

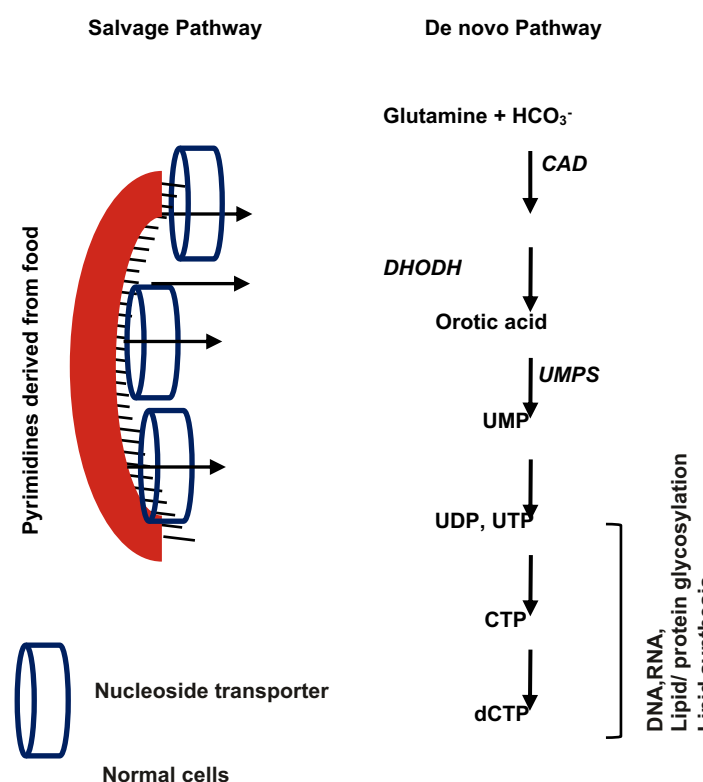
Emvododstat (PTC299) Targets De Novo Pyrimidine Synthesis in Acute Myeloid Leukemia

Sujan Piya^{1*}, Huaxian Ma^{1*}, Kensuke Kojima², Vivian Ruvalo^{1*}, Mahesh Basyal^{1*}, Marina Konopleva¹, Marla Weetall³, Josephine Sheedy³, Balmiki Ray³, Michael Andreeff^{1*}, Gautam Borthakur¹

Departments of ¹Leukemia and ^{*}Section of Molecular Hematology and Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX, ² Department of Leukemia, Saga University, Saga, Japan and ³ PTC Therapeutics, South Plainfield, NJ

Abstract

De novo nucleotide synthesis is a dynamic process that can address the enormous demand for nucleotides and other macromolecules required Acute myeloid leukemia (AML) proliferation^{1, 2, 3,4}. Hence, we hypothesized that targeting *de novo* nucleotide synthesis would lead to depletion of the nucleotide pool and pyrimidine starvation in leukemic cells compared to their non-malignant counterparts and impact proliferative and differentiation pathways. Emvododstat (PTC299) is an inhibitor of dihydroorotate dehydrogenase (DHODH), a rate limiting enzyme for de novo pyrimidine nucleotide synthesis that is currently in a clinical trial for the treatment of AML.



Objective

The goals of these studies were to demonstrate that emvododstat effects on leukemia growth are due to inhibition of *de novo* pyrimidine nucleotide synthesis for leukemic growth. A comprehensive analysis mitochondrial function, metabolic signaling in PI3K/AKT pathways, apoptotic signatures and DNA damage responses were evaluated. Clinical relevance of emvododstat efficacy was confirmed in an AML-PDX model.

Materials & Methods

Human T-ALL / Patient's derived T-ALL and PDX were used for study. Emvododstat (PTC299) used to target DHODH. Flow cytometry based Annexin binding assay for determination of activity of Emvododstat. Immunoblotting (WB) and Mass cytometry (CyTOF) for proteomic analysis. Disseminated mouse model – tail vein injection of ALL in NOD-*scid* IL2Rgamma^{null} (NSG) mice for preclinical study.

Results

Emvododstat exhibited broad spectrum activity in AML with IC₅₀s in very low nanomolar range (2-100 nM).

