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ELEVENTH ANNUAL MEETING

PYRIMETHAMINE SHOWS SYNERGY AND EFFICACY IN COMBINATION WITH VENETOCLAX IN PRECLINICAL MODELS OF THERAPY RESISTANT MDS/AML

B. Rivera-Peña[1], S. Chakraborty[1], Y. Shi[2], H. Zhang [1], R. Zhao[1], G. Choudhary[1], S. Gordon-Mitchell[1], K. Pradhan[1], B. Fu[1], I. Tosic[3], C. Schinke[4], A. Varshavsky[5], J. Feld [6], M. Goldfinger[1], M. Carbajal[1], N. Ramachandra[1], M. Konopleva[1], D. Frank[4], Y. Sauntharajah[5], A. Verma[1], A. Shastri[1]

1. Department of Oncology, Division of Hema-Oncology, Albert Einstein College of Medicine, Bronx, NY, USA.
2. Department of Pathology, Albert Einstein College of Medicine & Montefiore Medical Center, Bronx, NY, USA.
3. Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA.
4. Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, Little Rock, AR, USA.
5. Department of Bone Marrow Transplant and Cellular Therapies, Fox Chase Temple University Hospital, Philadelphia, PA, USA.
6. Tisch Cancer Institute, Mount Sinai Icahn School of Medicine, New York, NY, USA.
7. Department of Hematology and Medical Oncology, Winship Cancer Institute-Emory University School of Medicine, Atlanta, GA, USA.
8. Translational Hematology and Oncology Research, Cleveland Clinic, Cleveland, OH, USA

EINSTEIN
Albert Einstein College of Medicine
Montefiore

INTRODUCTION

- MDS and AML arise after accumulation of genetic and epigenetic alterations in hematopoietic stem cells (HSCs) that can potentially give rise to leukemic stem cells (LSCs).
- Therapies for MDS and AML have failed to eliminate LSCs which are the basis of disease relapse.
- Our previous studies show that HSCs exhibit overexpression of the signal transducer and activator of transcription 3 (STAT3) (Shastri, 2017).
- Overexpression of STAT3 is observed in MDS and AML patients and it is predictive of a significantly adverse prognosis.
- We previously showed that an antisense oligonucleotide inhibitor of STAT3 can get incorporated into HSCs, inducing apoptosis and downregulation of STAT3 when compared to health controls (Shastri, 2018).
- An initial screen identified pyrimethamine (PYR), an FDA approved anti-parasitic drug, as a STAT3 inhibitor that could inhibit STAT3 transcriptional activity at concentrations readily achieved in patients (Nelson, 2011).

AIM

- Molecular targeting of aberrant LSCs may be a potential strategy for developing agents capable of eliminating LSCs.
- HMA and Ven resistance is a large unmet need in MDS/AML and can occur due to the persistence of relapse associated with aberrant HSCs that overexpress STAT3.
- **Our study evaluates the efficacy of PYR as a treatment for MDS and AML. Furthermore, we assess PYR efficacy in a hypomethylating agent (HMA) and venetoclax (Ven)-resistant model.**

METHOD

Cell Viability Assays

- **THP1, MDSL, KT1, KG1, CMK, cell lines**
  - PYR 0.5 µM, 1 µM or 10 µM for 72 hrs
- **HMA resistant THP1, Ven resistant MOLM13 AML cell lines**
  - PYR 1 µM, 5 µM or 10 µM alone or combination Aza 10 µM or Ven 1 µM, or 10 µM for 48 hrs

Apoptosis Analysis

- **THP1, MDSL cell lines**
  - PYR 0.5 µM, 1 µM, 2.5 µM, 5 µM and 10 µM for 72 hrs
  - FACS Annexin V and PI

Western Blot analysis

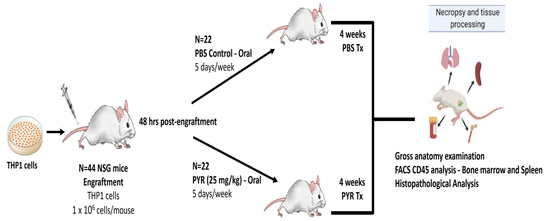
Drug Synergy analysis

- Analysis and visualization of PYR and Aza or Ven drug combination response data.

