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Epigenetic drug combination induces remission in mouse xenograft models of pediatric acute myeloid leukemia

Anilkumar Gopalakrishnapillai, Ph.D.¹, E. Anders Kolb, M.D.¹, Suzanne M. McCahan, Ph.D.², and Sonali P. Barwe, Ph.D.^{1,3}

¹Nemours Center for Childhood Cancer Research, Alfred I. duPont Hospital for Children, Wilmington, DE 19803, USA

²Nemours Bioinformatics Core Facility, Alfred I. duPont Hospital for Children, Wilmington, DE 19803, USA

Abstract

Aberrations in epigenetic modifications contribute to leukemogenesis in childhood acute myeloid leukemia (AML). We combined DNA hypomethylating agent azacitidine with histone deacetylase inhibitor panobinostat in preclinical models of childhood AML. Synergistic cytotoxic effect upon treatment with azacitidine and panobinostat with combination indices < 1.0 was observed.

Azacitidine and panobinostat increased median survival by 26 and 6 days respectively in MV4;11 xenografted mice. Mice treated with both drugs showed a drastic reduction in leukemic burden leading to complete remission sustained for the duration of the experimental period lasting more than 519 days. Reduced leukemic burden and prolonged survival was also observed in AML-193 xenografted mice treated with azacitidine-panobinostat combination. Differential gene expression profiling was performed on AML cells treated with azacitidine, panobinostat or azacitidine-panobinostat combination. Functional mapping of transcripts uniquely regulated by the azacitidine-panobinostat combination in MV4;11 cells identified p53 as an upstream regulator. A comparison of the uniquely modulated transcripts by azacitidine-panobinostat combination in MV4;11 cells versus AML-193 and THP-1 cells, bearing mutated p53, also revealed p53 as the topmost upstream regulator. Finally, expression of mutant p53 in MV4;11 cells reduced sensitivity to azacitidine-panobinostat combination, suggesting that p53 may be a predictor of response to epigenetic therapy in pediatric AML.

Keywords

acute myeloid leukemia; azacitidine; panobinostat; epigenetics; xenograft; pediatric

³Corresponding Author: Sonali P. Barwe, Nemours Center for Childhood Cancer Research, Alfred I. duPont Hospital for Children, 1701 Rockland Road, Wilmington, DE 19803, USA, Telephone: (302) 651-6542, Fax: (302) 651-4827, barwe@medsci.udel.edu.

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INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous disease characterized by the presence of diverse molecular abnormalities resulting in differentiation arrest and uncontrolled proliferation in immature myeloid precursors. In addition to cytogenetic and genomic aberrations, dysregulation of the epigenome in the form of DNA promoter hypermethylation and aberrant histone H3 hypoacetylation is also observed in AML patients [1, 2]. Recent genomic analyses identified mutations in genes encoding epigenetic modifiers- *IDH1/2*, *DNMT3A*, *TET2* (involved in the regulation of DNA methylation) and *ASXL1*, *EZH2*, *KDM6A* (involved in the modulation of chromatin modifications) [3, 4]. Distinct epigenetic profiles can differentiate the hematopoietic stem cells from the leukemia stem cells and can be used to classify AML patients into individual prognostic groups related to cytogenetics [5]. The epigenome is amenable to change, and strategies to target epigenetic modifications in AML have been utilized. Epigenetic drugs such as DNA hypomethylating agents and histone deacetylase (HDAC) inhibitors maintain normal hematopoietic stem cell self-renewal, while inducing terminal differentiation and selective anti-leukemic effect [6]. These agents are currently in clinical trials for adult AML either as single agents or in combination.

Differences between adult and pediatric AML epigenomes are vast likely due to the age-induced epigenetic drift observed in many neoplasms [2]. For example, mutations in *IDH1/2*, *DNMT3a*, *TET2* genes found in adult AML patients are rare in pediatric AML [7, 8]. Children do have high incidence of mutations/translocations in the *MLL* gene, a histone methyltransferase, suggesting a distinct role for dysregulated epigenetic modifications in leukemogenesis in children [9, 10]. Therefore, there is a need to determine if pediatric AML patients would respond in a similar way to adults. Our data showing sustained remission and reduced leukemic burden following combination therapy of azacitidine and panobinostat represents the first preclinical evidence of efficacy of this combination in pediatric AML. Differential gene expression analysis and functional mapping of genes uniquely modulated by the combination, identified p53 as an upstream regulator. Expression of R248W mutant p53 in MV4;11 cells reduced the sensitivity of these cells to azacitidine-panobinostat combination. Moreover, the combination induced complete remission in MV4;11 xenografts with wild-type p53 suggesting that presence of p53 may predict efficacy of this combination.