Cell Line	Mutations	IC50	ED50
HL-60	NRASm (Q61L), P53 null, CDKN2A	59.7 nM	979.5 nM
KBM5	BCRABL, TP53, ASXL1	18.2 nM	408.4 nM
KBM5:STI	BCRABL, TP53, ASXL1	2.7 nM	108.8 nM
K562	CDKN2A(del471), P53	16.12 nM	145.16 nM
MOLM13	ITD (m/w), t(9;11)	5.6 nM	28.5 nM
MOLM14	ITD, t(9;11)	21.31 nM	55.56 nM
MV4;11	ITD (m/m), t(4;11)	13.15 nM	18.34 nM
OCI-AML3	NPMc+, DNMT3Am (R822C), NRASm	4.43 nM	41.16 nM
OCI-AML2	NPMwt, DNMT3Am (R635W)	8.09 nM	82.19 nM
U937	NRASm (G12D), CDKN2A(del471)	11.71 nM	22.85 nM
THP-1	FLT3WT, CALM-AF10	14.3 nM	45.2 nM

# Primary sample	Bulk (CD45+)	Stem and progenitor (CD34+CD38-)
#1:NF1,PTPN11,NRAS, TP53,U2AF1	24.03 nM	18.26 nM
#2:NRAS, KIT, FLT3	83 nM	85 nM

Table 1: AML/Primary AML cells were treated with Emvododstat in different concentration for 3 days or 5-7 days respectively and determine the IC₅₀ and ED₅₀ values,

Emvododstat induced apoptosis in cytarabine-resistant AML, and induced differentiation, and reduced blast proliferation. In primary AML

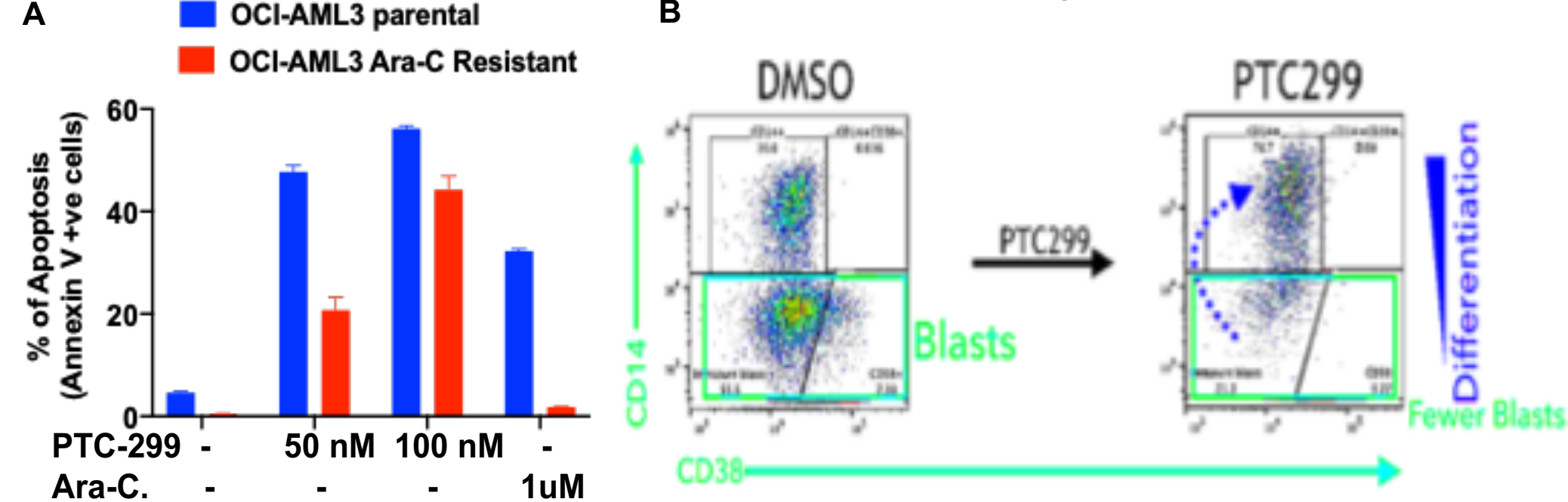


Figure 1: (A)OCI-AML parental or Ara-C resistant cells were treated with emvododstat (PTC299) and Ara-C for 72hours and subjected to flow cytometry after staining for Annexin V. (B)-Primary AML treated with emvododstat (PTC299) and subjected to flow cytometry after staining for CD34, CD45 and CD14.

