

# Decitabine priming enhances the anti-leukemic effects of exportin 1 (XPO1) selective inhibitor selinexor in acute myeloid leukemia

**Running head:** Decitabine and XPO1 inhibition in AML

\*Parvathi Ranganathan<sup>1,2</sup>, \*Xueyan Yu<sup>1,2</sup>, Ramasamy Santhanam<sup>1,2</sup>, Jessica Hofstetter<sup>1,2</sup>, Alison Walker<sup>1,2</sup>, Katherine Walsh<sup>1,2</sup>, Bhavana Bhatnagar<sup>1,2</sup>, Rebecca Klisovic<sup>1,2</sup>, Sumithira Vasu<sup>1,2</sup>, Mitch A. Phelps<sup>3</sup>, Steven Devine<sup>1,2</sup>, Sharon Shacham<sup>4</sup>, Michael Kauffman<sup>3</sup>, Guido Marcucci<sup>1,2</sup>, William Blum<sup>1,2</sup> and Ramiro Garzon<sup>1,2</sup>

<sup>1</sup>Division of Hematology, Department of Internal Medicine; <sup>2</sup>The Comprehensive Cancer Center; <sup>3</sup>The College of Pharmacy, The Ohio State University, Columbus, OH 43210; <sup>4</sup>Karyopharm Therapeutics Inc, Natick, MA, USA.

**\* P.R. and X.Y contributed equally to this study.**

Corresponding author:

Ramiro Garzon, MD

The Ohio State University, Comprehensive Cancer Center

Biomedical Research Tower, Room 1084

460 West 12th Avenue, Columbus, OH 43210, USA

e-mail: [ramiro.garzon@osumc.edu](mailto:ramiro.garzon@osumc.edu)

Phone :614-685-9180

**Keywords:** Decitabine, XPO1, AML

**Category:** Myeloid Neoplasia

**Abbreviations Used:** AML: acute myeloid leukemia, DAC: decitabine.

## **Abstract**

The prognosis of AML is poor, highlighting the need for novel treatments. Hypomethylating agents, including decitabine are used to treat elderly AML patients with relative success. Targeting nuclear exporter receptors (Exportin 1, XPO1) is a novel approach to restore tumor suppressor (TS) function in AML. Here, we show that sequential treatment of AML blasts with decitabine followed by selinexor (XPO1 inhibitor) enhances antileukemic effects of selinexor. These effects could be mediated by the re-expression of a subset of TS (*CDKN1A* and *FOXO3A*) that are epigenetically silenced via DNA methylation, and cytoplasmic-nuclear trafficking is regulated by XPO1. We observed a significant upregulation of *CDKN1A* and *FOXO3A* in decitabine versus control treated cells. Sequential treatment of decitabine followed by selinexor in MV4-11 xenograft model significantly improved survival compared to selinexor alone. Based on these preclinical results, a Phase 1 clinical trial of decitabine followed by selinexor in elderly AML has been initiated.

### **Key points:**

- 1. Decitabine priming increases anti-leukemic effects of selinexor in AML *in vitro* and *in vivo*.**
- 2. Decitabine priming allows for decreasing the dose of selinexor in patients, thus increasing tolerability without affecting anti-leukemic activity.**

## Introduction

Acute myeloid leukemia (AML) is a clonal hematopoietic disorder characterized by genetic and epigenetic alterations leading to a block in differentiation and accumulation of leukemic blasts in blood and bone marrow (BM).<sup>1</sup> Epigenetic silencing of genes involved in hematopoietic differentiation plays a critical role in myeloid leukemogenesis.<sup>2,3</sup> Gene silencing caused by DNA hypermethylation can be reversed pharmacologically by inhibition of DNA methyltransferases (DNMTs) using azanucleosides such as decitabine.<sup>2,3</sup> Our group recently reported a 47% complete response rate with a 10-day regimen of low dose decitabine as a single agent in previously untreated older AML patients (>60 years).<sup>4</sup> Although patients' survival was not significantly better than that obtained with more intensive chemotherapy regimens (i.e., "7+3"), the single agent decitabine regimen was well tolerated and had low toxicity. These results therefore suggest the opportunity to capitalize on this relatively non-toxic treatment and make it more effective by incorporating this compound into novel molecularly targeted approaches. Exportin 1 (XPO1) is a nuclear receptor exporter involved in the active transport of a number of cargo proteins, including transcription factors and tumor suppressor proteins (TSP; i.e., p53 and p27), cell-cycle regulators (i.e., Cdkn1a) and RNA molecules.<sup>5,6</sup> Recent data indicate that TSP can be exported from the nucleus and thereby inactivated in cancer by hyperactive nuclear export.<sup>7</sup> Indeed, XPO1 over-expression has been reported in several solid tumors and leukemias, including AML and it is associated with worse outcome.<sup>8,9</sup> Thus, XPO1 inhibition may result in increased levels of active TSP and orally bioavailable selective inhibitors of nuclear export (SINE) that covalently bind and inhibit XPO1 are being explored as a novel therapeutic approach

