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POSTER ABSTRACTS

631.CHRONIC MYELOID LEUKEMIA: BIOLOGY AND PATHOPHYSIOLOGY, EXCLUDING THERAPY

BCL-Xl Represents a Novel Therapeutic Target in Type 2 Mutant Calr-Driven Myeloproliferative Neoplasms

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Myeloproliferative neoplasms (MPNs) are clonal disorders that arise in the hematopoietic stem cell (HSC) compartment and that result in the excess production of mature blood cells of the myeloid lineage. All MPNs are unified by their reliance on the constitutive activation of the JAK/STAT pathway. Nevertheless, JAK inhibitors have proven insufficient to significantly improve disease outcome, highlighting the need to further understand the molecular mechanisms driving these diseases and to discover novel therapeutic targets. Calreticulin (CALR), an endoplasmic reticulum (ER) chaperone protein, has been found to be mutated in ~40% of essential thrombocytosis (ET) and myelofibrosis (MF) cases. The most common mutations in CALR are type 1 (52 base pair deletion; CALRdel52) and type 2 (5 base pair insertion; CALRins5) mutations, which both confer distinct clinical and prognostic characteristics. We have previously demonstrated that mutant CALR-driven MPNs rely not only on aberrant JAK/STAT signaling but also on the upregulation of BCL-2 family proteins and activation of the unfolded protein response (UPR) pathway (ASH abstract #147859, 2021). Specifically, we have shown that CALRins5 mutations lead to activation of and dependency on the activating transcription factor 6 (ATF6) branch of the UPR. In parallel, we found that CALRins5 cells exhibit differential up-regulation of the anti-apoptotic protein BCL-xL, but not BCL-2 (ASH abstract #3588, 2021). Based on these findings, we hypothesized that the active form of ATF6, which functions as a transcription factor, may directly transcriptionally activate BCL-xL leading to its up-regulation specifically in CALRins5 but not CALRdel52 MPN cells, and that the ATF6/BCL-xL axis may represent an important pro-survival mechanism that can be targeted for therapeutic gain in CALRins5 MPN patients.

To test this hypothesis, we first validated that BCL-xL is significantly up-regulated in CALRins5 versus CALRdel52 and CALR wild type (CALRwt) human cell lines and peripheral blood mononuclear cells (PBMCs) from MPN patients. Because BCL-xL can also be regulated downstream of JAK/STAT signaling, we next tested whether the increased expression of BCL-xL in CALRins5 cells is due to ATF6 activation, JAK/STAT activation, or both. In cells treated with JAK inhibitor ruxolitinib, we found that while BCL-xL expression levels decreased nearly 90% in ruxolitinib treated CALRdel52 cells, this decrease was much more modest in CALRins5 cells, suggesting JAK/STAT activation is only partially responsible for BCL-xL up-regulation in CALRins5 MPN cells. Conversely, in ATF6 knockout cells, we found that deletion of ATF6 did not affect BCL-xL levels in CALRwt or CALRdel52 cells, but did significantly abrogate BCL-xL up-regulation in CALRins5 cells. This suggests that in CALRins5 cells, while JAK/STAT signaling contributes in part to BCL-xL up-regulation, ATF6 is the key mediator of BCL-xL expression. CUT&RUN studies further revealed that ATF6 directly regulates BCL-xL transcription.

Lastly, we sought to test whether inhibition of BCL-xL alone is sufficient to induce apoptosis in CALRins5 cells. BH3 profiling studies revealed that CALRins5 cells are more primed for apoptosis via BCL-xL peptide antagonism compared to CALRdel52 cells. We next evaluated the activity of A-1331852, a BCL-xL selective inhibitor, alone or in combination with ruxolitinib, in

human cell lines and PMBCs from MPN patients. We observed that CALRins5 cells displayed decreased viability in response to A-1331852 alone and in combination treatments, suggesting an increased sensitivity to BCL-xL inhibition.
In conclusion, we demonstrate that CALRins5-driven MPN cells display an enhanced sensitivity to BCL-xL inhibition, which may represent an effective therapeutic approach for CALRins5+ MPN patients.

Disclosures No relevant conflicts of interest to declare.

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