Targeting glutamine metabolism leads to terminal differentiation in acute myeloid leukemia (AML)

Robert D. Leone, Im-Meng Sun, Min-Hee Oh, Roshan Chikarmane, Radhika Viswanathan, Sanjeda Patwari, Matthew Arwood, Gabriel Ghiaur, Jonathan Powell
Bloomberg~Kimmel Institute for Cancer Immunotherapy at Johns Hopkins School of Medicine, Baltimore, MD 21287

Introduction

- Arrested cellular differentiation is a hallmark of acute myeloid leukemia (AML)
- Differentiation therapies aim to abrogate tumorigenicity by reactivating differentiation processes in AML
- AML relies heavily on exogenous glutamine[1]
- 6-diazo-5-oxo-L-norleucine (DON) is a non-specific glutamine inhibitor that decreases AML growth[2]
- Recent reports demonstrate that pharmacologic blockade of the glutamine-requiring pyrimidine synthesis/hexosamine pathway can induce terminal differentiation in AML[3]

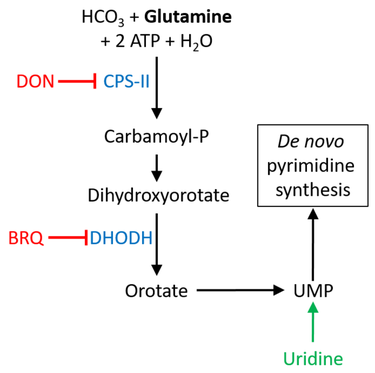

Figure 1. Disruption of the glutamine-requiring pyrimidine synthesis/ hexosamine pathway induces AML differentiation. Breqinar sodium (BRQ) inhibits DHODH and induces differentiation of human AML cell lines.[3] Supplementation of uridine restores downstream metabolites and reverses differentiation induced by BRQ. Glutamine is rate limiting for *de novo* pyrimidine synthesis.

Hypothesis: Glutamine antagonism induces terminal differentiation in AML

Objectives

- Determine the capacity of glutamine blockade to induce terminal differentiation in AML cells
- Assess the growth potential of DON- differentiated cells
- Investigate the role of *de novo* pyrimidine synthesis and *O*-linked glycosylation (hexosamine) pathway in DON-induced AML differentiation
- Investigate mechanisms of AML differentiation in response to glutamine blockade
- Assess differentiation therapy of AML in mouse models using the DON prodrug JHU083[4]

Results

Glutamine blockade with DON leads to differentiation of human AML cell lines

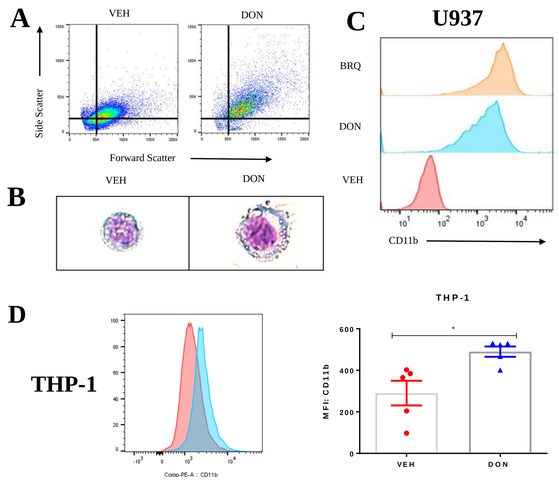

Figure 2. U937 human AML cells exposed to 5 μM DON for five days shows an increase size and granularity on (A) a population level (B) a single-cell level. (C) Exposure of AML to DON shows upregulation of myeloid differentiation marker CD11b in a manner analogous to , 0.25 μM BRQ exposure. (D) DON induces CD11b upregulation on THP-1 AML cells.

DON-treated AML cells show attenuated colony formation

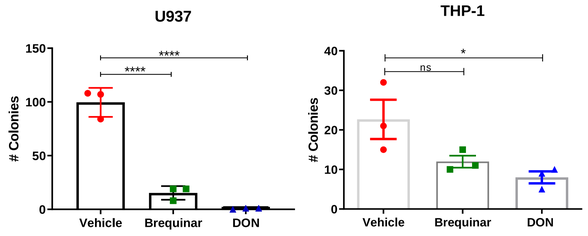

Figure 3. (A-B) Colony growth of U937 cells (A) or THP-1 cells (B) pre-treated with vehicle, BRQ, or DON for 5 days and subsequently grown in methylcellulose growth media for 11 days.

DON leads to differentiation of AML via an α-ketoglutarate-dependent mechanism

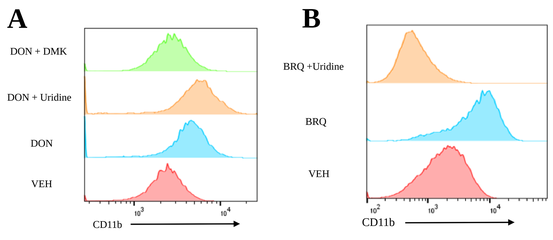

Figure 4. Analysis of human U937 AML cells by flow cytometry shows that DON-induced upregulation of CD11b is reversible with the cell-permeable α-ketoglutarate analogue DMK but not with uridine (A), at a concentration of uridine that strongly reverses that BRQ-induced upregulation of CD11b (B).