Exogenous uridine and orotate rescue the apoptosis phenotype induced by Emvododstat

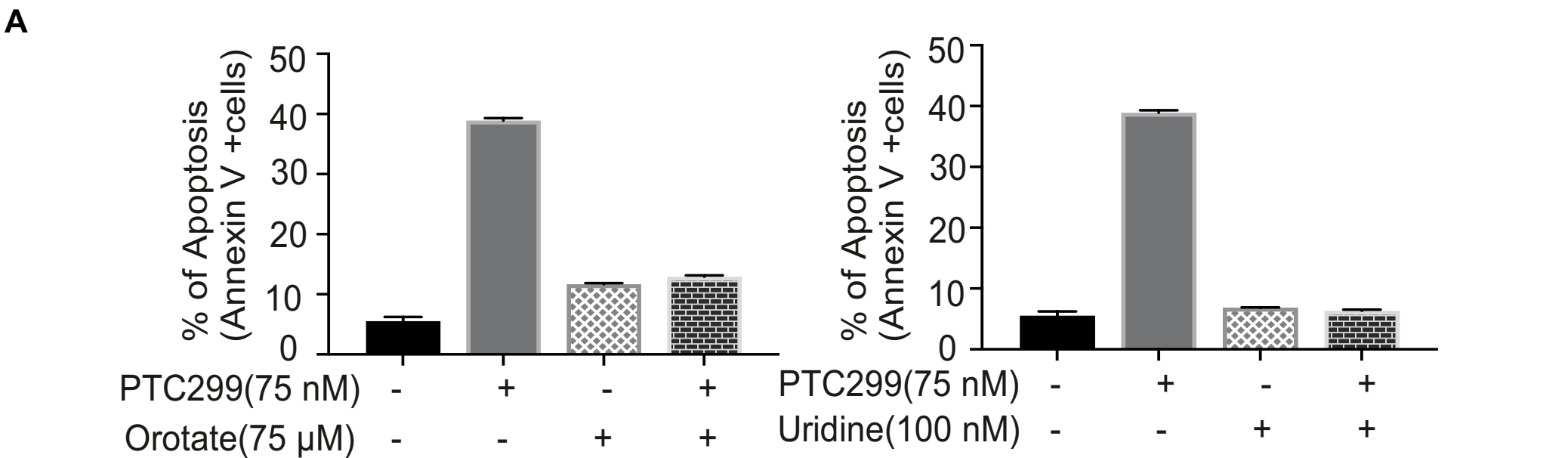


Figure 3:OCI-AML cells were treated with emvododstat (PTC299) with or without (A) orotate (B) uridine for 72 hours and measure percentage of apoptosis by flow cytometry.