Primary MDS patient samples

- FACS cell differentiation analysis after PYR treatment

***in vivo* CDX model strategy**



RESULTS

Figure 1

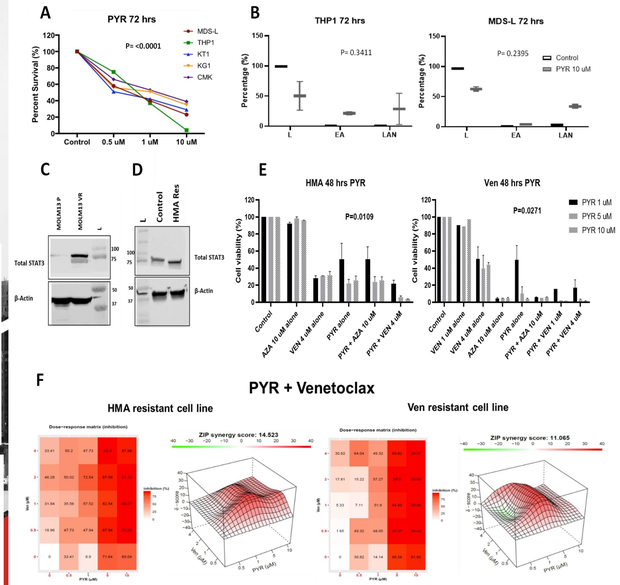


Figure 1. PYR can inhibit proliferation and induce apoptosis in MDS and AML cell lines, including HMA and Ven resistant cell lines. **A.** MDS and AML cell lines were treated with PYR 0.5uM, 1uM & 10 uM respectively and viability assessed at 72 hrs with Cell-Titer blue assay. **B.** THP-1 and MDS-L cell lines were treated with PYR 0.5 uM, 1 uM, 2.5 uM, 5 uM and 10 uM respectively for 72 hrs. Cells were stained with Annexin V and PI analyzed by FACS. L = Live cells; EA = Early Apoptotic cells; LAN = Late Apoptotic and Necrotic cells **C.** Ven resistant cell MOLM13 AML cell line shows increased total STAT3 protein expression **D.** HMA resistant THP1 AML cell line shows increased total STAT3 protein expression **E.** HMA and Ven resistant cell lines were treated with PYR 1uM, 5uM or 10 uM alone or in combination with Aza 10 uM or Ven 1uM, or 10 uM. Viability was assessed at 48 hrs with Cell-Titer Glo assay. **F.** Drug synergy analysis between PYR and Venetoclax combination in HMA and Ven resistant cell lines.

Figure 2

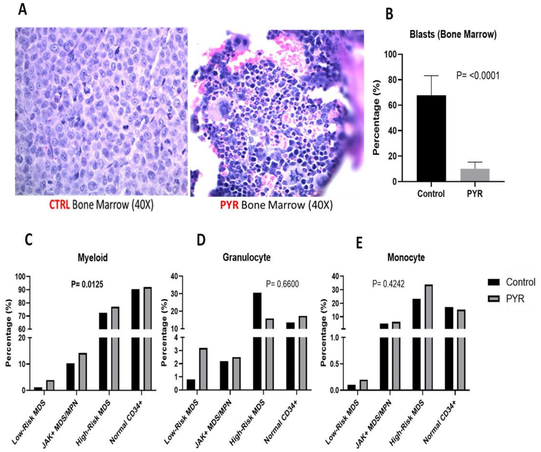


Figure 2. PYR efficacy in an *in vivo* CDX model and in primary MDS patient samples. **A.** PYR treated and Control mouse Bone Marrow H&E stain (40X). **B.** *in vivo* CDX model bone marrow blast percentage after PYR treatment and Control **C.** Myeloid differentiation percentage in primary MDS patient samples treated with PYR. **D.** Granulocyte differentiation percentage in primary MDS patient samples treated with PYR. **E.** Monocyte differentiation percentage in primary MDS patient samples treated with PYR.

REFERENCES

Nelson E.A., Sharma S.V., Settleman J., Frank D.A. A chemical biology approach to developing STAT inhibitors: Molecular strategies for accelerating clinical translation. *Oncotarget.* 2011;2:518–524.

A. Shastri, M. Teixeira, S. Bhattacharya, N. Ramachandra, R. Lopez, G. Ravipati, T. et al. Therapeutic Targeting of MDS & AML Stem Cells with an Antisense Inhibitor of STAT3, Leukemia Research. 55, Supplement 1,2017

A. Shastri, G. Choudhary, M. Teixeira, S. Gordon-Mitchell, N. Ramachandra, L. Bernard, S. Bhattacharyya, et al. Antisense STAT3 inhibitor decreases viability of myelodysplastic and leukemic stem cells. *J Clin Invest.* 2018;128(12):5479-5488.

Ianevski, A., Giri, K. A., Aittokallio, T., 2022. SynergyFinder 3.0: an interactive analysis and consensus interpretation of multi-drug synergies across multiple samples. Nucleic Acids Research.

CONCLUSIONS

- Results show that MDS/AML cell lines, including HMA and Ven resistant leukemic cell lines, are sensitive to PYR treatment, in a dose dependent manner within the therapeutic index of STAT3 inhibition (5-10 uM).
- PYR and Ven drug combination show a significant reduction in cell proliferation and likely drug synergy.
- Our current findings support PYR synergy with Ven and the combination could be a potential therapeutic option for patients with MDS/AML after prior treatment with hypomethylating agents for MDS and AML.
- Our preclinical data provide evidence of synergy between PYR and venetoclax and rationale to move this combination forward into an early phase clinical trial in HMA-resistant MDS.

CONTACT INFORMATION

Bianca Rivera-Peña, PhD
bianca.riverapena@einsteinmed.edu
Aditi Shastri, MD
ashastri@montefiore.org
Amit Verma, MD
amit.verma@einsteinmed.edu

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