in solid tumors and hematologic malignancies.<sup>10,11</sup> Our group recently reported the anti-leukemic activity of SINEs in vitro and in vivo in AML.<sup>11</sup> SINEs displayed potent anti-proliferative properties at submicromolar concentrations with regard to apoptosis, cell-cycle arrest and myeloid differentiation in AML cell lines and patient blasts.<sup>11</sup> Finally, using the *FLT3*-ITD positive MV4-11 xenograft murine model, we show that treatment of mice with oral SINE significantly prolongs survival of leukemic mice.<sup>11</sup> These preclinical studies have supported the development of a large Phase 1 clinical trial with selinexor (last generation SINE) in patients with advanced hematological malignancies, including AML. Preliminary data indicate that selinexor is well tolerated, safe and active in refractory/relapsed AML patients.<sup>12</sup> However, considering the molecular complexity of AML, it is unlikely that this disease can be cured with monotherapy and therefore we asked whether adding an already established effective drug such as decitabine to selinexor will enhance or improve its anti-leukemic effects in AML.

## **Methods**

### **Cell lines**

AML cell lines, MV4-11, MOLM-13 and OCI-AML3 were purchased from DSMZ, Germany. All cell lines were cultured in RPMI supplemented with 10% FBS and 100U/mL penicillin and 100ug/mL streptomycin.

### **Primary AML samples**

Newly diagnosed untreated and frozen BM AML patient samples were obtained from the Ohio State University Leukemia Tissue Bank after getting informed consent approved by the cancer institution review board.

Further detailed methods are provided in the Figures legends and in the supplement section.

### **Results and Discussion**

We initially treated two AML cell lines (OCI-AML3 and MV4-11) concomitantly with decitabine and selinexor at two-fold dilutions of their individual  $IC_{50}$  values, and measured cell proliferation using the WST-1 assay at 48 hours. The combination index (CI) was calculated according to the Chou-Talalay method.<sup>13</sup> The CIs for the different combinations were around 1 or above 1, indicating no synergy (Supplemental Figure 1). Since decitabine is a cell-cycle dependent nucleoside analogue, we reasoned that these results could be explained by the cell cycle arrest induced by selinexor. Next we asked whether sequential treatment would be more effective. For this approach, we used the WST-1 assay where cells were exposed to decitabine ( $IC_{50}$  2 fold dilutions, 4 $\mu$ M – 250 nM) for 24hrs followed by selinexor ( $IC_{50}$  2 fold dilutions, 400nm – 25nM) for 24hrs (Supplemental Table S1). This sequential treatment resulted in CI values below 1, indicating synergy as calculated by the Chou-Talalay method (Figure 1A and Supplemental Table S2). These results were validated using a third AML cell line, MOLM-13 (Supplemental Figure 2A). In addition, a significant induction of apoptosis was observed (Supplemental Figure 3).