MATERIALS AND METHODS

Cell culture and transfection

AML-193 (CRL-9589), MV4;11 (CRL-9591) and THP-1 (TIB-202) cells were obtained from American Type Culture Collection (ATCC), (Manassas, VA). THP-1 cells were cultured in RPMI culture medium supplemented with 10% fetal bovine serum (FBS), 2 mM/L L-glutamine, 25 U/mL penicillin, and 25 µg/mL streptomycin. MV4;11 cells were cultured in IMDM culture medium with supplements listed above. AML-193 cells were cultured in IMDM with 5% FBS, 0.5 ng/ml insulin, 5 ng/ml transferrin receptor and 5 ng/ml GM-CSF.

For generation of MV4;11 cells expressing mutant p53, pCMV-Neo-Bam p53 R248W (Addgene plasmid # 16437), a gift from Bert Vogelstein [11] was used. MV4;11 cells (2.5×10^6) were transfected by nucleofection using Amaxa Nucleofector (Lonza, Basel, Switzerland), Kit V, program A-030. Transfected clones were isolated by single cell dilution cloning and antibiotic selection (500 µg/ml G418), followed by screening by immunoblotting.

Cell viability assay and IC50 determination

Viability of AML cell lines was determined using the Cell Titer Blue cell viability assay, Promega (Madison, WI) following manufacturer's protocol. Briefly, 50,000 cells plated in 96-well plates were incubated with DMSO (vehicle), azacitidine (S1782, Selleck Chemicals, Houston, TX), panobinostat (P-3703, LC Laboratories, Boston, MA) or a combination of the two at the indicated concentrations for 72h. Cell Titer Blue reagent was added to the cells following treatment and the fluorescence corresponding to the percentage of live cells was captured four hours later by using a VICTOR Multilabel Plate Reader, PerkinElmer (Waltham, MA). The viability of vehicle-treated cells was considered to be 100% for calculation of percent cell viability of treated cells. IC50 for each cell line and drug was calculated using Prism 6, GraphPad Software (San Diego, CA) from 3–5 trials of cell viability determinations performed in triplicates.

Immunoblotting

Cells were lysed in a buffer containing 20 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1% v/v Triton X-100, 1 mM EDTA, 1 mM EGTA, 1 mM sodium glycerol phosphate, 1 mM sodium orthovanadate, 1 mM PMSF, and 5 µg/ml each of antipain, pepstatin and leupeptin. The lysates were sonicated and clarified by centrifugation at 16,000 g for 10 min at 4°C. Total protein in cell lysates was estimated by Bio-Rad DC Reagent, Bio-Rad (Hercules, CA) following manufacturer's instructions. One hundred µg of total protein was resolved by SDS-PAGE, transferred to nitrocellulose membrane, and immunoblotted using anti-p53 antibody (7F5), followed by HRP-conjugated anti-rabbit secondary antibody diluted in Tris-buffered saline containing 5% w/v non-fat dried milk and 0.1% v/v Tween-20. Enhanced chemiluminescent lighting system, PerkinElmer Life Sciences (Boston, MA) was used for detection. Antibodies were purchased from Cell Signaling Technology (Lexington, KY).

