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)

Timothy Z. Lehmberg, BA, Rebecca Valentin, MD, Stacey M. Fernandes, BS, Jennifer R. Brown, MD, PhD, Matthew S. Davids. MD, MMSc

4395

Department of Medical Oncology, Dana-Farber Cancer Institute (DFCI), Harvard Medical School, Boston, MA, USA

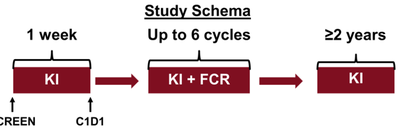
BACKGROUND

- **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 member proteins following treatment with a drug of interest. This is quantified by treating cells with BH3-only peptides and measuring cytochrome 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 dependency 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 regimen 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**

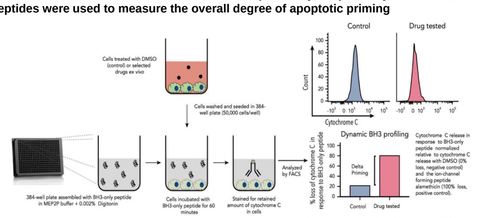
METHODS

- **Mononuclear cells from CLL patients enrolled in 2 separate clinical trials of FCR plus either IBR (iFCR) or DUV (dFCR) were isolated before receiving therapy (screen) and after a week of small molecule monotherapy (C1D1)**
- **Pre-Treatment cells were co-cultured with NK.TERT stromal cells (1:10 ratio) with 1 μM of ibrutinib or duvelisib or DMSO controls**

Study Schema



- **Dynamic BH3 profiling (DBP) was performed as previously reported (Montero et al., Cell, 2015) by measuring the release of cytochrome C in gently permeabilized CLL cells in response to BH3-only peptides using a BD FACS Fortessa**
- **The BH3 BAD peptide and venetoclax (VEN) were used to measure BCL-2 dependence, BH3 Hrk peptide was used to measure BCL-xL dependence, and the synthetic peptides MS1 and FS1 were used to measure MCL-1 and BFL-1 dependency, respectively. BH3 BIM and Puma peptides were used to measure the overall degree of apoptotic priming**



Reviewed in Valentin et al., Blood, 2018

- **Viability assays were performed using Annexin V/Hoechst. Cells were fixed post staining with 4x Annexin V Fixative (4% Formaldehyde, 0.5% Glutar-aldehyde, 1X Annexin Staining Buffer) and analyzed by FACS**
- **Statistical analyses were by unpaired and paired t-test with a two-tailed nominal p ≤ 0.05 considered as significant. Given the hypothesis-generating goal of this project, multiplicity was not corrected for**

RESULTS

Ibrutinib and Duvelisib Increase Apoptotic Priming and BCL-2 Dependence in Both Peripheral Blood (PB)-Derived CLL Cells

FIG. 1: *IN VIVO AND EX VIVO* EXPERIMENTAL WORKFLOW OF KI+FCR CORRELATIVE STUDIES

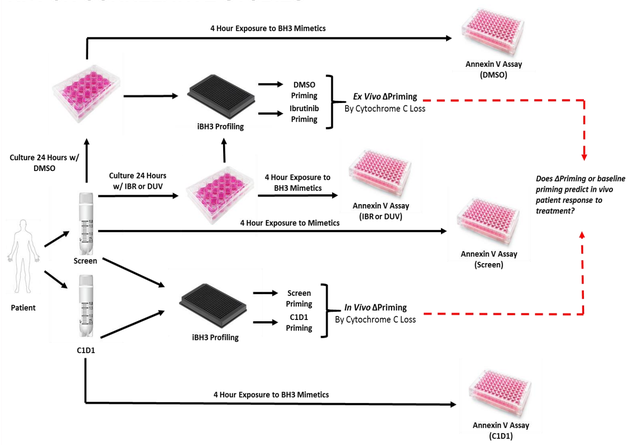
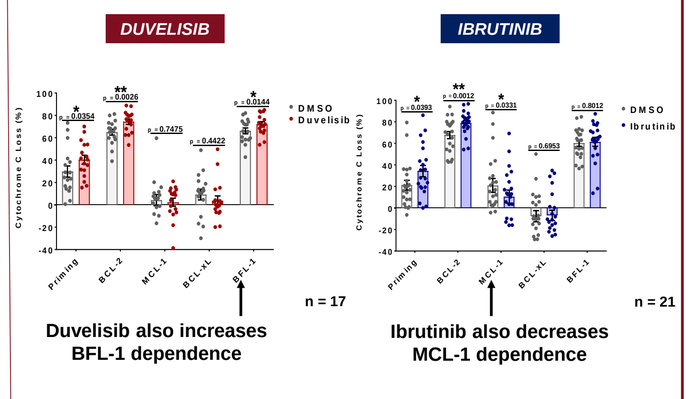


FIG. 2: *EX VIVO* TREATMENT OF PB-DERIVED CLL CELLS WITH IBRUTINIB OR DUVELISIB INCREASES APOPTOTIC PRIMING AND BCL-2 DEPENDENCE



In Vivo Treatment with Duvelisib or Ibrutinib Selectively Enhances Sensitivity to BCL-2 Mimetics

FIG. 3*: IN VIVO* TREATMENT WITH DUVELISIB OR IBRUTINIB SELECTIVELY ENHANCES SENSITIVITY TO BCL-2 MIMETICS BUT NOT MCL-1 or BCL-xL MIMETICS: DOSE RESPONSE CURVES