Results

Emvododstat alter the mitochondrial function/ dynamic metabolic landscape

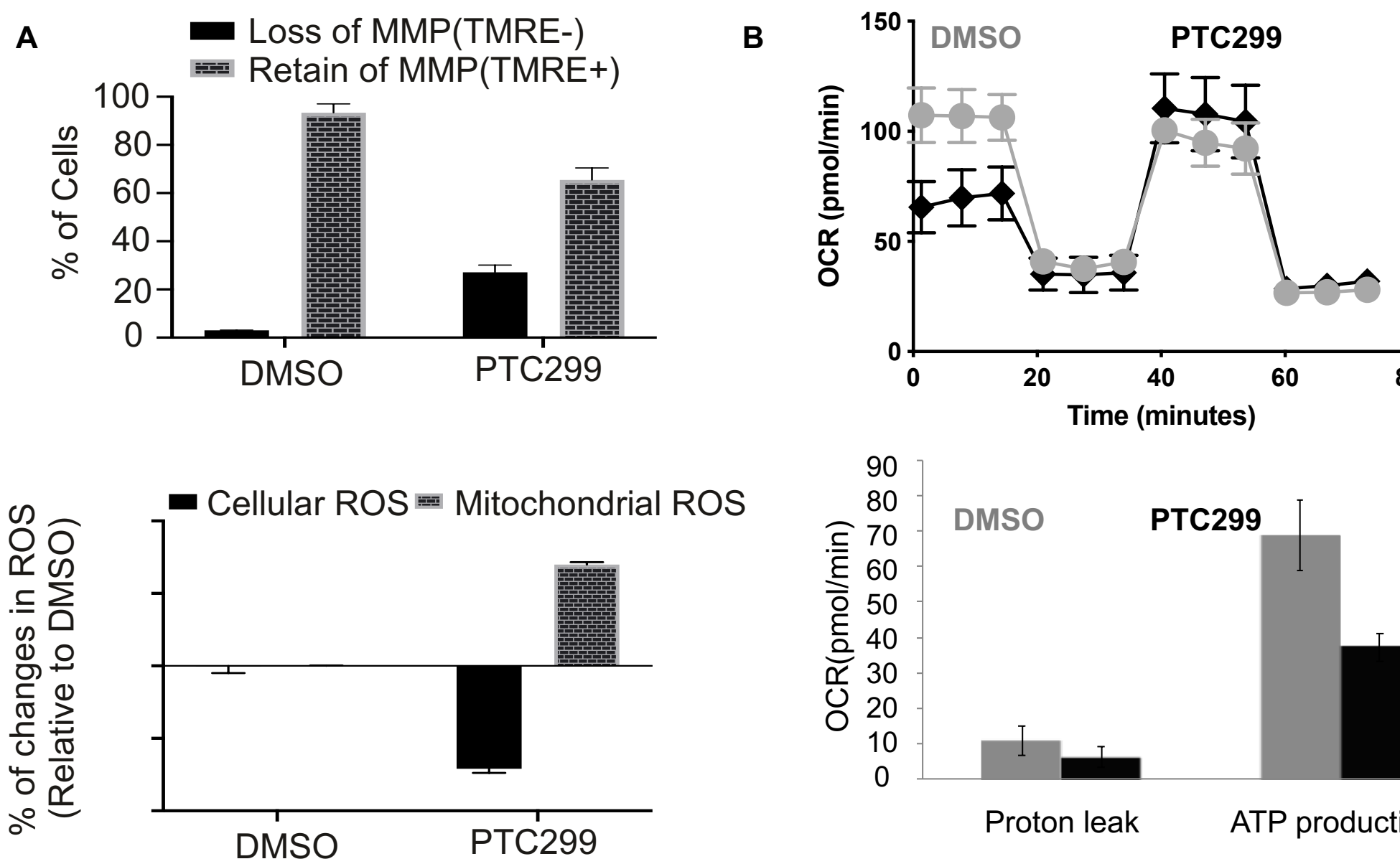


Figure 2:OCI-AML cells were treated with emvododstat (PTC299) for 24 hours and subjected to (A) measure membrane potential and ROS (B) sea horse to measure the oxygen consumption rate and ATP production.

Emvododstat alters the proteins of Apoptosis /DNA damage responses and downregulates PI3/AKT pathway