Xenograft studies

NSG-B2m mice (Stock#010636) purchased from Jackson Laboratories (Bar Harbor, ME) were transplanted with leukemia cells (3.5×10^6) via tail-vein injections as described previously [12]. Mice were maintained in the Nemours Life Science Center following the guidelines established by the Nemours Institutional Animal Care and Use Committee (IACUC). Disease progression was monitored by flow cytometry (see below) of mouse peripheral blood drawn periodically by submandibular bleeds. When the average percentage of engraftment was 0.4%, the mice were randomized into four treatment groups– vehicle (5% dextrose), azacitidine (2.5 mg/Kg), panobinostat (2.5 mg/Kg), and azacitidine + panobinostat (2.5 mg/Kg each). The mice were dosed with four cycles lasting five days a week with two days rest. An additional rest period of one-week was included between cycles 2 and 3. Mice were euthanized by carbon-di-oxide asphyxiation using a method consistent

with the euthanasia guidelines of the American Veterinary Medical Association, when they exhibited disease symptoms: increased leukemic burden, persistent weight loss or hind-limb paralysis. All studies involving mice were approved by the Nemours IACUC.

Flow cytometry

Mouse blood was stained with FITC-conjugated anti-human CD45 and APC-conjugated anti-mouse CD45 antibodies from Affymetrix eBioscience (San Diego, CA). The red blood cells (RBC) were lysed by using a multi-species RBC lysis buffer (Affymetrix eBioscience) containing ammonium chloride. The number of cells stained specifically for either antibody (CD45+ cells) within a cell population gated for blasts (based on their forward and side scatter parameters) was determined by flow cytometry using a BD Accuri C6 flow cytometer, BD Biosciences (San Jose, CA). The percentage of human cells in mouse peripheral blood was calculated using this formula – (No. of human CD45+ cells)/(No. of human CD45+ cells + No. of mouse CD45+ cells) × 100).

Gene expression analysis

The concentrations of azacitidine and panobinostat that allow at least 75% cell viability at 48 h post treatment even in the combination were pre-determined for each cell line. MV4;11 cells were treated with vehicle (0.1% DMSO), 0.5 µM azacitidine, 1.5 nM panobinostat or 0.5 µM azacitidine plus 1.5 nM panobinostat for 48 h. AML-193 cells were treated with vehicle (0.1% DMSO), 5 µM azacitidine, 5 nM panobinostat or 5 µM azacitidine plus 5 nM panobinostat for 48 h. THP-1 cells were treated with vehicle (0.1% DMSO), 3 µM azacitidine, 5 nM panobinostat or 3 µM azacitidine plus 5 nM panobinostat for 48 h. Five replicates of each were used. Total RNA was isolated using Trizol Reagent, Invitrogen, ThermoFisher Scientific (Waltham, MA) following manufacturer's protocol. RNA quality was verified with a 2100 Bioanalyzer, Agilent Technologies (Santa Clara, CA). Targets were prepared from 100 ng RNA using GeneChip® WT Plus Reagent Kit, Affymetrix (Santa Clara, CA). Labeled RNA samples were hybridized to GeneChip Human Gene 2.0 ST Array after the addition of the GeneChip Expression 3' Amplification Reagents Hybridization Controls. Labeled sense targets were hybridized overnight in the GeneChip Hybridization Oven 645 at 60 rpm and 45°C for 16–20 h. The arrays were washed and stained with the GeneChip Hybridization Wash and Stain Kit (Affymetrix) utilizing the GeneChip Fluidic Station 450 (Affymetrix). The stained arrays were scanned with the GeneChip scanner 3000 7G (Affymetrix). The array images were processed and quality assessed using Affymetrix GeneChip Command Console Software (AGCC) and Affymetrix Expression Console Software. The microarray data was deposited in the Gene Expression Omnibus of the National Center for Biotechnology Information (NCBI) and assigned accession number GSE83983. Here is a link for reviewer access <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=ehcregqipnabted&acc=GSE83983>

Log2 expression values for transcript clusters were calculated with the RMA (Robust Multichip Average) algorithm using the oligo Bioconductor package [13]. Transcript clusters with a statistically significant (1% FDR) change in expression after treatment were identified using limma (a linear model approach) with Benjamini and Hochberg's method to control the false-discovery rate [14–16]. Hierarchical clustering was performed using Euclidean

distance and average linkage with MeV (MultiExperimentViewer) [17]. Transcript clusters uniquely modulated by the combination treatment were imported into the Ingenuity Pathway Analysis (IPA) software (Qiagen, Valencia, CA) for canonical pathway analysis and identification of upstream regulators.