Results

Glutamine blockade with DON induces global epigenetic remodeling

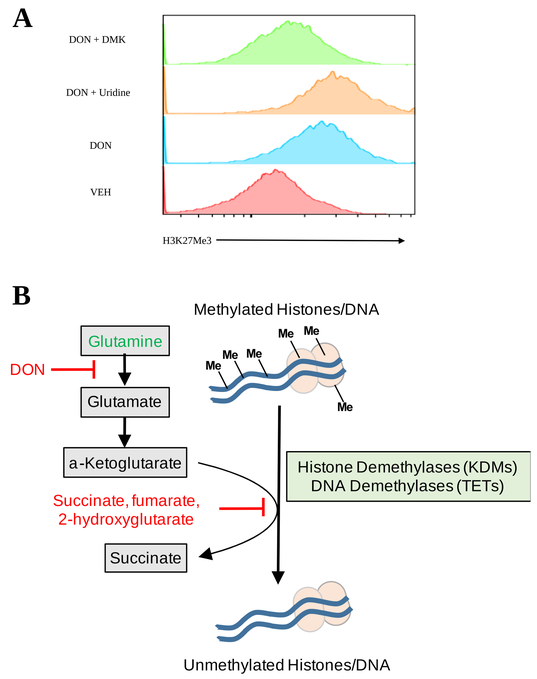

Figure 5. (**A**) DON-induced upregulation of H3K27Me3 reverses with 4 mM DMK but not with 100 μM Uridine. (**B**) Histone and DNA demethylases require α-ketoglutarate. α-Ketoglutarate is a downstream metabolite of glutamine, the formation of which can be blocked by DON.

Glutamine blockade with DON leads to dramatic upregulation of myeloid lineage transcription factors

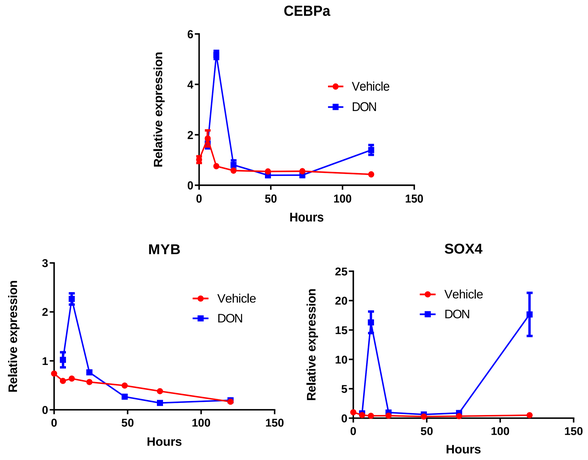

Figure 6. Time course of mRNA expression during *in vitro* DON treatment of U937 cells.

Results

In vivo glutamine antagonism with JHU083, a DON prodrug, induces differentiation and epigenetic remodeling of U937 AML

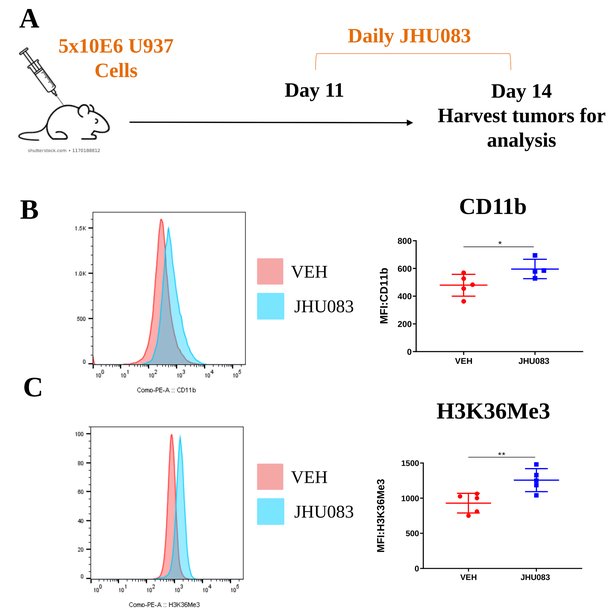

Figure 7. (A) 5x10E[6] U937 cells were subcutaneously implanted in the right flanks of NSG mice. Mice were treated with JHU083 (1 mg/kg) daily for 5 days starting on day 11 post-implantation. (**B-C**) Explanted tumors were evaluated by FACS for differentiation markers (CD11b) and changes in global epigenetic marks (H3K36Me3).

Conclusions

Blocking glutamine metabolism in AML:

1. Promotes differentiation in AML cell lines
2. Significantly suppresses growth of AML cells
3. Triggers AML differentiation that is independent of pyrimidine synthesis and hexosamine pathways
4. Causes epigenetic remodeling and differentiation that is mediated by α-ketoglutarate
5. Leads to acute increases in the expression of myeloid-determining transcription factors
6. Enhances differentiation and epigenome remodeling in mouse models of AML using JHU083

References

1. Wise, D.R. and Thompson, C.B. Glutamine addiction: a new therapeutic target in cancer. *Cell*, (2010);35(8): 427-433.
2. Griffiths, M., Keast, D., Patrick, G., Crawford, M. and Palmer, T.N. The role of glutamine and glucose analogues in metabolic inhibition of human myeloid leukaemia in vitro. *The International J.of Biochem.*, (1993);25(12):1749-1755.
3. Sykes, D.B., Kfoury, Y.S., Mercier, F.E., et al. Inhibition of dihydroorotate dehydrogenase overcomes differentiation blockade in acute myeloid leukemia. *Cell*, (2016):167(1):171-186.
4. K. M. Lemberg, J. J. Vornov, R. Rais, B. S. Slusher, We're Not "DON" Yet: Optimal Dosing and Prodrug Delivery of 6-Diazo-5-oxo-L-norleucine. *Molecular cancer therapeutics* **17**, 1824-1832 (2018).