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

Matthew Jarvis, PhD[1], Gopi Mittapalli, PhD[1], Emily Creger[1], Chelsea Nora, MS[1], Maureen Ibanez, MS[2], Deepti Bhat, MS[2], Brian Hofilena[1], Josh Stewart, PhD[2], Elizabeth McMillan, PhD[2], Hadi Falahatpisheh, PhD[2], Chi-Ching Mak, PhD[1], Michael A. White, PhD[2], John S. Hill, PhD[1], Carine Bossard, PhD[1]

[1]Biosplice Therapeutics, Inc., San Diego, CA 92121, [2]Formerly Biosplice Therapeutics, Inc.

Abstract #5019

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[1-2].
- Non-selective inhibition or knockout of DYRK1A have demonstrated anti-tumor activity both in vitro and in vivo[3-8].
- However, there has been a paucity of potent, specific, and orally bioavailable DYRK1A inhibitors that did not target the related cdc2-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 activity 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.**

Results

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

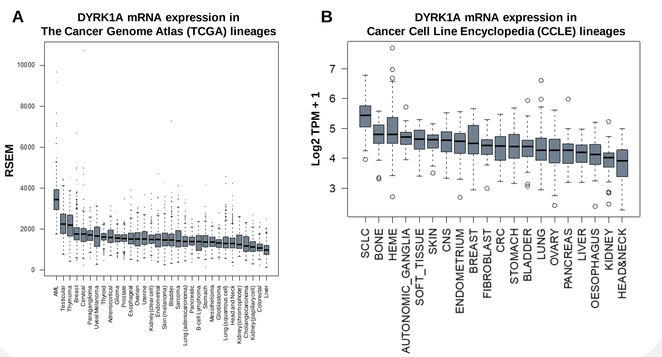


Fig. 2: Hematological and SCLC lineages are predicted to be highly sensitive to DYRK1A inhibition

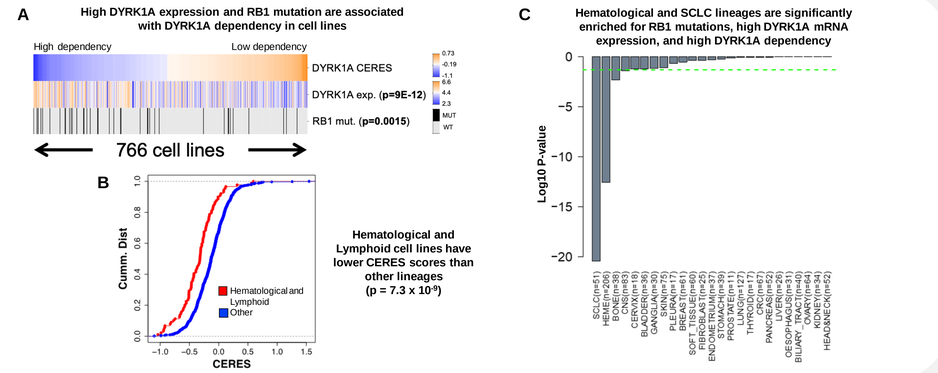
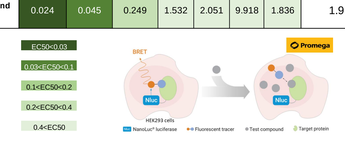


Fig. 3: Profile of small molecule selective DYRK1A inhibitors

A

Compound ID Number	Target Engagement EC50 (μM)							Full kinome ≥90% inhibition
	DYRK1A	DYRK1B	DYRK2	CLK1	CLK2	CLK3	CLK4	
Compound 1	0.134	0.757	3.241	0.746	8.025	9.98	0.939	0.8
Compound 2	0.019	0.026	0.077	0.483	0.575	9.980	0.418	2.3
Compound 3	0.048	0.096	0.577	1.400	2.630	9.259	1.403	1.0
Compound 4	0.024	0.045	0.249	1.532	2.051	9.918	1.836	1.9



B

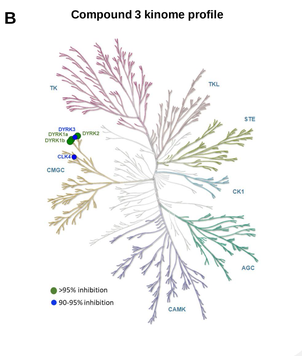


Fig. 4: Hematological cell lines are sensitive to DYRK1A small molecule inhibition

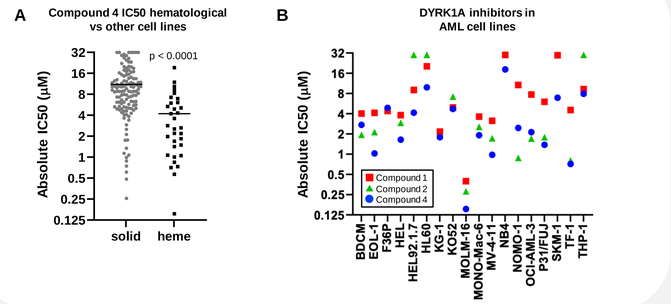
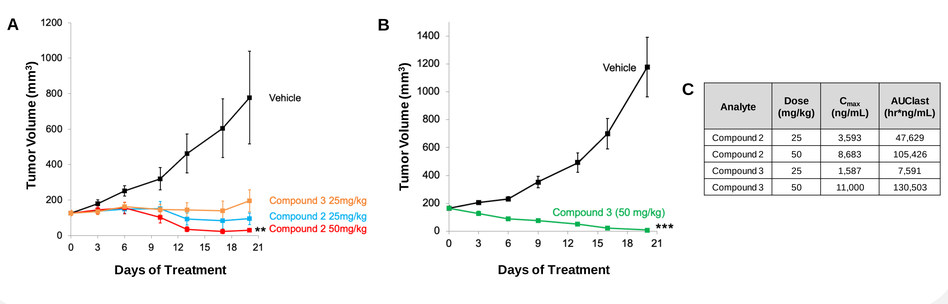