Thus, we hypothesized that these effects could be mediated by the re-expression of a subset of nuclear tumor suppressors (i.e., *CDKN1A* (*p21*) and *FOXO3A*)<sup>14,15</sup> that are epigenetically silenced via DNA methylation and whose cytoplasmic-nuclear trafficking is regulated by XPO1<sup>6</sup> (Figure 1B). Supporting this hypothesis we observed that decitabine treatment increased *CDKN1A* and *FOXO3A* expression levels at both the RNA and protein level in the AML cell lines OCI-AML3, MV4-11 and MOLM-13 (Figure 1C and Supplemental Figure 2B). These two genes were also found up-regulated in cell lines and BM samples from AML patients treated with hypomethylation agents.<sup>14,15</sup> To delineate further the mechanism of this enhanced anti-leukemic activity, we over-expressed *CDKN1A* using a lentiviral vector in both OCI-AML3 and MV4-11 cells (to mimic the priming effect of decitabine) and subsequently treated them with selinexor. Similar to the decitabine/selinexor treatment, ectopic *CDKN1A* and *FOXO3A* over-expression in OCI-AML3 and MV4-11 cells followed by selinexor treatment at lower than IC<sub>50</sub> values resulted in significantly decreased cellular proliferation (Figure 1D and 1E) and increased apoptosis (Supplemental Figures 4 and 5) as compared to selinexor alone. Expression levels of lentiviral induced *CDKN1A* and *FOXO3A* were validated by real-time PCR (Supplemental Figure 6). We then tested the efficacy of the combination *in vivo* using an established xenograft mouse model of AML. MV4-11 cells obtained from spleens of primary MV4-11 xenografts were transplanted into non-obese diabetic/severe combined immunodeficient (NOD-SCID) gamma (NSG) mice via tail vein. Mice were monitored closely for clinical signs of leukemia as described in methods. One week after leukemia cell injection, the mice were given either vehicle control, decitabine alone (0.4mg/kg, intra peritoneal (i.p.) twice weekly (BIW)), selinexor alone (20mg/kg, oral

gavage, BIW), selinexor alone (10mg/kg, oral gavage, BIW) or decitabine (0.4mg/kg i.p. BIW, given Mondays and Tuesdays followed by selinexor (10mg/kg, oral gavage BIW), given on Wednesdays and Fridays. All treatments were given for three weeks after leukemia cell injection. Single agent selinexor at 10mg/kg or decitabine alone had no effect in prolonging the survival of mice with respect to the control mice (vehicle control). In contrast, decitabine (0.4mg/kg) followed by selinexor (10mg/kg), significantly increased mice survival even when compared with selinexor alone at higher dose (20mg/kg; 47 vs. 36.5 days,  $p < 0.008$ ; Figure 2A). On day 25, separate cohorts of vehicle and drug treated mice were sacrificed; spleens harvested and picture taken for comparative study of spleen size. The decitabine-selinexor combination treated mice exhibited smaller spleens size than the other groups and controls (Supplemental Figure 7). Finally, we validated the efficacy of the sequential drug treatment *in vitro* using primary AML blasts. In Supplementary Table S3 we report patient's cytogenetic and molecular characteristics and concentrations of decitabine and selinexor treatments. The initial priming by decitabine followed by selinexor treatment significantly decreased the percentage of live cells compared to either decitabine treatment alone (Figure 2B,  $p = 0.005$ ) or selinexor treatment alone (Figure 2B,  $p = 0.02$ ) as measured by apoptosis.

In summary, we show here that decitabine priming increased the anti-leukemic effects of selinexor in AML *in vitro* and *in vivo*. It should be underscored that the decitabine priming enhanced the antileukemic activity of selinexor at lower doses compared to the treatment of selinexor alone at higher doses. This is relevant to patients since it is then possible to use lower doses of selinexor to increase tolerability without compromising the



anti-leukemia activity. Based on these preclinical results, we have launched a Phase 1 clinical trial of decitabine followed by selinexor in newly diagnosed unfit elderly ( $\geq 60$  years) AML and primary refractory/relapsed AML at The Ohio State University (OSU-13182, NCI ClinicalTrials.gov identifier: NCT02093403).

### **Acknowledgments**

We thank Donna Bucci and David Lucas for providing us with primary AML samples from the OSU Leukemia Tissue Bank. Supported by NIH grants; P50CA140158, R01 CA188269 and Leukemia and Lymphoma Society Special Fellow Award (P.R).

### **Authorship Contributions**

P.R, X.Y, R.S and J.H performed all the experiments, P.R., A.W, K.W, R.K, B.B, S.D., S.V., W.B, M.K, M.P., S.S., G.M., and R.G. designed research, analyzed data and manuscript, P.R and R.G. wrote the manuscript.

### **Conflict of Interests**

**M.K and S.S** are employees of Karyopharm therapeutics, a clinical stage biopharmaceutical company that develops selective inhibitors of nuclear export-targeted therapeutics.