Statistical analysis

Prism 6 GraphPad software was used for plotting the percentage of human leukemic cells in mouse peripheral blood over time and fitting to a non-linear regression curve. The time at which this percentage reached 0.5% (disease detection) was interpolated from this curve. The length of remission was calculated as the number of days from treatment initiation to disease detection. Kaplan-Meier survival curves were compared by Log-rank (Mantel-Cox) test. Median survival was calculated as the number of days since treatment start when the percentage of surviving mice was 50%. A p-value <0.05 was considered statistically significant.

Average cell viability following drug treatment from three independent experiments calculated as a percentage of control was plotted in Microsoft Excel. p-value was calculated using the students paired t-test.

RESULTS

Epigenetic modifiers synergistically induce cytotoxicity in AML cell lines

The viability of three pediatric AML cell lines following exposure to azacitidine and panobinostat was determined. Panobinostat was more efficient in inducing cytotoxicity than azacitidine for all cell lines tested with IC50s ranging from 3.35 to 43.04 nM, while azacitidine IC50s ranged from 3.11 to 17.98 μ M (Fig. 1 and Table I). The combination index was calculated as the sum of the ratios of the dose required for each drug to reduce cell viability by 50% when used in combination to the dose required when used alone. A combination index value equal to 1.0 indicates additive effects, while a value less than 1.0 signifies synergism. For all cell lines tested, the combination index was < 1.0, indicating that the two drugs synergistically induced cytotoxicity in AML cells.

Azacitidine and panobinostat combination induces complete remission in mice bearing MV4;11 xenografts

The effect of the azacitidine-panobinostat combination on leukemic burden and survival was tested in MV4;11 transplanted mice. The maximum-tolerated dose (2.5 mg/Kg) was calculated by dose escalation in mice. Similar to reports from clinical trials, drug-induced toxicity in mice caused fatigue and diarrhea in mice resulting mainly from panobinostat treatment [18]. However, the mice recovered when treatment ended. Disease progression and engraftment of human cells in NSG-B2m mice transplanted with MV4;11 cells was monitored by periodic determination of the percentage of human leukemic cells versus mouse cells in peripheral blood by flow cytometry. The mice were randomly recruited to four treatment groups when the average percentage of engraftment was 0.4% – vehicle, azacitidine, panobinostat, and azacitidine + panobinostat. The length of remission was calculated as the number of days from treatment initiation until the engraftment percentage

reached 0.5%. Panobinostat treatment alone was not successful in maintaining low disease burden, where as azacitidine sustained remission for 52 days from the time treatment started (Fig. 2A, C). Panobinostat alone increased the median survival by 6 days compared to vehicle treated mice, while azacitidine treatment improved survival by 26 days (Fig. 2B, C). Treatment with azacitidine-panobinostat combination induced complete remission and the mice were disease-free until sacrifice > 519 days post treatment start.

Similar to MV4;11 xenografts, panobinostat failed to control leukemic burden in AML-193 xenografts, while azacitidine treatment maintained remission for 37 days post treatment start (Fig. 3A, C). The azacitidine-panobinostat combination sustained remission much longer, until 114 days post start of treatment. Panobinostat did not show an increase in median survival, while azacitidine had a 39-day survival advantage compared to vehicle-treated mice (Fig. 3B,C). Unlike MV4;11 xenografts, mice transplanted with AML-193 cells and treated with azacitidine-panobinostat combination did not attain complete remission and the percentage of human CD45+ cells in mouse blood increased over time. These mice survived 79 days longer than the vehicle-treated mice, demonstrating that the combination was more beneficial than either drug alone (Fig. 3C). Thus, taken together, consistent with the *in vitro* data, azacitidine and panobinostat synergize to reduce leukemic burden and prolong survival in two different AML xenografts in mice.

