PKM and *PKLR* mRNA expression in CD34⁺ cells derived from patients with myelodysplastic syndromes

MDS-673

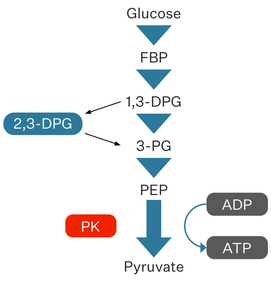
Erin Tsai[1], Leonardo Rivadeneyra[1], **Parija Patel**[1], Saliha Yilmaz[1], Megan Wind-Rotolo[1]

[1]Agios Pharmaceuticals, Inc., Cambridge, MA, USA

BACKGROUND

- Pyruvate kinase (PK) is a key enzyme in the glycolytic pathway, catalyzing the final step of glycolysis (**Figure 1**)[1]

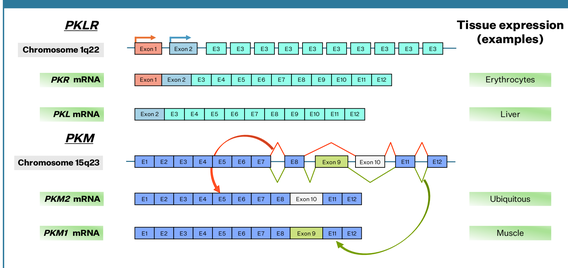
Figure 1. Pyruvate kinase catalyzes the final step of glycolysis[1]



ADP, adenosine diphosphate; ATP, adenosine triphosphate; DPG, diphosphoglyceric acid; FBP, fructose bisphosphate; PEP, phosphoenolpyruvate; PG, phosphoglycerate; PK, pyruvate kinase.

- PK is encoded by two different genes (*PKLR* and *PKM*), producing four protein isoforms as a result of tissue-specific promoters or alternative splicing (**Figure 2**):[2,3]
  - PKR (red blood cell [RBC]-specific);[2,3]
  - PKL (predominantly expressed in the liver);[2,3]
  - PKM1 (dominant in tissues with high metabolic demand);[2,4] and
  - PKM2 (prevalent in differentiating cells)[2,4]

Figure 2. Four PK isoforms are encoded by 2 genes[2,5–7]



E, exon; mRNA, messenger RNA; PK, pyruvate kinase; PKL, pyruvate kinase liver isoenzyme; PKLR, pyruvate kinase L/R; PKM, pyruvate kinase M1/2; PKM1, pyruvate kinase muscle isoenzyme 1; PKM2, pyruvate kinase muscle isoenzyme 2; PKR, pyruvate kinase RBC-specific; RBC, red blood cell.

- During early stages of hematopoiesis in healthy individuals, *PKM2* is the dominant isoform compared with *PKR*.[8] Subsequently, *PKM2* levels decrease upon RBC differentiation, with *PKR* becoming the dominant isoform[8] (**Figure 3**)

Figure 3. *PKM2* and *PKR* isoform expression in normal hematopoiesis[8]



Transcriptomes from hematopoietic and erythroid progenitors were evaluated, and mRNA levels of *PKL*, *PKR*, *PKM1*, and *PKM2* were measured at different stages of hematopoiesis.[8]

BasoE, basophilic erythroblast; CD, cluster of differentiation; CFU-E, colony forming unit–erythroid; CMP, common myeloid progenitor; MEP, megakaryocyte erythroid progenitor; MPP, multipotent progenitor; mRNA, messenger RNA; OrthoE, orthochromatic erythroblast; PK, pyruvate kinase; PKL, pyruvate kinase liver isoenzyme; PKM1, pyruvate kinase muscle isoenzyme 1; PKM2, pyruvate kinase muscle isoenzyme 2; PKR, pyruvate kinase RBC-specific; PolyC, polychromatic erythroblast; ProE, proerythroblast; RBC, red blood cell; Retic, reticulocyte.

