Extrinsic Interactions in the Microenvironment In Vivo Activate an Antiapoptotic Multidrug-Resistant Phenotype in CLL

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In this manuscript the authors Jayappa and colleagues from the University of Virginia address a potential mechanism of multidrug resistance in patients with chronic lymphocytic leukaemia (CLL).

They used the early activation marker CD69 and chemokine receptor CXCR4 to identify CLL cells that were recently engaged in microenvironmental interactions. They found that irrespective of patients' treatment status, the $CD69^{Pos}/CXCR4^{Low}$ CLL cells, considered to be activated and recently emigrated from the lymph node, exhibited significant overexpression of multiple anti-apoptotic proteins including Bcl2, Mcl-1 and Bcl-xL, as compared to $CD69^{Neg}/CXCR4^{High}$ counterparts, representing cells that were not recently engaged in microenvironmental interactions. The same results were observed in the $CD5^{High}/CXCR4^{Low}$ population, also representing activated CLL cells.

Furthermore, the authors found that classical NF-kB signalling pathway was significantly upregulated in $CD6^{Pos}/CXCR4^{Low}$ CLL cells as compared to $CD69^{Neg}/CXCR4^{High}$ counterparts. The authors subsequently employed cytotoxicity assays and observed that CLL cells expressing a marker of microenvironmental interaction ($CD69^{Pos}$) exhibited significant apoptosis resistance to several BH-domain antagonists tested individually as compared to their $CD69^{Neg}$ counterparts. The author concluded that diverse microenvironmental stimuli converge on NF-kB signaling, leading to NF-kB-dependent overexpression of multiple anti-apoptotic proteins and multi-drug resistance.

Next, the authors followed patients during venetoclax treatment and observed that surviving cells were enriched for leukemic B-cells displaying markers of activation. The authors suggested that those cells were selected during venetoclax treatment. They subsequently performed a focused combination drug screen with venetoclax as an anchor drug, combined with clinically tested or FDA approved drugs reported to decrease the threshold for apoptosis. They observed that combination of venetoclax with an inhibitor of NEDD8-activating enzyme (pevonedistat/MLN4924), proteasome (bortezomib), or MALT1 (MI-2), all of which also inhibit f NF-κB signalling, were synergistically effective in overcoming CLL multi-drug resistance. From these observations the authors were able to conclude that simultaneous inhibition of multiple anti-apoptotic proteins was required to overcome resistance of $CD69^{Pos}/CXCR4^{Low}$ CLL cells.

This manuscript provides mechanistic insight into the association between activation marker expression and CLL patient outcomes. Based on this results it may be inferred that functional assays using patient samples are required to predict treatment outcome and to timely counteract unwanted resistance to new targeted treatments.