study shows increased overall priming in lymphoblast as compared with myeloblast and in pediatric as compared to blasts from adult leukaemia specimens. These findings offer a functional window to further explain differences in outcomes in leukaemia in different ages as well as lineages. Innovative trial designs incorporating cellular vulnerabilities to anti-apoptotic proteins using functional BH3 profiling as an accessory biomarker will enable improved treatment outcomes with reduced toxicity.

Disclosures No relevant conflicts of interest to declare.

Figure 1

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