### **References**

1. Estey E, Dohner H. Acute myeloid leukaemia. *Lancet*. 2006;368(9550):1894-1907.

2. Mizuno S, Chijiwa T, Okamura T, *et al.* Expression of DNA methyltransferases DNMT1, 3A, and 3B in normal hematopoiesis and in acute and chronic myelogenous leukemia. *Blood*. 2001;97(5):1172-1179.
3. Plass C, Oakes C, Blum W, Marcucci G. Epigenetics in acute myeloid leukemia. *Semin Oncol*. 2008;35(4):378-387.
4. Blum W, Garzon R, Klisovic RB, *et al.* Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine. *Proc Natl Acad Sci U S A*. 2010;107(16):7473-7478.
5. Mutka SC, Yang WQ, Dong SD, *et al.* Identification of nuclear export inhibitors with potent anticancer activity in vivo. *Cancer Res*. 2009;69(2):510-517.
6. Turner JG, Sullivan DM. CRM1-mediated nuclear export of proteins and drug resistance in cancer. *Curr Med Chem*. 2008;15(26):2648-2655.
7. Kanai M, Hanashiro K, Kim SH, *et al.* Inhibition of Crm1-p53 interaction and nuclear export of p53 by poly(ADP-ribosyl)ation. *Nat Cell Biol*. 2007;9(10):1175-1183.
8. Shen A, Wang Y, Zhao Y, Zou L, Sun L, Cheng C. Expression of CRM1 in human gliomas and its significance in p27 expression and clinical prognosis. *Neurosurgery*. 2009;65(1):153-159; discussion 159-160.
9. van der Watt PJ, Maske CP, Hendricks DT, *et al.* The Karyopherin proteins, Crm1 and Karyopherin beta1, are overexpressed in cervical cancer and are critical for cancer cell survival and proliferation. *Int J Cancer*. 2009;124(8):1829-1840.
10. Etchin J, Sun Q, Kentsis A, *et al.* Antileukemic activity of nuclear export inhibitors that spare normal hematopoietic cells. *Leukemia*. 2013;27(1):66-74.

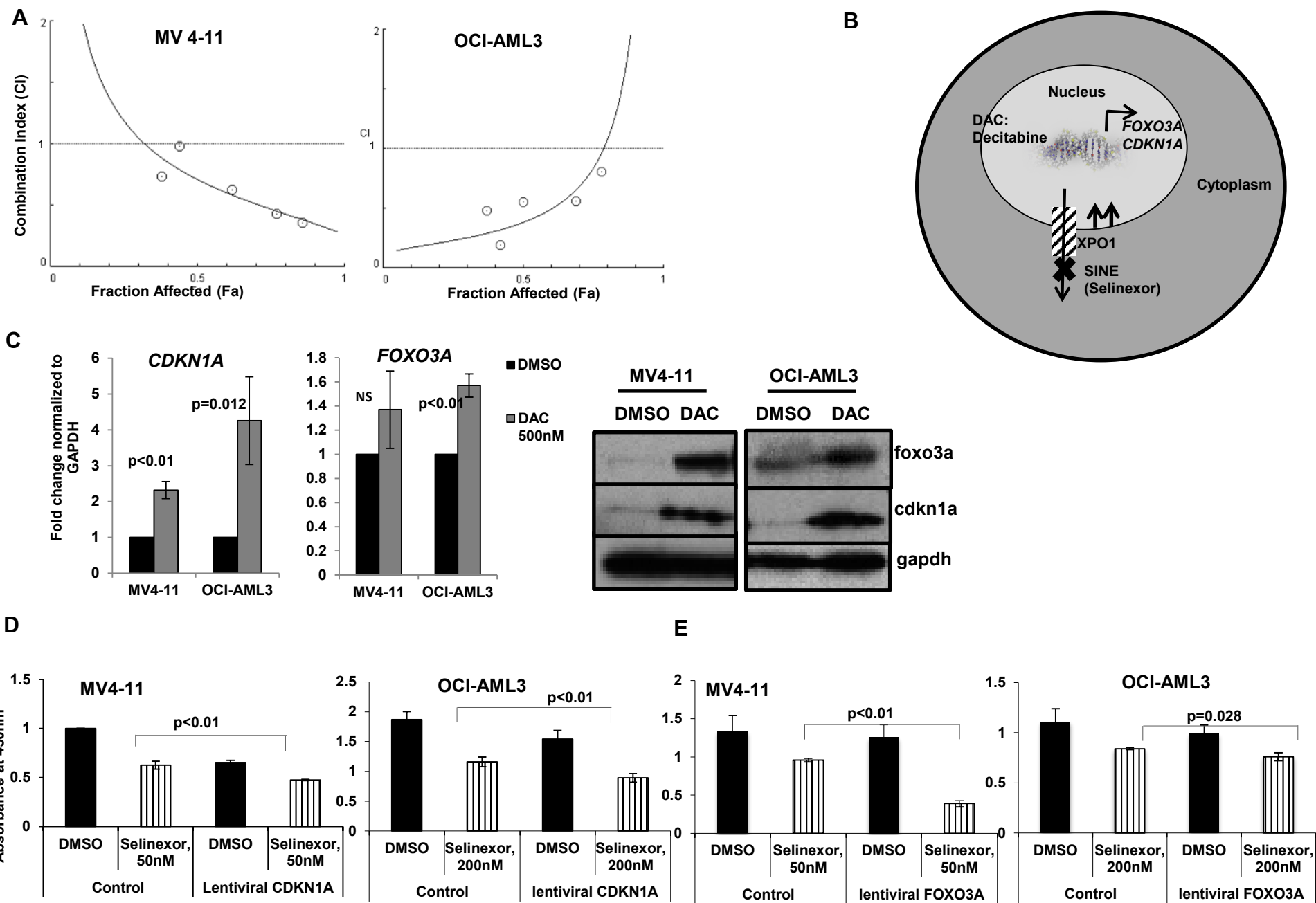
11. Ranganathan P, Yu X, Na C, *et al.* Preclinical activity of a novel CRM1 inhibitor in acute myeloid leukemia. *Blood*. 2012;120(9):1765-1773.
12. Garzon R, Flinn I, Berdeja J, *et al.* A phase 1 dose escalation study of the oral selective inhibitor of nuclear export (SINE) selinexor (KPT-330) in patients (pts) with relapsed / refractory acute myeloid leukemia (AML). *Haematologica*. June 2014, 99:1-796; Abstract no. 5591.
13. Chou T-C. Drug Combination Studies and Their Synergy Quantification Using the Chou-Talalay Method. *Cancer Res*. 2010;70(2):440-446.
14. Schmelz K, Wagner M, Dorken B, Tamm I. 5-Aza-2'-deoxycytidine induces p21WAF expression by demethylation of p73 leading to p53-independent apoptosis in myeloid leukemia. *Int J Cancer*. 2005; 114(5):683-695.
15. Thepot S, Lainey E, Cluzeau T, *et al.* Hypomethylating agents reactivate FOXO3A in acute myeloid leukemia. *Cell Cycle*. 2011; 10(14):2323-2330.