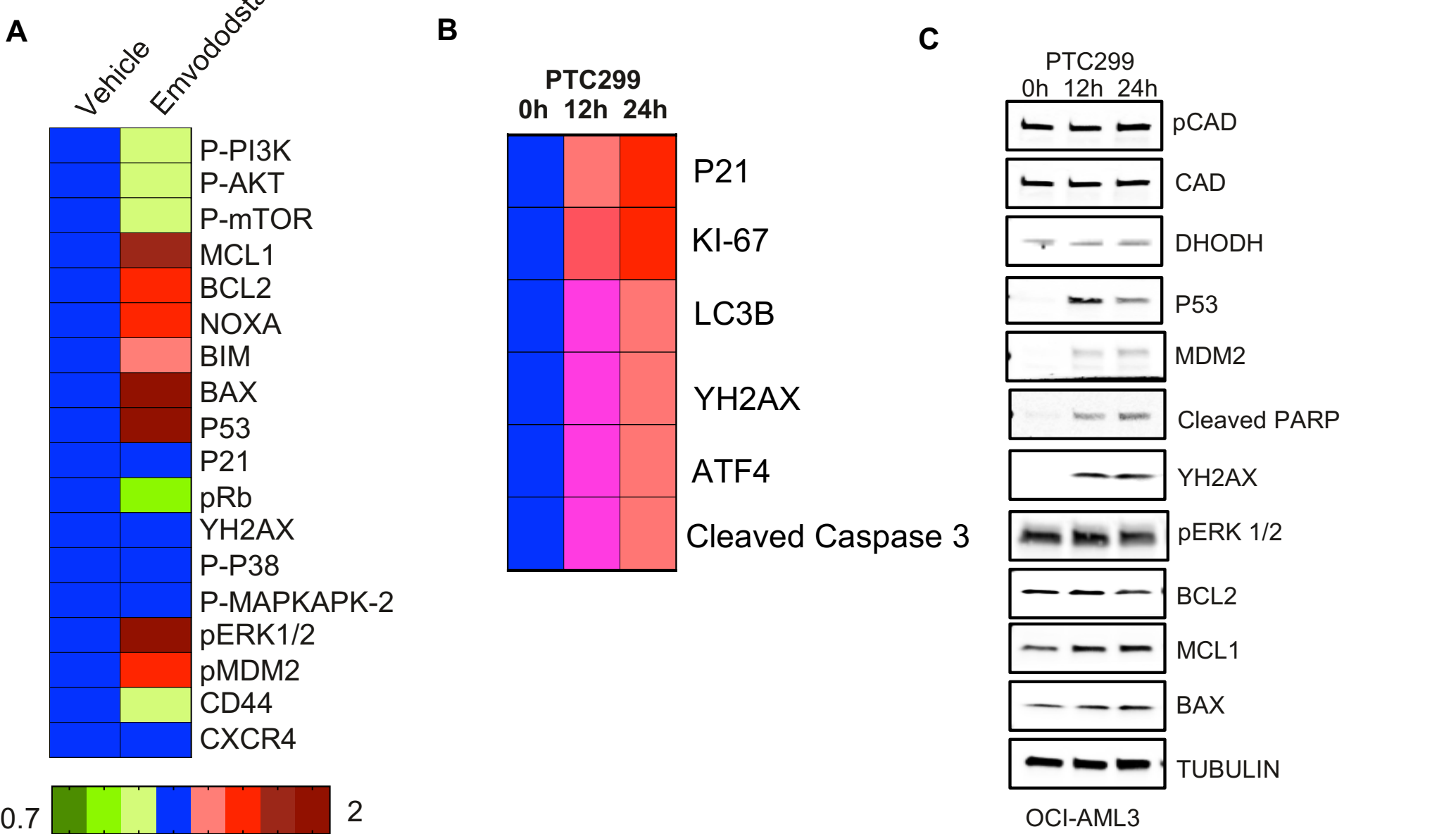


Figure 4: OCI-AML cells were treated with emvododstat (PTC299) for 24 hours or indicated time and subjected to (A)CyTOF (B)flow cytometry after staining for specified protein or (C)-immunoblotting for different proteins.

