# (PF651) CLK INHIBITOR CTX-712 POTENTIATES THE ACTIVITY OF ABT-199 AND AZACYTIDINE IN MYELOID LEUKEMIA

**Topic:** 09. Myelodysplastic syndromes - Biology & translational research

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## Background

Alternative splicing (AS) is an essential mechanism in post-transcriptional mRNA processing and produces various mature mRNAs with different structures and functions. Dysregulation of AS plays a critical role in myeloid malignancy progression through abnormal expression or mutation of splicing factors. CDC2-like kinases (CLKs) are key regulators of AS via phosphorylation of serine/arginine (SR) domain rich spicing factors, including SRSF2, U2AF1 and ZRSR2, which are frequently mutated in myelodysplastic syndromes (MDS) and a subset of acute myeloid leukemia (AML). Therefore, we hypothesis that CLK might be a potential target for the treatment of MDS and AML.

## Aims

To study the effect of CLK inhibitor CTX-712 alone and in combination with ABT-199 and/or azacytidine on MDS and AML.

## Methods

Human bone marrow samples were obtained from MD Anderson Cancer Center tissue bank following institutional guidelines. CD34+ cells were selected using CD34 MicroBead kit. StemSpan SFEM II was used for CD34+ cell in vitro culture. RNA sequencing was used for RNA expression and alternative splicing analysis. Flow cytometry was used for apoptosis analysis. Western blot was used for protein analysis.

## Results

We observed significantly increased CLK2 ($p<0.05$) and CLK4 ($p<0.001$) mRNA expression in CD34+ cells from 55 prior untreated MDS patients compared with 20 healthy donors. CTX-712 is a potent pan-CLK inhibitor. To investigate the therapeutic potential of CTX-712, primary CD34+ cells from 3 MDS patients were expand in vitro and then treated with CTX-712 for 72 hours. The IC-50s for the 3 patients were: pt1 (CREBBP, SF3B1, SMC3) 26.8nM; pt2 (TERT, IKZF1) 119.9nM; pt3 (ASXL1, KRAS, SRSF2, TET2, STAG2) 45nM. Induced apoptosis was observed in a dose dependent manner.
AML cell lines MOLM3 and MOLM13-AZA (azacytidine resistant derivatives) were then treated with CTX-712 for 72 hours. The IC-50s were 35.5nM and 34.3nM respectively with no difference between the two cell lines. By western blot analysis, decreased phosphorylation of SRSF4 and SRSF6 were observed in a dose dependent manner in MOLM13 cells at 6 hours of CTX-712 treatment.
MOLM13 cells were then treated with CTX-712 combined with BCL-2 inhibitor ABT-199 and /or azacytidine for 72 hours. Synergistic apoptotic induction effect was observed: 40% of apoptosis was induced with 60nM of CTX-712 treatment, 41% and 56% with 3nM and 10nM of ABT-199 treatment alone; 86% and 94% were observed for the combination treatment. Synergistic apoptotic induction effect was also observed using CTX-712 combined azacytidine with or without ABT-199: 48% of apoptosis was induced with 60nM CTX-712 treatment, 27% with 300nM of azacitidine, 31% with 3nM of ABT-199 treatment. 80% of apoptosis was observed for the combination of CTX-712 and azacitidine. 96% for the combination of all the three drugs. To understand the mechanisms of the synergistic effect of these combinations, we performed RNA-seq analysis on MOLM13 cells. We observed CTX-712 treatment decreased the BCL2 and BCL2A1 expression, increased the BAX and BAK1 expression. Splicing analysis showed that CTX-712 alone or in combination induced increased splicing events, and skipped exon was the major event induced by CTX-712. ABT-199 and azacytidine treatment alone had no significant effect on RNA splicing.

## Summary/Conclusion

Taken together, our results suggested that the CLK inhibitor alone or in combination with ABT-199 and/or azacytidine could be a novel therapeutic approach for the treatment of MDS and AML.