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POSTER ABSTRACTS

605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Transcriptional Changes in Patients with CLL Starting Venetoclax Therapy Identifies Inflammatory and Adaptive Stress Responses

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Venetoclax is a selective BCL2 inhibitor that was recently approved for CLL treatment. Venetoclax inhibits the pro-survival protein BCL2 and induces deep and durable responses in a significant proportion of CLL patients. Herein, we aimed to study gene expression changes in leukemic cells from CLL patients during the ramp-up period of venetoclax treatment and to assess the transcriptional effect of escalating doses of BCL2 inhibition on tumor biology. We performed RNA sequencing on CD19+ selected tumor cells from 29 patients, ramped-up according to the standard Venetoclax schedule, with weekly increments from 20 mg to 400mg. Four samples were collected per patient, at baseline, and after completing one week on 50mg,100mg, and 400mg. Twelve (41%) patients switched from BTK inhibitor therapy to venetoclax due to disease progression on the BTKi. Those patients discontinued BTKi the day before they started Venetoclax ramp-up. 19 (59%) patients were either treatment-naïve or relapsed with at least months of treatment free-intervals before starting venetoclax.

Circulating CD19+ cell counts, typically composed of >95% CLL cells, were enumerated using flow-cytometry. during the ramp-up period, revealed gradual reduction in CLL burden with escalating doses. In the off-BTKi group, median CLL cell counts started to decline on the 100mg dose and were reduced to 98% of baseline at the end of the ramp-up. In the other patients, reductions in median counts were already apparent after one week on 20mg and reached 99% at the end of the ramp-up.

Principal component analysis (PCA) of transcriptome data grouped samples by patient, as we have commonly observed in such unsupervised analysis. After batch correcting the patient effect, PCA clearly separated on treatment timepoints from baseline (Figure). Interestingly, patients who recently stopped BTKi had distinct distribution on PCA plots in comparison to the other patients (Others). Indicating distinct transcriptomic changes in patients based on their recent treatment histories.

To identify the impact of venetoclax on the CLL transcriptome, we carried out Gene set enrichment analysis using the Hallmark and C3 gene sets from the molecular signature database. Gene sets were identified using a false discovery rate (FDR) of < 0.05, and a normalized enrichment score (NES) of $\geq |1.8|$. Gene sets were ranked by NES. In the off-BTKi patient group, 27 gene sets were identified as statistically significantly enriched at 100mg and 400 mg doses. In the "other" patients group, 15 gene sets were found significantly enriched at the same doses. Eight gene sets relating to inflammatory, interferon, and reactive oxygen species responses, cytokine signaling, and fatty acid metabolism. In the off-BTKi we prominently found enriched gene sets regulated by TP53 and related to DNA repair, cell cycle checkpoints, hypoxia and apoptosis. Consistent with an oxidative stress response we identified upregulation of NRF2, NFE2, TCF11, and BACH motif gene sets. The apparent more pronounced transcriptional response to venetoclax in the off-BTKi group prompted us to look more closely at changes in gene. At baseline, expression of BCL2L2 and NOXA was significantly lower in the off-BTKi patients in comparison to the "others" patient group. Surprisingly, on the 50mg dose expression of both the pro-apoptotic and anti-apoptotic BCL2 family members was significantly increased in the off-BTKi group in comparison to the others group; at the 100mg dose only pro-apoptotic genes remained significantly overexpressed in the off-BTKi group.

In conclusion, venetoclax induced a distinct and prominent transcriptional response in CLL patients indicating broad impact on tumor biology, even at doses below 400mg. Intriguingly, CLL cells from patients who had progressed on BTKi therapy and switched to venetoclax showed a more extensive stress response that included activation of DNA damage and anti-oxidant pathways. It will be interesting to correlate the identified transcriptional changes with the depth and durability of the venetoclax response.

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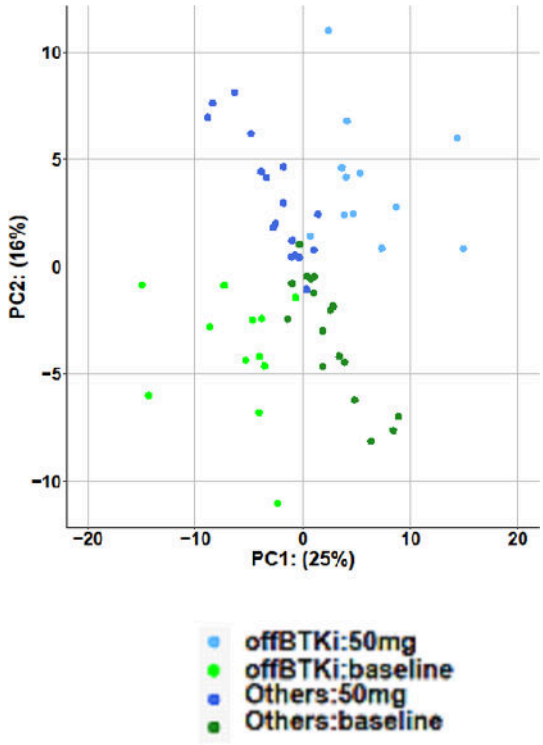

Figure 1

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