Results

Emvododstat improves survival of AML-PDX mice

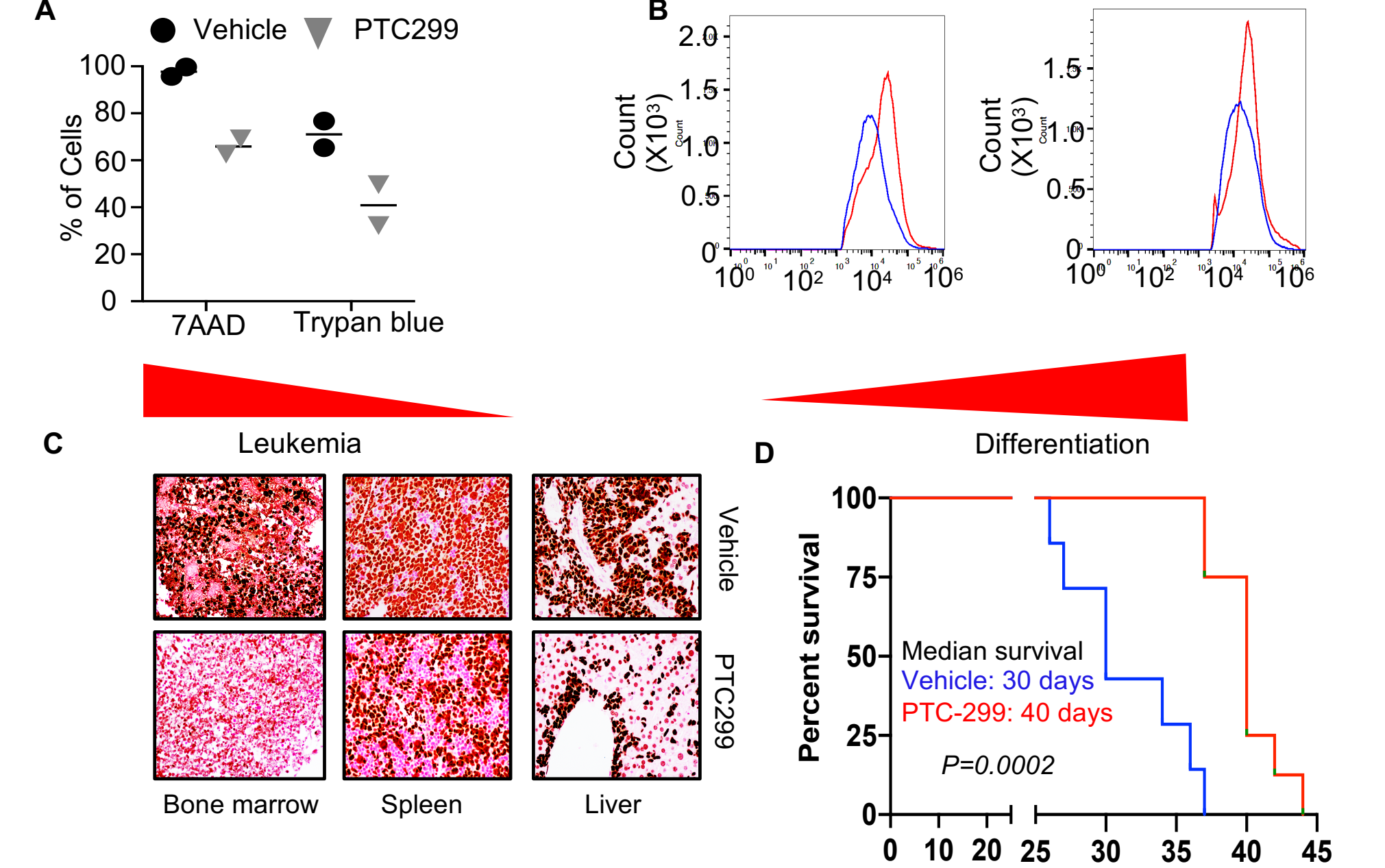


Figure 5: AML was established on NSG mice and treated with emvododstat (10 mg/kg /5 times a week for 3 weeks. Shown are (A) viability of AML(B)differentiation (C)immunostaining of KU-80 and (D) Kaplan-meir survival curve.

Summary/Conclusion

- Emvododstat was broadly active against diverse AML with different genetic abnormalities.
- Emvododstat alters the proteins of Apoptosis /DNA damage responses and downregulates PI3/AKT pathway.
- Emvododstat triggers differentiation, apoptosis and/or inhibition of proliferation of AML.
- Emvododstat as inhibitor of DHODH show promising therapeutic potential in preclinical AML-PDX model.

References

- Thomas D, Majeti R. *Blood* 2017; **129**(12): 1577-1585. e-pub ahead of print 2017/02/06; doi: 10.1182/blood-2016-10-696054
- Quek L, Otto GW, Garnett C, Lhermitte L, Karamitros D, Stoilova B *et al. The Journal of experimental medicine* 2016; **213**(8): 1513-1535. e-pub ahead of print 2016/07/06; doi: 10.1084/jem.20151775
- Villa E, Ali ES, Sahu U, Ben-Sahra I. *Cancers (Basel)* 2019; **11**(5). e-pub ahead of print 2019/05/22; doi: 10.3390/cancers11050688
- DeBerardinis RJ, Chandel NS. *Sci Adv* 2016; **2**(5): e1600200. e-pub ahead of print 2016/07/08; doi: 10.1126/sciadv.1600200