Ultra-selective DYRK1A inhibitors as a new therapeutic approach for the treatment of hematological malignancies

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Background

- The dual-specificity tyrosine-regulated kinase 1A (DYRK1A) can phosphorylate multiple targets involved in key cellular processes that have been associated with the hallmarks of cancer, including proliferation, survival, cell cycle regulation, and the DNA damage response.5–8
- Non-selective inhibition or knockout of DYRK1A have demonstrated anti-tumor activity both in vitro and in vivo.4
- However, there has been a paucity of potent, specific, and orally bioavailable DYRK1A inhibitors that did not target the related cdk2-like kinases (CLKs) and glycogen synthase kinase 3B.
- In this study, we describe a set of highly selective DYRK1A inhibitors and used them to evaluate the specific contribution of DYRK1A to tumor biology and evaluate the therapeutic potential of DYRK1A inhibition in cancer as a single agent and in combination with standard of care venetoclax.

Conclusions

- We have developed multiple small molecule DYRK1A inhibitors that are highly specific and potent for DYRK1A and DYRK1B without inhibiting CLK family members.
- Hematological and SCLC cell lines are predicted to be more dependent on DYRK1A (DepMap) and more sensitive to DYRK1A inhibition, and acute myeloid leukemia (AML) cell lines treated with DYRK1A-B specific inhibitors showed a significant loss in viability.
- BioSplice’s DYRK1A inhibitors showed good oral bioavailability and significant tumor growth inhibition in MV-4-11 AML xenograft model.
- Inhibition of DYRK1A alone, and in combination with compounds like venetoclax, support further evaluation of DYRK1A inhibitors as a therapeutic strategy for some cancers, especially hematological malignancies (including AML) and possibly in solid tumors such as SCLC.

Fig. 1: Hematological and small cell lung cancer (SCLC) malignancies in TCGA and the CCLE have high DYRK1A expression

Fig. 2: Hematological and SCLC lineages are predicted to be highly sensitive to DYRK1A inhibition

Fig. 3: Profile of small molecule selective DYRK1A inhibitors

Fig. 4: Hematological cell line sensitivity to DYRK1A small molecule inhibition

Fig. 5: DYRK1A inhibition significantly reduces tumor growth in MV-4-11 xenograft model

Fig. 6: DYRK1A inhibition synergizes with venetoclax treatment in AML cell lines

Figure Legends and Methodology

- Fig. 1: (A, B) Bioplot showing the median RSEM values for DYRK1A mRNA expression in TCGA primary tumor cohorts. (B) Bioplot showing the median Log-transformed TPM values for DYRK1A mRNA expression in tumor-derived cell line images in the CCEL. Raw data downloaded from the publicly available TCGA and the CCEL resources.
- Fig. 2: (A) Heatmap showing the association between DYRK1A/CRES scores, DYRK1A mRNA expression and RB1 mutation using the BioViz.net model. DepMap CRES scores and CCRE mRNA expression data were used for the analysis. Cells in the table are colored by the significance of the association, p-values represent the significance of the association between the CRES score and either DYRK1A mRNA expression or RB1 mutation status. (B) Cumulative distribution plot highlighting the difference in CRES scores between non-melanoma and melanoma cell lines (n = 101) and other cell lines (blue; n = 876). (C) Bar plot showing the Log10-adjusted p-value of an enrichment analysis for the CRES score. (D) Boxplot showing the median RNA expression for CLK family members. (E) Dot plot showing the difference in CRES values across MLL lineage cell lines (n = 18) for 2 DYRK1A inhibitors (compound 1 red, compound 2 green, and compound 4 blue).
- Fig. 3: Table listing the target engagement of CLK/DYRK family members by four small molecule DYRK inhibitors. (A) Table listing the target engagement of CLK/DYRK family members by four small molecule DYRK inhibitors. (B) Table listing the target engagement of CLK/DYRK family members by four small molecule DYRK inhibitors. (C) Table listing the target engagement of CLK/DYRK family members by four small molecule DYRK inhibitors.
- Fig. 4: (A) Dot plot showing the difference in IC50 values between hematological lineage cell lines (n = 33) and other cell lines (n = 127) after a 4-day treatment, 10-point dose response from a top concentration of SM13328 (Cellfitt-Glo® luminescent assay). Significance determined through Mann-Whitney test. (B) Dot plot showing the difference in IC50 values across MLL lineage cell lines (n = 18) for 2 DYRK1A inhibitors (compound 1 red, compound 2 green, and compound 4 blue).
- Fig. 5: (A) Line plot showing the tumor volume in female athymic nude Foxn1 mice implanted with MV-4-11 cells in the right flank and treated with the indicated DYRK1A inhibitors (n = 6 mice per group). Significance values represent Kruskal-Wallis tests followed by multiple comparison procedures with Dunn’s test. ** p < 0.01; *** p < 0.0005. Each data point represents mean tumor volume ± SEM. (B) Table highlighting the EC50 and AUC for each small molecule inhibitor in panels A and B of Figure 5. Exposure values represent data collected at day 21 of each experiment. Compounds were administered daily and PO.
- Fig. 6: (A) A table showing synergy scores between DYRK1A Inhibitors and Venetoclax in five AML cell lines. In addition to a KG1a line that was made to be venetoclax-resistant. Cells are colored by synergy score cutoff values. (B) Bar plots showing synergy scores between DYRK inhibitors in combination with venetoclax in five AML cell lines, in addition to a KG1a line that was made to be venetoclax-resistant. Red horizontal line highlights a synergy score of 10, above which synergy is observed. Synergy scores were calculated using the Loewe model with Chou-ati software based on growth inhibition after a 4-day treatment following a dose-response matrix (9 x 9) at 13, 0, 0.1, 1, 10 to 10 μM.

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