## Figure Legends

**Figure 1. Decitabine priming enhances selinexor anti-leukemic effects in vitro.** (A) Combination index (CI) plots of decitabine (DAC) with selinexor. The effect of the combinations was assessed by WST-1 assay after initial priming with DAC (24hrs) followed by selinexor treatment for 24hrs. The doses for both drugs were chosen according to their individual  $IC_{50}$  (2 fold dilutions), which were determined using WST-1 assay (Supplemental Table 1). Since DAC is given for 48 hours, we determined the DAC  $IC_{50}$  at 48 hours. The  $IC_{50}$  for selinexor was determined at 24 hours since the treatment with this drug was shorter. The effects of the combinations were calculated using

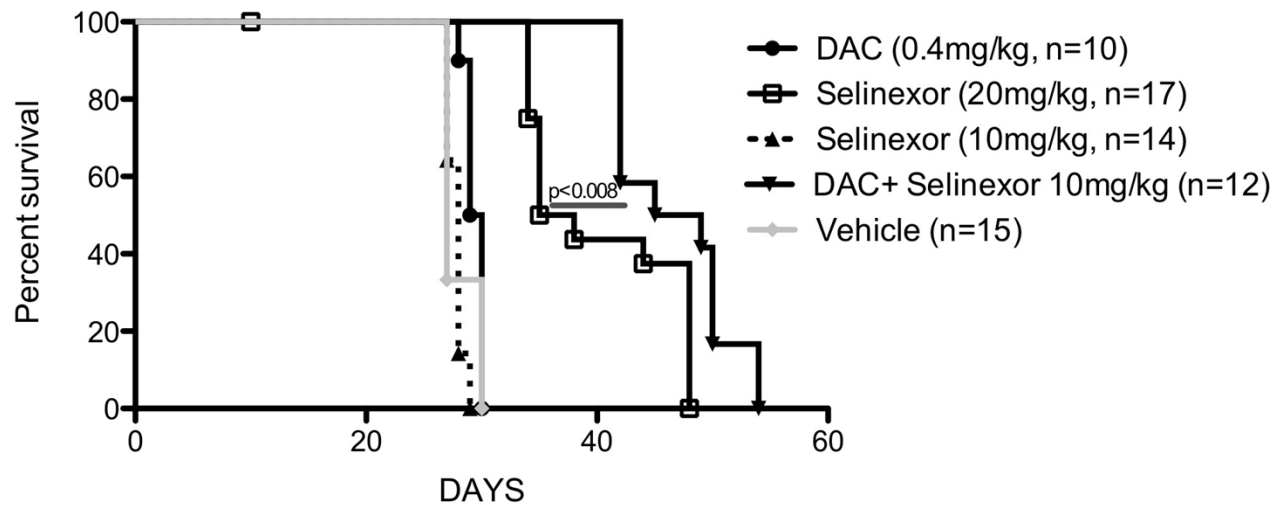
CalcuSyn software, where  $CI < 1$  indicates synergy,  $CI = 1$  is additive and  $CI > 1$  is antagonistic. (B) Schematic illustrating hypothesis. (C) Fold change in *CDKN1A* and *FOXO3a* mRNA expression by real-time PCR (24 hours) and western blot (48 hours) of the same to show change in protein expression. (D) WST-1 assay of cell lines treated with control or lentiviral vector expressing *CDKN1A* (*p21*) followed by selinexor treatment. (E) WST-1 assay of cell lines treated with control or lentiviral vector expressing *FOXO3A* followed by selinexor treatment. Selinexor was introduced to cell culture 24 hours after transfection. Assays were performed at indicated time points.