Modulation of gene expression profile by azacitidine and panobinostat in AML cells

In order to understand the mechanism of synergism between azacitidine and panobinostat, differential gene expression analysis was performed in MV4;11 cells treated with vehicle, azacitidine, panobinostat or azacitidine-panobinostat combination. 38, 2546 and 3679 transcript clusters were differentially expressed (1% FDR) when comparing (1) vehicle and azacitidine treatments, (2) vehicle and panobinostat treatments and (3) vehicle and azacitidine-panobinostat treatments, respectively. A Venn diagram of these differentially expressed transcript clusters showed 1577 transcript clusters were unique to the vehicle vs azacitidine-panobinostat comparison (Fig. 4A). These transcript clusters didn't reach significance when comparing vehicle and azacitidine treatments or vehicle and panobinostat treatments. Hierarchical clustering with these 1577 transcript clusters resulted in grouping of samples according to treatment (Fig. 4B). This also illustrated that the expression levels of some of the transcript clusters were higher in vehicle treated cells (veh1-5) and some were higher in azacitidine-panobinostat treated cells (ap16-29). The pattern of expression in azacitidine treated cells (aza6-10) and panobinostat treated cells (pan11-15) was consistent within treatment groups, but not as distinct from vehicle treated cells.

The 1577 transcript clusters (consisting of 1301 annotated transcript clusters) uniquely modulated by the combination were imported into IPA. Analysis of the genes represented by these transcript clusters identified p53 as the most significant upstream regulator ($p\text{-value} = 2.88 \times 10^{-16}$), although the TP53 gene transcript itself was not modulated. The activation z-score assessing the match of observed and predicted up/down regulation patterns of target molecules in the dataset was 2.758. 158 transcript clusters (out of 1301) uniquely modulated by the combination were transcriptionally regulated by p53 and contributed to activation of the p53 pathway.

In an effort to correlate the *in vitro* drug sensitivities and *in vivo* response with gene expression profiles, differential gene expression analysis was performed in AML-193 and THP-1 cells in addition to MV4;11 cells.. Venn diagrams of differentially expressed transcript clusters identified 1623 and 2295 transcript clusters that were uniquely regulated by the combination in AML-193 and THP-1 cells, respectively (Fig. 5A,B). IPA analysis of the transcript clusters with a 1% FDR did not recognize p53 to be an activated upstream regulator in AML-193 or THP-1 cells.

The 1577 transcript clusters uniquely modulated by the azacitidine-panobinostat combination in MV4;11 cells were compared to those uniquely modulated by the drug combination in AML-193 (1623 transcript clusters) and THP-1 cells (2295 transcript clusters). 1263 transcript clusters were uniquely modulated in MV4;11 cells compared to AML-193 and THP-1 cells (Fig. 5C). Pathway mapping of these transcripts detected p53 as the most significant upstream regulator with a p-value of 4.56×10^{-15} and activation z-score = 2.770. Interestingly, MV4;11 cells possess wild-type p53 [19] while both AML-193 and THP-1 cells have mutated p53 [20]. These data suggested that azacitidine-panobinostat combination was likely to be more efficient in inducing death in AML cells bearing wild-type p53.

Overexpression of dominant negative mutant of p53 in MV4;11 cells desensitizes to azacitidine-panobinostat treatment

In order to determine if the presence of mutated p53 reduces sensitivity to the azacitidine-panobinostat combination, MV4;11 cells expressing R248W dominant negative mutant of p53 were generated. Figure 6A shows increased levels of p53 protein in cells expressing R248W. MV4;11 R248W cells were used to compare their sensitivity towards azacitidine-panobinostat combination. These cells showed higher cell viabilities when treated with varying doses of azacitidine-panobinostat (Fig. 6B). These data indicate that the presence of wild-type p53 may enhance the sensitivity to epigenetic drugs.

DISCUSSION

Single agent testing of demethylating agents and HDAC inhibitors in children with refractory AML is underway [21]. Clinical application of these agents is largely driven by strategies developed for adult malignancies [5, 18, 22]. In order to develop these promising therapies further in children, a strong preclinical rationale is required. The use of cell line-derived xenograft models of AML for preclinical testing has been established [23]. In this study, we demonstrated the efficacy of the combination of azacitidine and panobinostat, as single agents or in combination, in NSG-B2m mice transplanted with two pediatric AML cell lines – MV4;11 and AML-193, suggesting that this drug combination may be an effective strategy in pediatric AML.

