Activation of Pyruvate Kinase by Mitapivat Potentially Rescues Ineffective Erythropoiesis in Models of Diamond Blackfan Anemia.

Jonathan R.A. de Wilde[1], Richard Pozzetto Huot[2], Veronica Riccardi[2], Yuefeng Tang[1,3], Alexis Bertrand[1], Riaz Rao[1], Marion Serra[1], Naomi Taylor[4], Vincent P. Schulz[5], Patrick G. Gallagher[6], Jeffrey M. Lipton[1,3,7], Roberta Russo[8,9], Achille Iolascon[8,9], Leo Rivadeneyra[10], Megan Wind-Rotolo[10], Mohandas Narla[11], Julien Papoin[1,3], Lucia de Franceschi[2,12]* and Lionel Blanc[1,3]*.

[1]The Feinstein Institutes for Medical Research, Northwell Health, Manhasset, NY, USA; [2]Department of Engineering for Innovative Medicine, University of Verona, Verona, Italy; [3]Zucker School of Medicine at Hofstra/Northwell, Hempstead, NY, USA; [4]Pediatric Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA; [5]Department of Pediatrics, Yale School of Medicine, New Haven, CT, USA; [6]Center for Perinatal Research, Nationwide Children's, Ohio State University, Columbus, OH, USA; [7]Division of Hematology/Oncology, Cohen Children's Medical Center, New Hyde Park, NY, USA; [8]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Naples, Italy; [9]CEINGE Biotecnologie Avanzate, Naples, Italy; [10]Agios Pharmaceuticals, Inc., Cambridge, MA, USA; [11]Lindsey F. Kimball Research Institute, New York Blood Center, New York, NY, USA; [12]Azienda Ospedaliera Universitaria Integrata, Verona, Italy. *Co-senior.

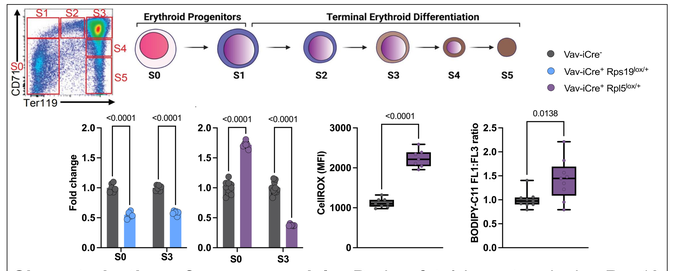
INTRODUCTION

Diamond-Blackfan anemia (DBA) is a rare congenital bone marrow failure syndrome, characterized by erythroid hypoplasia. It is predominantly caused by pathogenic variants in genes encoding ribosomal proteins and related factors, leading to imbalance in ribosome subunits, cellular stress, and premature cell death. Treatment options for DBA are limited, and a significant proportion of patients is transfusion-dependent due to corticosteroid resistance. Pyruvate kinase (PK) activation therapy has not yet been explored in DBA.

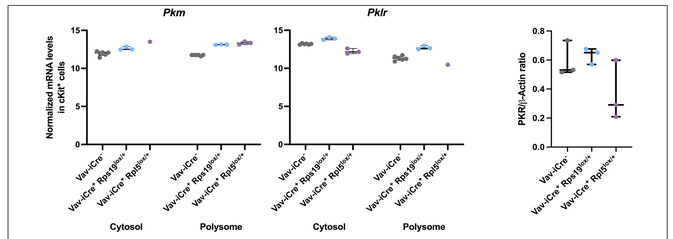
AIM

Using our novel mouse models of DBA, representing two of the most commonly affected ribosomal protein genes (i.e., *RPS19* and *RPL5*), we evaluated how ribosomal haploinsufficiency affected metabolism in hematopoietic progenitor cells and maturing erythroblasts. We then assessed the effect of *ex vivo* treatment with the PK activator, mitapivat, both on murine cells derived from both the mouse models as well as on human cells derived from DBA patients.

