

## **Supplemental methods:**

### **RNA sequencing**

Globin mRNA was substantially depleted from total RNA samples using the GlobinClear-Human Kit (Life Technologies # AM1980). The globin-depleted RNA was quantitated using a NanoDrop spectrophotometer and then was converted into cDNA libraries using the TruSeq Stranded Total RNA-RiboZero Gold Sample Prep Kit (Illumina, #RS-122-2303). Final cDNA libraries were analyzed for size distribution using an Agilent Bioanalyzer (DNA 1000 kit, Agilent # 5067-1504), quantitated by qPCR (KAPA Library Quant Kit, KAPA Biosystems # KK4824), then normalized to 2 nM in preparation for sequencing. Using an Illumina TruSeq Paired-End Cluster Kit V3 (Illumina # PE-401-3001), a clustered flowcell was generated using the normalized cDNA libraries as templates. Sequencing was performed using an Illumina HiSeq 2000, generating 50 base, paired end reads. Data alignment, processing, and analysis are described in the supplemental methods.

### **RNA sequencing data processing**

Raw RNASeq reads in all FASTQ files were mapped to human reference genome (UCSC build hg19) with OmicSoft Sequence Aligner (OSA) inside Array Studio (version 7.2.2.34, OmicSoft Corporation). Default parameters were used for OSA alignment except that the SearchNovelExonJunction and DetectIndels options were enabled. Using the human gene model in the UCSC RefGene database (December 2012 version), the Array Studio software extracted raw counts and computed FPKM values (1) both at the transcript level and at the gene level.

A threshold level of expression was applied to the log<sub>2</sub> FPKM values to remove genes with low and highly variable expression values close to the level of detection. Housekeeping genes selected using Normfinder (2) were then used to adjust the log<sub>2</sub> FPKM values; and a quality control filter was applied to exclude data from potentially degraded samples. Pairs of samples which showed a linear regression

correlation coefficient  $r^2 < 0.7$  between C1D1 and C1D8 visits were thus excluded. In the samples from the remaining 31 patients, we evaluated BCL2 family gene expression changes between C1D1 and C1D8. Significance was determined using a paired t-test.

#### **Expected % apoptosis of duvelisib and venetoclax combination**

Expected % apoptosis of duvelisib and venetoclax combination was calculated as following. First raw viability was normalized and then expected % viability was calculated using following formula: “ % viability of drug duvelisib \* % viability of drug venetoclax / 100”. Finally expected % apoptosis was calculated as “100-(Expected % apoptosis)” as described before(3).

#### **Oncomine Analysis**

Analysis of Bcl-2 transcripts expression in previously published datasets were done using Oncomine Gene browser (ThermoFisher Scientific). Bcl-2 gene expression among previously published research articles curated in Oncomine under names - Haferlach (4) , Visone (5), Basso (6) , Fabris (7), Falt (8), Fernandez (9), Haslinger (10), Saiya-Cork (11), Alizadeh (12), and Rosenwald (13) were used.

## Supplemental references:

1. Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat Methods*. 2008;5:621-8.
2. Andersen CL, Jensen JL, Orntoft TF. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Res*. 2004;64:5245-50.
3. Cervantes-Gomez F, Lamothe B, Woyach JA, Wierda WG, Keating MJ, Balakrishnan K, et al. Pharmacological and Protein Profiling Suggests Venetoclax (ABT-199) as Optimal Partner with Ibrutinib in Chronic Lymphocytic Leukemia. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2015;21:3705-15.
4. Haferlach T, Kohlmann A, Wieczorek L, Basso G, Kronnie GT, Bene MC, et al. Clinical utility of microarray-based gene expression profiling in the diagnosis and subclassification of leukemia: report from the International Microarray Innovations in Leukemia Study Group. *J Clin Oncol*. 2010;28:2529-37.
5. Visone R, Rassenti LZ, Veronese A, Taccioli C, Costinean S, Aguda BD, et al. Karyotype-specific microRNA signature in chronic lymphocytic leukemia. *Blood*. 2009;114:3872-9.
6. Basso K, Margolin AA, Stolovitzky G, Klein U, Dalla-Favera R, Califano A. Reverse engineering of regulatory networks in human B cells. *Nat Genet*. 2005;37:382-90.
7. Fabris S, Mosca L, Todoerti K, Cutrona G, Lionetti M, Intini D, et al. Molecular and transcriptional characterization of 17p loss in B-cell chronic lymphocytic leukemia. *Genes Chromosomes Cancer*. 2008;47:781-93.
8. Falt S, Merup M, Gahrton G, Lambert B, Wennborg A. Identification of progression markers in B-CLL by gene expression profiling. *Exp Hematol*. 2005;33:883-93.
9. Fernandez V, Salamero O, Espinet B, Sole F, Royo C, Navarro A, et al. Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. *Cancer Res*. 2010;70:1408-18.
10. Haslinger C, Schweifer N, Stilgenbauer S, Dohner H, Lichter P, Kraut N, et al. Microarray gene expression profiling of B-cell chronic lymphocytic leukemia subgroups defined by genomic aberrations and VH mutation status. *J Clin Oncol*. 2004;22:3937-49.
11. Saiya-Cork K, Collins R, Parkin B, Ouillette P, Kuizon E, Kujawski L, et al. A pathobiological role of the insulin receptor in chronic lymphocytic leukemia. *Clin Cancer Res*. 2011;17:2679-92.
12. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*. 2000;403:503-11.
13. Rosenwald A, Alizadeh AA, Widhopf G, Simon R, Davis RE, Yu X, et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. *J Exp Med*. 2001;194:1639-47.