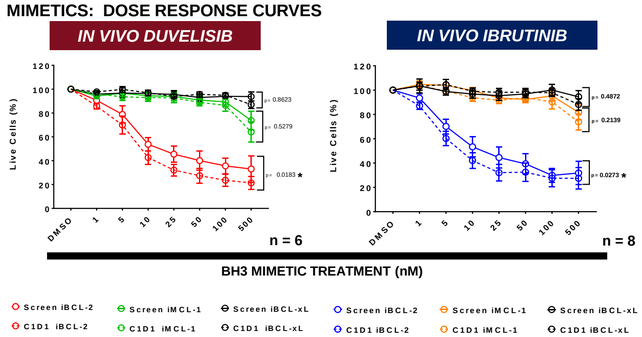
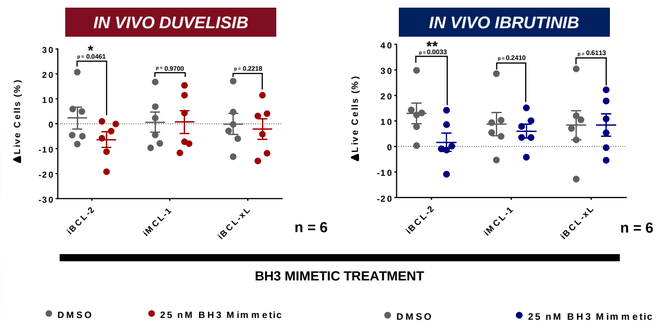


FIG. 4*: IN VIVO* TREATMENT WITH DUVELISIB OR IBRUTINIB INCREASES SENSITIVITY TO BCL-2 MIMETICS BUT NOT MCL-1 or BCL-xL MIMETICS



In Vivo Dynamic BH3 Profiling Predicts Depth of Response to KI + FCR

FIG. 5 : ΔBCL-2 DEPENDENCE IS HIGHER IN PATIENTS ACHIEVING A CR THAN PATIENTS ACHIEVING A PR ON DUVELISIB + FCR

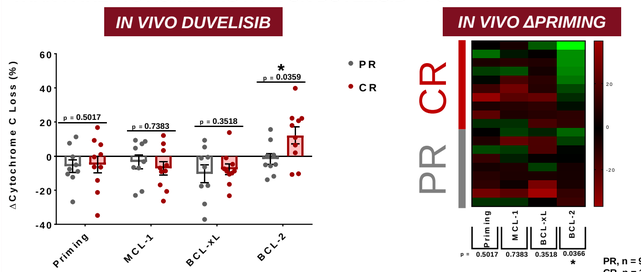
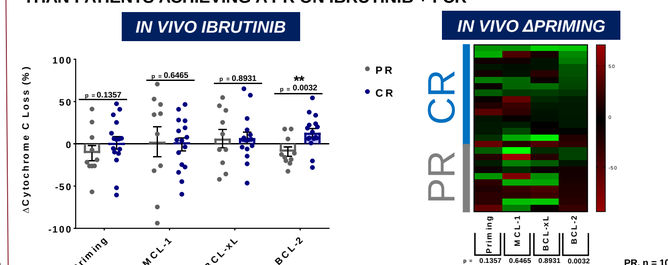
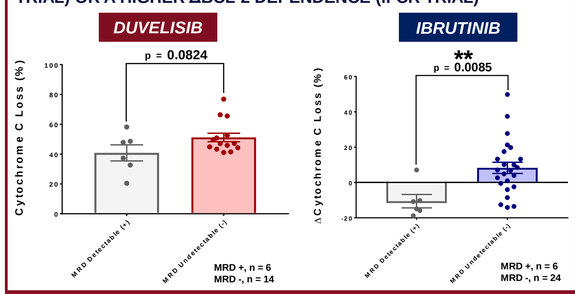


FIG. 6 : ΔBCL-2 DEPENDENCE IS HIGHER IN PATIENTS ACHIEVING A CR THAN PATIENTS ACHIEVING A PR ON IBRUTINIB + FCR



In Vivo Dynamic BH3 Profiling Predicts MRD Status

FIG. 7: PATIENTS WHO GO ON TO ACHIEVE MRD UNDETECTABILITY HAVE A HIGHER PRE-TREATMENT BCL-2 DEPENDENCE (dFCR TRIAL) OR A HIGHER ΔBCL-2 DEPENDENCE (iFCR TRIAL)



CONCLUSIONS

- ***Ex vivo* treatment of CLL cells with IBR or DUV leads to an increase in apoptotic priming and BCL-2 dependence**
- ***Ex vivo* treatment of CLL cells with IBR leads to a decrease in MCL-1 dependence, while DUV leads to an increase in BFL-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* ΔBCL-2 dependence is higher 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 ΔBCL-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**

DISCLOSURES

- R. Valentin: travel reimbursement from Roche and Abbvie
- JR. Brown:
  - **Consultancy:** Pfizer, Gilead, Sun, Novartis, AbbVie, Celgene, Astra-Zeneca, Janssen, Pharmacyclics, TG Therapeutics, Verastem, Genentech, Acerta, Loxo, Sunesis, BeiGene
  - **Research Funding:** Gilead, Sun, Verastem
- MS. Davids:
  - **Consultancy/Advisory Boards:** Pharmacyclics, Verastem, Abbvie, Genentech, Janssen, Astra-Zeneca, Acerta, MEI Pharma, Gilead, Syros, Sunesis
  - **Research Funding:** Pharmacyclics, Verastem, Genentech, TG Therapeutics, BMS, Surface Oncology, MEI Pharma, Astra-Zeneca
  - **Grant Funding:** LLS Clinical Scholar Award

LEUKEMIA & LYMPHOMA SOCIETY®

Corresponding author: matthew_davids@dfci.harvard.edu