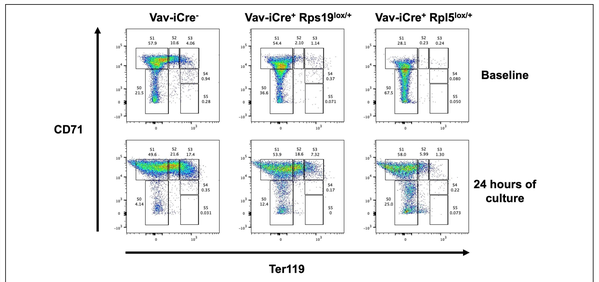
RESULTS



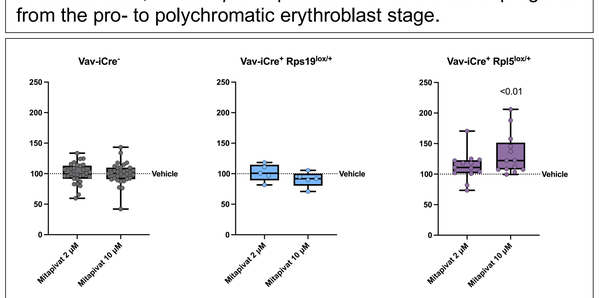
Characterization of mouse models. During fetal hematopoiesis, *Rps19*-haploinsufficient mice deplete their pool of stem and progenitor cells, whilst erythroid progenitors are affected in *Rpl5*-haploinsufficiency. This is paired by distinct cell death mechanisms: apoptosis in *Rps19* versus ferroptosis in *Rpl5.* The latter is supported by, among others, increased levels of oxidative stress (CellROX) and lipid peroxidation (BODIPY-C11 FL1:FL3 ratio).



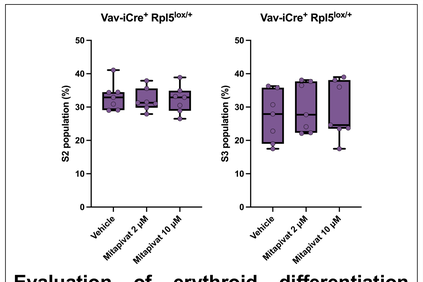
Altered regulation of PK isoforms. Both PKM and PKR appear to be affected on the transcriptional, translational and protein level.



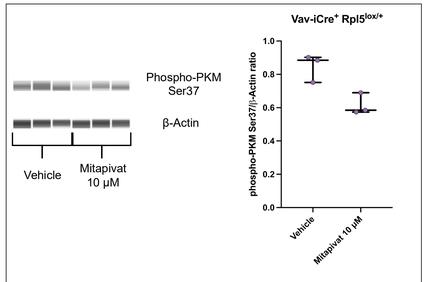
Erythroid culture reflects phenotype of mouse models. Whereas *Rps19*-haploinsufficient cells develop almost similarly to control cells, whilst *Rpl5*-haploinsufficient cells fail to progress from the pro- to polychromatic erythroblast stage.



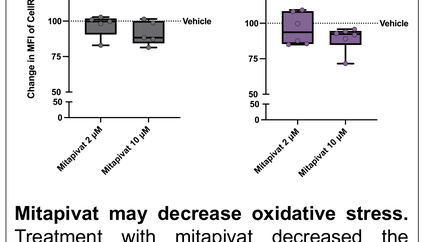
***Rpl5*-haploinsufficient cells benefit from mitapivat treatment.** Mitapivat treatment did not improve proliferation in control or *Rps19*-haploinsufficient cell culture. However, mitapivat did promote survival of *Rpl5*-haploinsufficient cells, which generally do not proliferate during the erythroid culture.



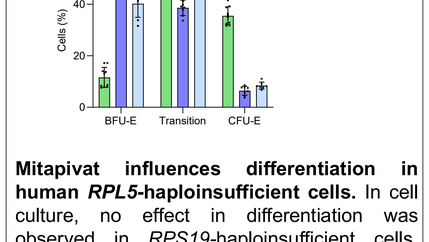
Evaluation of erythroid differentiation upon mitapivat treatment. Mitapivat did not evidently influence erythroid differentiation of the murine *Rpl5*-haploinsufficient cells during erythroid cell culture.