- Dysregulation of hematopoiesis and ineffective erythropoiesis are hallmarks of myelodysplastic syndromes (MDS)[9,10]
- Preclinical data have shown glycolytic pathway dysfunction and reduced PK activity in RBCs from patients with anemia due to lower-risk MDS compared with healthy controls (HC)[11]
- Separately, ex-vivo data from 10 patients with various subtypes of MDS demonstrated a significant decrease in activity of PK relative to hexokinase activity when compared with HC participants[12]
- Collectively, these findings support glycolytic dysfunction in MDS and the potential of PK activation in lower-risk MDS

OBJECTIVE

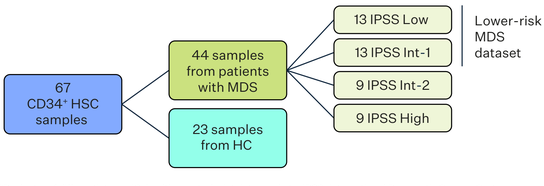
- **To evaluate the expression of different PK isoforms (*PKR*, *PKL*, *PKM1*, and *PKM2*) in CD34⁺ hematopoietic stem cells (HSC) of patients with MDS versus HC**

METHODS

Bioinformatics

- Bulk RNA sequencing data from a publicly available dataset[13] were obtained from **67** bone marrow-derived CD34⁺ HSC samples; **44** from patients with MDS and **23** HC (BioProject PRJNA435879)[14] (**Figure 4**)
  - Patient samples were generally obtained within 6 months of diagnosis of MDS

Figure 4. Distribution of patient samples



CD, cluster of differentiation; HC, healthy controls; Int, intermediate; IPSS, International Prognostic Scoring System; MDS, myelodysplastic syndromes.

- Data were processed using the following steps:
  - Quality control with FastQC (version 0.11.8)[15]
  - Flexible read trimming using Trimmomatic (version 0.39)[16]
  - Transcriptome mapping and alignment with the reference genome using Spliced Transcripts Alignment to a Reference (STAR) (version 2.7.1)[17]
- Following data processing, PK isoform transcripts were quantified by quantified by RNA-Seq by Expectation-Maximization (RSEM) (version 1.3.1),[18] and reported as transcripts per million (TPM)

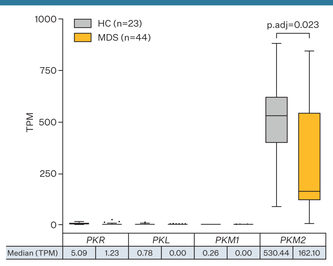
Data analysis

- Patients with MDS were analyzed both as a group overall (n=44) and as a subgroup of patients categorized as lower-risk MDS
  - The **lower-risk MDS group** was categorized based on the International Prognostic Scoring System (IPSS) classification and consisted of individuals designated to Low and Intermediate (Int)-1 risk categories[13] (n=26)
- Differential expression of transcripts was conducted using DESeq2, with reporting of adjusted p-values per the Benjamini–Hochberg methodology to account for multiple comparisons[19]

RESULTS

- *PKM2* was the dominant isoform detected in CD34⁺ HSC; levels of expression were significantly higher in HC when compared with patients with MDS (p. adjusted=0.023) (**Figure 5**)
- When considering the lower-risk MDS subgroup compared with HC, significantly lower expression of *PKM2* was also noted (p. adjusted=0.017) (**Figure 6**)
- The mRNA transcripts *PKL*, *PKR*, and *PKM1* were low to undetectable across both HC and MDS samples (all medians <5.09 TPM; n=67)*

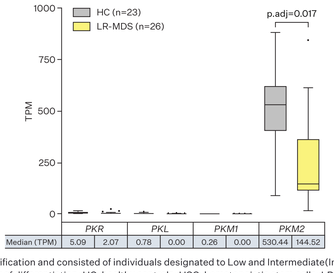
Figure 5. *PKR*, *PKL*, *PKM1*, and *PKM2* mRNA expression in CD34⁺ HSC samples from HC versus patients with MDS



Median (TPM)	PKR		PKL		PKM1		PKM2	
	5.09	1.23	0.78	0.00	0.26	0.00	530.44	162.10

adj, adjusted; CD, cluster of differentiation; HC, healthy controls; HSC, hematopoietic stem cells; MDS, myelodysplastic syndromes; mRNA, messenger RNA; PKL, pyruvate kinase liver isoenzyme; PKM1, pyruvate kinase muscle isoenzyme 1; PKM2, pyruvate kinase muscle isoenzyme 2; PKR, pyruvate kinase RBC-specific; RBC, red blood cell; TPM, transcripts per million.