Fig. 5: DYRK1A inhibition significantly reduces tumor growth in MV-4-11 xenograft model



C

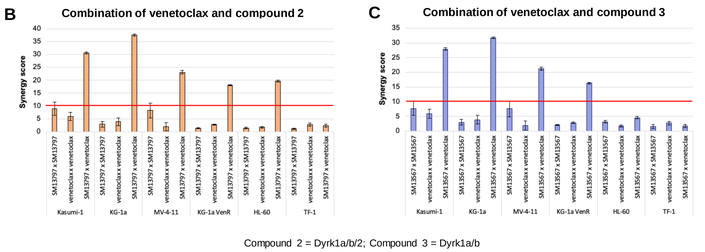
Analyte	Dose (mg/kg)	C_{max} (ng/mL)	AUClast (hr*ng/mL)
Compound 2	25	3,593	47,629
Compound 2	50	8,683	105,426
Compound 3	25	1,587	7,591
Compound 3	50	11,000	130,503

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

A

venetoclax in combination with:	Kasumi	MV-4-11	HL60	TF-1	KG1a	KG1a-VenR
Compound 2	30.56	23.11	19.64	2.35	37.61	18.04
Compound 3	27.91	21.18	4.59	1.70	31.76	16.32

	Synergy Score
High Synergy	>20
Low Synergy	10 to 20
Additivity	-10 to 10
Antagonism	< -10



Compound 2 = Dyrk1a/b/2; Compound 3 = Dyrk1a/b

Figure Legends and Methodology

- **Fig. 1**: (**A**) Boxplot showing the median RSEM values for DYRK1A mRNA expression in TCGA primary tumor cohorts. (**B**) Boxplot showing the median Log2-transformed TPM values for DYRK1A mRNA expression in tumor-derived cell line lineages in the CCLE. Raw data downloaded from the publicly available TCGA and the CCLE resources.
- **Fig. 2**: (**A**) Heatmap showing the association between DYRK1A CERES scores, DYRK1A mRNA expression and RB1 mutation using the Elastic Net model. DepMap CERES scores and CCLE 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 CERES score and either DYRK1A mRNA expression or RB1 mutation status. (**B**) Cumulative distribution plot highlighting the difference in CERES scores between hematological and lymphoid linage cell lines (red; n = 101) and other cell lines (blue; n = 878). (**C**) Bar plot showing the Log10-adjusted p-value of an enrichment score based on RB1 mutations status, DYRK1A mRNA expression, and DYRK1A CERES score for CCLE cell line lineages. The number of cell lines in each lineage is shown following the lineage label in the x-axis. Green line indicates p = 0.05.
- **Fig. 3**: (**A**) Table listing the target engagement of CLK/DYRK family members by four small molecule DYRK inhibitors. Target engagement assay EC_{50} values were determined using the Promega NanoBRET TE Intracellular Kinase Assay platform in transiently transfected HEK293T cells. IC_{50} values were determined from 10-point dose response curves using non-linear regression curve fit. Full kinome data is from Thermo Fisher Scientific SelectScreen™ Profiling Service using compounds at 1μM. (**B**) Dendrogram of the human kinome. Kinases inhibited more than 90% and 95% by Biosplice compound 3 are highlighted with a blue and green circle, respectively and labeled accordingly.
- **Fig. 4**: (**A**) Dot plot showing the difference in compound 4 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 30μM (CellTiter-Glo® luminescent assay). Significance determined through Mann-Whitney test. (**B**) Dot plot showing the difference in IC50 values across multiple AML lineage cell lines (n = 18) for 3 DYRK1A inhibitors (compound 1 in red, compound 2 in green, and compound 4 in blue).
- **Fig. 5**: (**A-B**) 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 DYRK inhibitors (n = 6 mice per group). Significance values represent Kruskal-Wallis tests followed by multiple comparison procedures with Dunn's test. ** is p < 0.01; *** is p < 0.0005. Each data point represents mean tumor volume ± SEM. (**C**) Table highlighting the C_{max} and AUC for each small molecule plotted 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-C**) 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 Chalice analyzer software based on growth inhibition after a 4-day treatment following a dose-response matrix (9 x 9) from a 15 μM or 50μM top concentration (CellTiter-Glo® luminescent assay). Error bars represent synergy score error.

References

1. Boni *et al.*, 2020
2. Deboever *et al.*, 2022
3. Lindberg and Meijer *et al.*, 2021
4. Bhansali *et al.*, 2021
5. Radhakrishnan *et al.*, 2016
6. Li *et al.*, 2019
7. Luna *et al.*, 2018
8. Pozo *et al.*, 2013
9. Massey *et al.*, 2021