Venetoclax resistance and acquired *BCL2* mutations in chronic lymphocytic leukemia

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Methods (Supplemental)

Whole exome and deep sequencing analysis

Whole exome sequencing was performed using SureSelectXT Human All Exon V5 library kit (Agilent Technologies, Santa Clara, CA) for library preparation per manufacturer instructions. Mutations were validated via targeted sequencing of BCL2 family genes (BCL2, BAX, BIM, BCL-XL and MCL1) using two different pools of custom oligo capture probes. Library preparation was performed using TruSeq Custom Amplicon Assay Kit v1.5. Libraries were sequenced in paired-end mode on a HiSeq 2000 (WES) or Miseq (Targeted Sequencing)

Bioinformatics

Burrows-Wheeler Aligner (BWA)-MEM alignment to genome (hg19) and VarScan v2.3.9 software were used to detect variants with the parameter setting at a minimum coverage of 10 with a minimum of 5 supporting reads. Furthermore SnpEff (v4.0) and Pindel (v0.2.5b5) were part of the bioinformatics pipeline. We identified mutations of potential interest via comparison of baseline and PD samples.

Molecular modeling and structural analysis

PDB structure 4MAN was obtained from Souers, et al. 2013[8]. Ribbon, stick and surface structures were represented with UCSF Chimera[9]. The G101V mutation within BCL2 was superimposed using the rotamers function, selecting a Dunbrack rotamer considering the highest probability. Venetoclax was modeled using Maestro[10] and was based on the provided ligand of PDB:4MAN. Venetoclax close contact areas to BCL2 were also determined by Maestro.