**Figure 2. Decitabine priming enhances selinexor anti-leukemic effects in vivo.** (A) Survival curve of NSG injected with MV4-11 xenografts and treated with indicated drugs. Survival comparison was made with log-rank test. (B) Primary AML patient blasts were treated with DAC for 16hrs followed by selinexor for 24 hrs. Controls included DMSO, DAC alone and selinexor treatments alone. Cell viability was measured using Annexin V/PI staining, and DMSO treated cells were normalized to 100% for comparison between treatment groups.

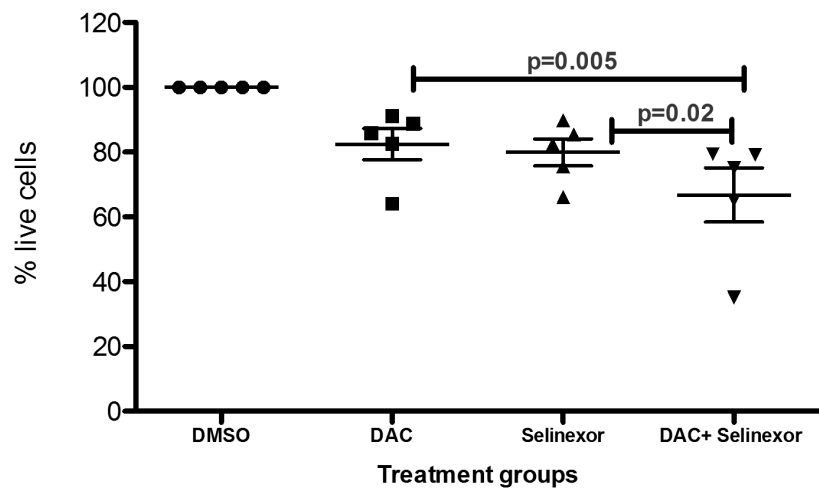
**Figure 1**

**Figure 2**

**A**



**B**





**blood**<sup>®</sup>

Prepublished online February 25, 2015;  
doi:10.1182/blood-2014-10-607648

## **Decitabine priming enhances the anti-leukemic effects of exportin 1 (XPO1) selective inhibitor selinexor in acute myeloid leukemia**

Parvathi Ranganathan, Xueyan Yu, Ramasamy Santhanam, Jessica Hofstetter, Alison Walker, Katherine Walsh, Bhavana Bhatnagar, Rebecca Klisovic, Sumithira Vasu, Mitch A. Phelps, Steven Devine, Sharon Shacham, Michael Kauffman, Guido Marcucci, William Blum and Ramiro Garzon

---

Information about reproducing this article in parts or in its entirety may be found online at:  
[http://www.bloodjournal.org/site/misc/rights.xhtml#repub\\_requests](http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests)

Information about ordering reprints may be found online at:  
<http://www.bloodjournal.org/site/misc/rights.xhtml#reprints>

Information about subscriptions and ASH membership may be found online at:  
<http://www.bloodjournal.org/site/subscriptions/index.xhtml>

---

Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include digital object identifier (DOIs) and date of initial publication.