High Sensitivity Testing Shows Multiclonal Mutations in Patients with CLL Treated with BTK Inhibitor and Lack of Mutations in Ibrutinib-Naive Patients

A Albitar, W Ma, MS1, I De Dios1, J Estella, MS1, M Farooqui, DO2, A Wiestner, MD, PhD2 and M Albitar, MD1
1NeoGenomics Laboratories, Irvine, CA,
2Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD

Background:
Patients with chronic lymphocytic leukemia (CLL) that develop resistance to Bruton’s tyrosine kinase (BTK) inhibitors are typically positive for mutations in BTK or phospholipase c gamma 2 (PLCγ2). Mutations in BTK at the C481S hotspot alter the active site of the mutant BTK to the effect that Ibrutinib is reversibly bound. PLCγ2 is downstream of BTK in the B-cell signaling pathway; mutations in PLCγ2 at either of the R665W, L845F, or S707Y hotspots result in a constitutively activated PLCγ2. In order to better understand the development of these resistance mechanisms in patients with CLL, we developed a high sensitivity (HS) assay utilizing branched and locked nucleic acids (BNA and LNA, respectively). We used this high sensitivity assay in combination with Sanger sequencing and next generation sequencing (NGS) and tested cellular DNA and cell free DNA (cfDNA) from patients with CLL.

Methods:
Using custom BNA or LNA oligos in a wild-type blocking polymerase chain reaction, then sequencing by Sanger and NGS methods, we achieved 100x greater sensitivity than Sanger. Sanger sequencing was capable of detecting <1 mutant allele in background of 1000 wild-type alleles (1:1000). Similar sensitivity was achieved with HS NGS. The assay is designed to cover BTK and PLCγ2 hotspots. Using this assay we tested peripheral blood samples from 44 Ibrutinib-naïve patients (Ib-) with CLL and 7 samples from CLL patients being treated with Ibrutinib (Ib+), that showed clinical evidence of disease progression. The same wild-type blocking was also used in NGS approach for confirmation. We performed wild-type blocking in a Nextera Rapid Capture Enrichment workflow for our custom 315 gene panel.

Results:
No BTK or PLCγ2 mutations were detected in any of the 44 ibrutinib-naive CLL patients. In contrast, all (N=7) tested patients with progressive disease on Ibrutinib showed one or more mutations in BTK or PLCγ2 using the HS method. Without the HS testing only 4 patients (57%) showed a mutation in BTK or PLCγ2. Two patients showed multiple mutant clones. One patient with double mutations in PLCγ2 (R665W and L845F) also showed triple independent mutations in BTK at codons C481 with HS testing. These mutations give rise to two distinct mutant proteins C481R (TGC>CGC) and C481S (TGC>AGC and TGC>TCC). NGS analysis confirmed that the the three BTK mutations are in three independent clones. A second patient showed initially a mutation in BTK (C481S), but subsequent sample showed a mutation in PLCg2 (R665W), in addition to the BTK mutation. All mutations detected in the peripheral blood cells were also detectable in cell-free DNA (cfDNA) using HS testing. However, without using HS testing, a BTK mutation was detected in cfDNA from a patient and this mutation was not detectable when cellular DNA was used.

Conclusions:
Our data suggests that ibrutinib-naïve patients with CLL do not have BTK or PLCγ2 mutations even when a highly sensitive assay is used. Emerging BTK or PLCγ2 mutant clones can be seen after therapy with the possibility of multiple clones emerging at the same time and may involve both BTK and PLCγ2 genes in the same patient. Furthermore, testing cfDNA is not only as informative as cellular DNA, but might show mutations earlier than cellular DNA. This may have clinical relevance in patients with lymphoma when only few lymphoma cells are in circulation.