Genomic evolution by serial single-cell sequencing identifies high tumor expression of LYN as a promoter of resistance in Waldenstrom's Macroglobulinemia undergoing ibrutinib monotherapy

Hao Sun[1,2,3,5], Romanos Sklavenitis-Pistofidis[2,3,4], Xia Liu[1,2,3], Nicholas Tsakmaklis[1,2,3], Maria Luisa Guerrera[1,2,3], Amanda Kofides[1,2,3], Andres Felipe Ramirez Gamero[1,3], Catherine A. Flynn[1,3], Christopher J. Patterson[1,2,3], Mu Hao[5], Lugui Qiu[5], Shayna R. Sarosiek[1,3], Jorge J. Castillo[1,3], Zachary R. Hunter[1,2,3], Shirong Liu[1,2,3], Steven P. Treon[1,2,3]

[1] Bing Center for Waldenström Macroglobulinemia, Dana-Farber Cancer Institute, Boston, MA, USA.
[2] Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA
[3] Harvard Medical School, Boston, MA, USA.
[4] Broad Institute of MIT and Harvard, Cambridge, MA, USA.
[5] Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China.

Ibrutinib, as a first-line treatment for Waldenstrom macroglobulinemia (WM), has demonstrated a good safety profile and efficacy, particularly in MYD88^{mut} patients. However, resistance to ibrutinib remains a significant clinical challenge.

To dissect the resistant tumor clones, we performed high-throughput droplet-based 5′-single-cell RNA sequencing paired with B-cell receptor (scBCR) sequencing on 75 longitudinal bone marrow samples collected at multiple timepoints from WM patients treated with ibrutinib monotherapy, with a median follow-up of 50 months

Three evolutionary patterns defined by copy number variations (CNV) were identified: 'clonal evolution', 'clonal de-evolution', and 'no evolution'. Whole exome sequencing (WES) of baseline and last-cycle samples confirmed the existence of these contrasting clonal evolution patterns. Notably, the 'clonal evolution' pattern was observed exclusively in patients with disease progression and showed a significant correlation with shorter progression-free survival (PFS). We further explored the dynamic transcriptional variations that might cause clones with static CNV to evolve ibrutinib resistance by comparing the 'winner clone' versus the 'loser clone' in patients with the 'clonal evolution' pattern. Analysis of commonly enriched upregulated and downregulated genes constructed a gene expression signature of response to ibrutinib, which was validated in a bulk RNA-seq cohort. Among these genes, the expression of the BCR signaling component LYN showed the highest upregulation. In vitro functional studies suggested that LYN contributes to the proliferation of WM cells and promotes ibrutinib resistance, pinpointing LYN as an informative biomarker and a novel therapeutic target in WM.