HemaSphere

EHA EUROPEAN HEMATOLOGY ASSOCIATION

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

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

Christian Hurtz[1], Gerald Wertheim[2], John Chukinas[3], Rahul Bhansali[4], Srividya Swaminathan[5], John Crispino[6], Junwei Shi[7], Sarah Tasian[3], Martin Carroll[8]

[1] Fels Institute for Personalized Medicine, Temple University, Philadelphia, United States;[2] Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, United States;[3] Oncology, CHOP, Philadelphia, United States;[4] Hematology Oncology, Upenn, Pehiladelphia, United States;[5] Systems Biology, City of Hope, Monrovia, United States;[6] Experimental Hematology, St. Jude Children's Research Hospital, Memphis, United States;[7] Cancer Biology, Upenn, Philadelphia, United States;[8] Medicine, Upenn, Philadelphia, United States

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*.

Image:

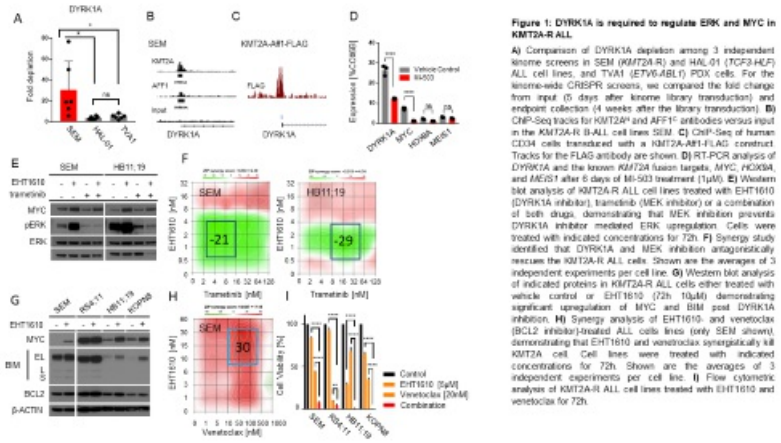

Figure 1: DYRK1A is required to regulate ERK and MYC in KMT2A-R ALL

A) Comparison of DYRK1A depletion among 3 independent kinome screens in SEM (*KMT2A*-R) and HAL-01 (TCF3-HLF) ALL cell lines, and TVM1 (*ETV6-ABL1*) PDX cells. For the kinome-wide CRISPR screens, we compared the fold change from input (5 days after kinome library transduction) and endpoint collection (4 weeks after the library transduction). **B)** ChIP-Seq tracks for KMT2A^{AR} and AFF1^{C} antibodies versus input in the *KMT2A*-R B-ALL cell lines SEM. **C)** ChIP-Seq of human CD34 cells transduced with a KMT2A-AR1-FLAG construct. Tracks for the FLAG antibody are shown. **D)** RT-PCR analysis of *DYRK1A* and the known *KMT2A* fusion targets, *MYC*, *HOXBA*, and *MEIS1* after 6 days of MI-503 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 rescues 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 cells 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.