Molecular Basis of Ibrutinib Resistance in Waldenstrom's Macroglobulinemia

Xu L, Tsakmaklis N, Yang G, Chen JG, Liu X, Chen J, Demos M, Patterson CJ, Dubeau T, Meid K, Gustine J, Castillo JJ, Advani R, Palomba M.L., Furman RR, Hunter ZR, and Treon SP. Bing Center for Waldenstrom's Macroglobulinemia, Dana Farber Cancer Institute; Harvard Medical School, Boston MA USA; Stanford University Medical Center, Stanford CA; Memorial Sloan Kettering Cancer Center, New York NY; and Weill Cornell Medical College, New York NY.

Ibrutinib is a small molecule that is approved by the U.S. FDA for the treatment of chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), and Waldenström's Macroglobulinemia (WM). In WM, mutated MYD88 supports the growth and survival of malignant lymphoplasmacytic cells (LPC) through BTK, while $CXCR4^{WHIM}$ mutations promote ibrutinib resistance (Yang et al, Blood 2013; Cao et al, Leukemia 2013). Ibrutinib irreversibly binds to Cys481 on BTK, and blocks its kinase activity. Despite high response rates and durable remissions in WM (Treon et al, NEJM 2015; Dimopoulos et al, ASH 2015), disease progression can occur in WM patients on active ibrutinib therapy. To investigate the molecular basis of ibrutinib resistance in WM, we first focused on BTK mutations at Cys481 that have been associated with ibrutinib resistance in CLL and MCL using Sanger sequencing and nested AS-PCR. To capture the known variants at BTK Cys481, three AS-PCR assays for $Cys481Ser^{G>C}$, $Cys481Ser^{T>A}$, and $Cys481Arg^{T>C}$ were developed with a sensitivity of detecting 0.1% of mutant alleles. Using these assays, we evaluated 8 WM patients who progressed on ibrutinib. Among these 8 patients, 5 had BTK Cys481 mutations: 3 were positive for $Cys481Ser^{G>C}$, and 2 were positive for all the three ($Cys481Ser^{G>C}$, $Cys481Ser^{T>A}$, and $Cys481Arg^{T>C}$) mutations. Cloning/sequencing analysis confirmed co-occurrence of multiple Cys481 mutations within individual WM patients and the presence of mutations at different alleles. Furthermore, targeted deep sequencing (>300X coverage) confirmed all BTK Cys481 mutations, and identified an additional mutation at Cys481 ($Cys481Tyr^{G>A}$) in both patients who were positive for $Cys481Ser^{G>C}$, $Cys481Ser^{T>A}$ and $Cys481Arg^{T>C}$. The estimated allele frequencies by targeted deep sequencing for individual BTK mutations ranged from 1-34%. In contrast, no BTK Cys481 mutations

were identified in 100 ibrutinib naive WM patients using the nested AS-PCR assays. Among the 8 WM patients included in this study, all had activating MYD88 mutations, and 4 had $CXCR4^{WHIM}$ mutations. All 4 patients with $CXCR4^{WHIM}$ mutations had BTK Cys481 mutations. We next utilized targeted deep sequencing to expand the mutation analysis to the entire coding regions of the BTK, as well as select genes relevant to BCR and MYD88 signaling. A missense mutation in CARD11 (L878F) was identified in one patient who lacked any BTK Cys481 mutations, while a missense mutation in PLCG2 (Y495H) was found in another patient with a $Cys481Ser^{G>C}$ mutation. The findings provide the first reported insights into the molecular mechanisms associated with ibrutinib resistance in WM, and highlight the emergence of multiple BTK mutated clones within individual patients who progress on ibrutinib.