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Lymphomas

BTK INHIBITION INDUCES DIFFERENTIAL RESPONSES OF BCR SIGNALING IN BASAL OR STIMULATED CONDITIONS IN MANTLE CELL LYMPHOMA

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Mantle cell lymphoma (MCL) is a rare B-cell malignancy with significant clinical heterogeneity. Although therapies targeting the BCR, such as the BTK inhibitor ibrutinib (Ibr), have a high initial response rate in MCL, relapses remain a challenge. We have recently shown that a deeper response of MCL cells to B-cell receptor (BCR) stimulation identified a subset of patients with a higher risk of progression.

To investigate the ability of Ibr to modulate the BCR signaling network, we analyzed the activating phosphorylation status of BCR-associated kinases (BAK), i.e., SYK, PLCγ2, STAT5, ERK 1/2, NF-κB p65, AKT, BTK, STAT3, in cells from peripheral blood of 29 MCL patients at diagnosis following Ibr treatment *in vitro*, in the basal condition and after BCR stimulation with anti-IgM -a condition that mimics antigen stimulation within the tumor microenvironment. We used phospho-specific flow cytometry, a multiparametric assay allowing functional signaling analysis at a single-cell level, combined with fluorescent cell barcoding.

In the basal condition, Ibr induced a significant average reduction of phosphorylation level for all BAKs but NF-κB p65 and STAT3. In the BCR-stimulated condition, we detected a significant average reduction of phosphorylation for all BAKs, including NF-κB p65, but STAT3. Comparison of the phosphorylation responses to Ibr for each BAK between the basal and BCR-stimulated conditions showed that the phosphorylation reduction in response to Ibr was significantly deeper in the BCR-stimulated condition compared with the basal one for SYK (P=0.011), ERK 1/2 (P<0.0001), NF-κB p65 (P<0.0001), and AKT (P<0.0001)(Figure 1).

In conclusion, our data show a differential sensitivity of BAKs to BTK inhibition in the basal condition and under BCR stimulation, thus highlighting that tumor microenvironment may influence the response of lymphoma cells to BCR-targeting therapies. Moreover, identifying novel BAKs that are inhibited by Ibr may form the rationale to target multiple signaling nodes to overcome resistance in MCL.

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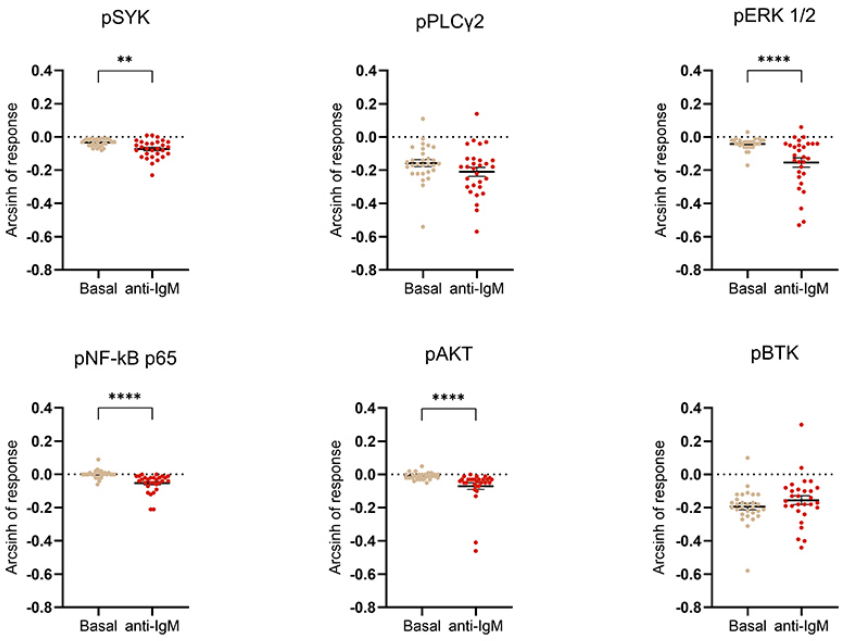

Figure 1 Response of B-cell receptor-associated kinases to Ibr treatment in vitro. Comparison of phosphorylation responses to Ibr in the basal and anti-IgM-stimulated conditions for each signaling protein in primary MCL cell samples (n=29). Cells were treated with 2uM Ibr for 1 hour at 37°C, followed by 10-minute treatment with 20 µg/ml goat F(ab') 2 anti-human IgM at 37°C. Phosphorylation responses were calculated referring to the basal and anti-IgM conditions, respectively. Comparison was performed using paired t or Wilcoxon test. **: P=0.011; ****: P<0.0001. Data were reported as mean + SEM.