Mitapivat affects PKM phosphorylation. Mitapivat decreased the Ser37 phosphorylated form of PKM, which may acquire nuclear localization and has been linked to ferroptosis.



Mitapivat may decrease oxidative stress. Treatment with mitapivat decreased the CellROX signal in *Rpl5*-haploinsufficient cells, suggesting decreases in ROS levels.



Mitapivat influences differentiation in human *RPL5*-haploinsufficient cells. In cell culture, no effect in differentiation was observed in *RPS19*-haploinsufficient cells, whilst in *RPL5*-haploinsufficient cells mitapivat enhanced the transition from BFU-E to CFU-E.

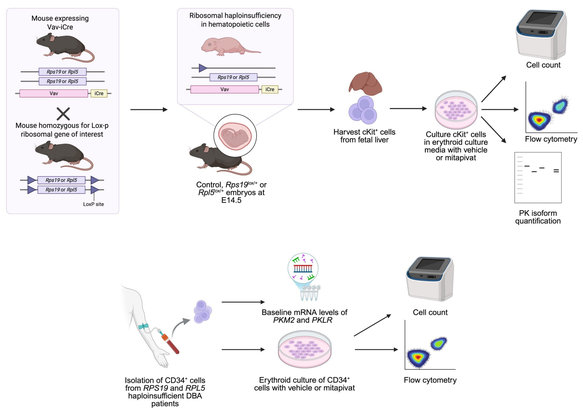
METHODS

Cells derived from the fetal liver of the *Rps19* and *Rpl5* haploinsufficient mice were studied in terms of metabolism, specifically focusing on two PK isoforms, PKM and PKR, using polysome sequencing and western blot analysis.

To study the effect of mitapivat, fetal liver-derived cKit$^+$ cells were cultured for 48 hours in presence of vehicle (DMSO 0.1%), mitapivat 2 µM or mitapivat 10 µM. The effects on proliferation and differentiation were assessed. Effects of mitapivat on oxidative stress (CellROX) and phosphorylation of PKM were assessed.

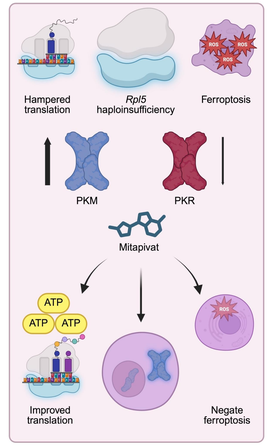
Patient-derived CD34$^+$ cells were cultured with mitapivat (2 µM) to study the effect on proliferation and differentiation.

Statistical significance (*P*-value <0.05) was assessed using a non-parametric T-test or Friedman test.



CONCLUSIONS

- ✓ Our *Rps19*- and *Rpl5*-haploinsufficient mouse models exhibit differential mechanisms of cell death, with ferroptosis in *Rpl5*-haploinsufficient erythroid progenitors.
- ✓ The PK isoforms are differently expressed, with an increase of PKM and decrease of PKR in the *Rpl5*-haploinsufficient cells.
- ✓ Erythroid culture demonstrates that *Rpl5*-haploinsufficient cells fail to progress to erythroblasts, in contrast to *Rps19*-haploinsufficient cells.
- ✓ In vitro treatment with mitapivat partially rescued proliferation of *Rpl5*-haploinsufficient cells, potentially by promoting PKM tetramerization and negating oxidative stress.
- ✓ Cultures with patient-derived CD34$^+$ cells support the potential beneficial effect of mitapivat in *RPL5*-haploinsufficient human erythroid progenitors.



ACKNOWLEDGEMENTS

We would like to thank all patients who participated in this study.

This study was supported by NIH NHLBI grant R35HL171168, an Agios research grant and the DBA-Italia foundation.

CONTACT INFORMATION

jdewilde@northwell.edu

lucia.defranceschi@univr.it

lblanc@northwell.edu