

## P324 PHARMACOLOGIC INHIBITION OF DYRK1A RENDERS HIGH-RISK KMT2A-R ALL SENSITIVE TO VENETOCLAX

**Topic:** 01. Acute lymphoblastic leukemia - Biology & Translational Research

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**Background:** *KMT2A*-rearranged (R) ALL is a high-risk disease with a frequency of 70% in infants and 10% in children and adults with ALL and is associated with chemoresistance, relapse, and poor survival. Current intensive multiagent chemotherapy regimens induce significant side effects, yet fail to cure many patients, demonstrating continued need for novel therapeutic approaches.

**Aims:** Determine if pharmacologic DYRK1A inhibition may be a novel treatment strategy for patients with *KMT2A*-R ALL.

**Methods:** To identify novel targets in *KMT2A*-R leukemia, we performed a domain-specific kinome-wide CRISPR screen and identified multiple kinases required for cell growth. We focused on DYRK1A as it met the following three criteria: 1) Growth inhibition upon kinase targeting was greater in *KMT2A*-R leukemic cells than in non-*KMT2A*-R cells, 2) DYRK1A was not found to be common essential gene assessed through the Cancer Dependency Map, 3) Small molecule inhibitors are available.

**Results:** We analyzed multiple ChIP-Seq experiments and identified that *KMT2A*-fusions directly bind to the *DYRK1A* promoter. Our RT-PCR and Western blot analyses demonstrate that *KMT2A*-R ALL cells treated with a menin inhibitor to disrupt the transcriptional activity of the *KMT2A*-R complex, downregulate DYRK1A, indicating direct regulation of DYRK1A by the *KMT2A*-fusion. We further observed that pharmacologic inhibition of DYRK1A with EHT1610 induced leukemic cell growth inhibition *in vitro* and *in vivo*, demonstrating that DYRK1A could be a new therapeutic target in *KMT2A*-R ALL cells. To further elucidate the mechanism of DYRK1A function, we treated several *KMT2A*-R ALL cell lines *in vitro* with EHT1610, which surprisingly resulted in the upregulation of MYC and hyperphosphorylation of the RAS/MAPK target ERK. Given that ERK hyperactivation stops B cell proliferation during early B cell development to allow them to rearrange their B cell receptor, we hypothesized that cell cycle inhibition upon ERK hyperactivation remains as a conserved mechanism of cell cycle regulation in *KMT2A*-R ALL. Strikingly, combining DYRK1A inhibition with the MEK inhibitor trametinib antagonistically rescued *KMT2A*-R ALL cell proliferation, indicating that ERK hyperactivation is the main driver of DYRK1A inhibitor mediated cell cycle arrest. Given that DYRK1A inhibitor does not induce apoptosis and cells restart cell proliferation after EHT1610 withdrawal we concluded that a DYRK1A monotherapy may not be an ideal new treatment option. However, it has been reported that increased MYC activity induces the accumulation of BIM in Burkitt's Lymphoma. Given the increased expression of MYC following DYRK1A inhibition we performed a new Western blot analysis and validated increased expression of BIM in our *KMT2A*-R ALL cell lines after EHT1610 treatment. To test if targeting the interaction of BIM with BCL2 will induce an apoptotic effect when combined with EHT1610, we treated four *KMT2A*-R ALL cell lines with increasing concentrations of EHT1610 and the BCL2 inhibitor venetoclax. Strikingly, the combination of DYRK1A inhibition with BCL2 inhibition synergistically killed *KMT2A*-R ALL cells and significantly reduced the leukemia burden *in vivo*.

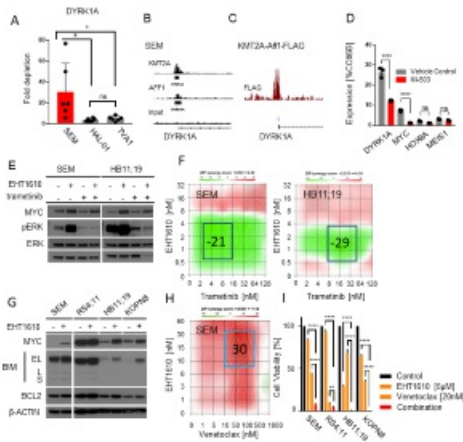
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## Image:



**Figure 1: DYRK1A is required to regulate ERK and MYC in KMT2A-R ALL**  
**A)** Comparison of DYRK1A depletion among 3 independent genome screens in SEM (*KMT2A-R*) and HAL\_01 (*TCF3-HLF*) ALL cell lines, and TVAL1 (*ETV6-ABL1*) PDX cells. For the lentiviral CRISPR screens, we compared the fold change from input (5 days after genome library transduction) and endpoint collection (4 weeks after the library transduction). **B)** ChIP-Seq tracks for KMT2A<sup>R</sup> and AFF1<sup>+</sup> antibodies versus input in the KMT2A-R ALL cell line SEM. **C)** ChIP-Seq of human CD34 cells transfected with a KMT2A-*AH1-FLAG* construct. Tracks for the FLAG antibody are shown. **D)** RT-PCR analysis of DYRK1A and the known *KMT2A* fusion targets, MYC, *NCX3B4*, and *IGCB1* after 6 days of Mi-523 treatment (1µM). **E)** Western blot analysis of KMT2A-R ALL cell lines treated with EHT1610 (DYRK1A inhibitor), trametinib (MEK inhibitor) or a combination of both drugs, demonstrating that MEK inhibition prevents DYRK1A inhibitor mediated ERK upregulation. Cells were treated with indicated concentrations for 72h. **F)** Synergy study identified that DYRK1A and MEK inhibition antagonistically rescue the KMT2A-R ALL cells. Shown are the averages of 3 independent experiments per cell line. **G)** Western blot analysis of indicated proteins in KMT2A-R ALL cells either treated with vehicle control or EHT1610 (72h, 10µM) demonstrating significant upregulation of MYC and BIM post DYRK1A inhibition. **H)** Synergy analysis of EHT1610 and venetoclax (BCL2 inhibitor)-treated ALL cell lines (only SEM shown), demonstrating that EHT1610 and venetoclax synergistically kill KMT2A cell. Cell lines were treated with indicated concentrations for 72h. Shown are the averages of 3 independent experiments per cell line. **I)** Flow cytometric analysis of KMT2A-R ALL cell lines treated with EHT1610 and venetoclax for 72h.

**Summary/Conclusion:** Our results validate DYRK1A as an important molecule to regulate cell proliferation via inhibition of MYC and ERK. Targeting DYRK1A results in the accumulation of BIM, which renders the cells sensitive to BCL2 inhibition via venetoclax. While further *in vivo* validation is needed, we predict that combining DYRK1A inhibition with venetoclax may be a novel precision medicine strategy for the treatment of *KMT2A-R* ALL.

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