Dynamic BH3 Profiling Predicts Patient Response and MRD Status in Chronic Lymphocytic Leukemia (CLL) Patients Undergoing Frontline Treatment with Kinase Inhibitor Plus FCR (KI + FCR)

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**BACKGROUND**
- Bruton Tyrosine Kinase (BTK) and phosphoinositide 3-kinase (PI3K) are well-established mediators of CLL survival in the microenvironment.
- Dynamic BH3 Profiling (DBP) measures the change in cellular sensitivity to apoptotic stimuli (priming) and dependency on BCL-2 family members following treatment with a drug of interest. This is quantified by treating cells with BH3-only peptides and measuring cytotoxic c-release from the mitochondria.
- Ex vivo inhibition of BTK and PI3K in CLL cells leads to an increase in apoptotic priming and BCL-2 dependence in the presence of stromal NK/TERT cells (Davids et al., 2011, Deng et al., 2017).
- The combination of the BTK inhibitor ibrutinib (IBR) or the PI3K inhibitor duvelisib (DUV) with FCR leads to high rates of MRD undetectability when given as frontline therapy for younger, fit CLL patients (Davids et al., ASH 2017, and EHA 2018), a strategy we call KI + FCR.
- Not all patients are able to achieve a deep response, demonstrating the need for predictive biomarkers to better match the most effective combination regimens to the right patient. Here, we evaluated the efficacy of dynamic BH3 profiling (DBP) for predicting the depth of response and likelihood of achieving MRD undetectability on two KI + FCR trials.

**METHODS**
- Mononuclear cells from CLL patients enrolled in 2 separate clinical trials of FCR plus either Ibrutinib or Duvelisib (VEN) were used to measure BCL-2 mimetics but not MCL-1 or BCL-xL sensitivity to apoptotic stimuli (priming) and dependency on BCL-2 mimetics.
- ibrutinib or duvelisib were used to measure BCL-2 mimetics, but not MCL-1 or BCL-xL dependency.
- Dynamic BH3 profiling (DBP) was performed as previously reported (Montero et al., Cell, 2010) by measuring the release of cytochrome c-gently perturb CLL cells in response to BH3-only peptides using a BD FACs Fortessa.
- The BH3-MIMET peptides and natural BH3 peptides were used to measure BCL-2 dependence. BH3 Mimetic peptides were used to measure BCL-2 mimetic but not MCL-1 or BCL-xL sensitivity. BCL-xL Dependence respectively. BH3 Mimetic were used to measure the overall degree of apoptotic priming.
- Viability assays were performed using Annexin V/Propidium Iodide. Cells were fixed prior staining with 6-Amino-4-Chloro-3-indolyl phosphate, 0.01% Glutaraldehyde, 1% Trypsin Staining Buffer and analyzed by FACs.
- Statistical analyses were by unpaired Student t-test with a two-tailed normal p ≤ 0.05 considered as significant.
- Complete remission (CR) defined as 100% lymphocyte count and no detectable circulating blasts. Partial response (PR) defined as a 50% decrease in lymphocyte count and no detectable circulating blasts.

**RESULTS**
- ibrutinib and duvelisib increase apoptotic priming and BCL-2 dependence in both peripheral blood (PB)-derived CLL cells.
- Dynamic BH3 profiling may serve as a predictive biomarker for response to FCR.
- In vivo dynamic BH3 profiling predicts depth of response to KI + FCR.

**CONCLUSIONS**
- Ex vivo treatment of CLL cells with IBR or DUV leads to an increase in apoptotic priming and BCL-2 dependence.
- In vivo treatment of CLL cells with IBR leads to a decrease in MCL-1 dependence, while DUV leads to an increase in BCL-1 dependence.
- In vivo treatment with IBR or DUV increases CLL sensitivity to BCL-2 mimetics, but not MCL-1 or BCL-xL mimetics.
- In vivo ABCL-2 dependence increases in patients achieving a CR than a PR for both KI-FCR regimens.
- Patients who achieve MRD-undetectability have higher pre-treatment BCL-2 dependence (dFCR) or ABCL-2 dependence (iFCR).
- Dynamic BH3 profiling may serve as a predictive biomarker for depth of response for patients treated using a KI + FCR regimen and merits further investigation in larger cohorts.

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