Figure 6. *PKR*, *PKL*, *PKM1*, and *PKM2* mRNA expression in CD34⁺ HSC samples from HC versus patients with lower-risk† MDS



Median (TPM)	PKR		PKL		PKM1		PKM2	
	5.09	2.07	0.78	0.00	0.26	0.00	530.44	144.52

†Based on the IPSS classification and consisted of individuals designated to Low and Intermediate(Int)-1 risk categories[13]
adj, adjusted; CD, cluster of differentiation; HC, healthy controls; HSC, hematopoietic stem cells; LR-MDS, lower-risk MDS; MDS, myelodysplastic syndromes; mRNA, messenger RNA; IPSS, International Prognostic Scoring System; PKL, pyruvate kinase liver isozyme; PKM2, pyruvate kinase muscle isoenzyme 2; PKR, pyruvate kinase RBC-specific; RBC, red blood cell; TPM, transcripts per million.

CONCLUSIONS

- **The *PKM2* mRNA transcript was the dominant PK isoform detected in CD34⁺ HSC, with lower expression in patients with MDS, including lower-risk, compared with HC**
- **Findings from this study on differential expression of PK isoforms during the early stages of hematopoiesis in MDS compared with HC enhance understanding of the potential for PK activation in MDS**
- **Tebapivat, a potent PK activator of PKR and PKM2, is currently under clinical investigation for treatment of anemia due to lower-risk MDS (NCT05490446)[20–22]**

Acknowledgments: Writing and editorial support was provided by Obsidian Healthcare Group Ltd, funded by Agios Pharmaceuticals, Inc.

Disclosures: This study was funded by Agios Pharmaceuticals, Inc. *The safety and efficacy of tebapivat are under investigation and have not been established. There is no guarantee that tebapivat will receive health authority approvals or become commercially available in any country for the uses under investigation.*

ET, LR, PP, SY, MW-R: Agios – employees and shareholders.

References: 1. Schormann N et al. *Protein Sci* 2019;28:1771–1784. **2.** Alquraishi M et al. *Free Radic Biol Med* 2019;143:176–192. **3.** Dayton TL et al. *EMBO Rep* 2016;17:1721–1730. **4.** Taniguchi K et al. *Sci Rep* 2015;5:8647. **5.** Bludau I, Aebersold R. *Nat Rev Mol Cell Biol* 2020;21:327–340. **6.** Homo sapiens pyruvate kinase L/R (PKLR). GenbankAccession No. NM_000298. Updated June 9, 2025. Last accessed June 26, 2025. https://www.ncbi.nlm.nih.gov/gene/5313. **7.** Homo sapiens pyruvate kinase M1/2 (PKM). GenBank Accession No. NM_002654. Updated June 21, 2025. Last accessed June 26, 2025. https://www.ncbi.nlm.nih.gov/gene/5315. **8.** Tsai E et al. Poster presented at EHA 2023, Frankfurt, Germany (and Virtual). June 2023. **9.** Merz AM, Platzbecker U. *Haematologica* 2025;110:330–338. **10.** Elias HK et al. *Oncogene* 2014;33:5139–5150. **11.** Fattizzo B et al. *Am J Hematol* 2024;99:1201–1204. **12.** de Wilde JRA et al. *Blood* 2023:142(Suppl 1):3222–3224. **13.** Im H et al. *Leuk Lymphoma* 2018;59:2952–2962. **14.** BioProject. 2018; Available from: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE111085. Updated March 27, 2019. Last accessed July 1, 2025. **15.** Babraham Bioinformatics. FastQC. 2023; Available from: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/. Updated March 1, 2023. Last accessed July 1, 2025. **16.** Bolger AM et al. *Bioinformatics* 2014;30:2114–2120. **17.** Dobin A et al. *Bioinformatics* 2013;29:15–21. **18.** Li B, Dewey CN. *BMC Bioinformatics* 2011;12:323. **19.** Love MI et al. *Genome Biol* 2014;15:550. **20.** ClinicalTrials.gov. 2025; Available from: https://clinicaltrials.gov/study/NCT05490446. Last accessed July 23, 2025. **21.** Zeidan AM et al. *Blood* 2024:144(Suppl 1):6708–6710. **22.** Al-Samkari H et al. *Blood* 2022:140(Suppl 1): 4076–4078.

Text footnotes: *Cumulative TPM across all 67 samples was <2.1 TPM.

Presented at Society of Hematologic Oncology (SOHO) 2025, September 3–6, 2025, Houston, Texas.