MV4;11 cells, which possess wild-type p53, had lower IC50s for azacitidine and panobinostat compared to AML-193 and THP-1 cells *in vitro*. MV4;11 cells expressing mutant p53 showed reduced sensitivity to azacitidine-panobinostat combination. These data are consistent with a previous study which showed that a spontaneously generated p53 mutant line of MV4;11 had reduced sensitivity to DNMT and HDAC inhibitor [24]. In our

study, mice transplanted with MV4;11 cells achieved complete remission when treated with the drug combination. It should be noted that AML-193 xenografts also responded to combination treatment, although complete remission was not attained. A clinical study showed that the presence of TP53 mutations correlated with poorer survival in AML patients treated with azacitidine [25]. Thus, p53 may be used as a biomarker to stratify patients who would better respond to epigenetic drug therapy. The incidence of TP53 gene mutation in AML is approximately 7% [3, 26], indicating that the majority of AML patients are likely to benefit from this epigenetic drug combination. AML patients with mutant p53 may benefit from supplementation of epigenetic therapy to their cytotoxic chemotherapy regimen. Studies are in progress in the laboratory to develop an integrative treatment regimen that combines chemotherapy with epigenetic therapy in preclinical models.

Other studies have shown that clinical response did not correlate with promoter hypomethylation or histone deacetylation [27]. Our data generates confidence that expanding the gene expression analysis methodology to include other cell lines – both responsive and non-responsive – should provide mechanistic insights into the synergistic induction of complete remission in MV4;11 cells and develop the basis for screening and identifying patients who will benefit from epigenetic therapy.

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Highlights

- Epigenetic drugs azacitidine and panobinostat synergistically induce AML cell death
- This combination prolonged survival in disseminated mouse models of pediatric AML
- Azacitidine panobinostat combination cured mice transplanted with MV4;11 cells
- p53 may be a predictor of response to epigenetic therapy in pediatric AML

Fig. 1. Dose response curves for azacitidine and panobinostat from 3–5 independent experiments performed in triplicates were generated using GraphPad Prism software. The calculated IC₅₀s are shown.

Fig. 2.

(A) The graph shows percentage of human CD45+ leukemic cells in mouse blood. At time 0, mice were injected with MV4;11 cells. The arrow indicates when drug treatment began. (B) Kaplan-Meier plot shows the percentage of mice surviving following treatment. The p-value for median survival between different treatment groups (by ANOVA) is <0.0001. The mice treated with the combination of azacitidine and panobinostat were disease-free until they were sacrificed 18 months post cell injection. (C) Table showing the length of remission and median survival in days for the mice treated with indicated drugs. n = number of mice per group. u.d. = undefined, n.a. = not applicable.

Fig. 3.

(**A**) The graph shows percentage of human CD45+ leukemic cells in mouse blood. At time 0, mice were injected with AML-193 cells. The arrow indicates the time when drug treatment was initiated. (**B**) Kaplan-Meier plot shows the percentage of mice surviving following treatment. The p-value for median survival between different treatment groups ($p = 0.0038$). (**C**) Table showing the length of remission and median survival in days for the mice treated with indicated drugs. n = number of mice per group. n.a. = not applicable.

Fig. 4.

(A) Venn diagram of differentially expressed transcript clusters (1% FDR) showing shared and unique transcript clusters in MV4;11 cells. 38, 2546 and 3679 transcript clusters were differentially expressed when comparing (1) vehicle and azacytidine treatments, (2) vehicle and panobinostat treatments and (3) vehicle and azacytidine-panobinostat treatments, respectively. 1577 transcript clusters were unique to the vehicle vs azacitidine-panobinostat comparison. **(B)** Hierarchical clustering of MV4;11 cells treated with vehicle (n=5, green bar, veh1-5), azacitidine (n=5, blue bar, aza6–10), panobinostat (n=5, red bar, pan11–15) or azacytidine-panobinostat combination (n=5, orange bar, ap16–20). Relative expression of transcript clusters uniquely regulated by the drug combination (1577) is shown.

Fig. 5.

(A) Venn diagram of differentially expressed transcript clusters (1% FDR) showing shared and unique transcript clusters in AML-193 cells. 1366, 2673 and 3898 transcript clusters were differentially expressed when comparing (1) vehicle and azacitidine treatments, (2) vehicle and panobinostat treatments and (3) vehicle and azacitidine-panobinostat treatments, respectively. 1623 transcript clusters were unique to the vehicle vs azacitidine-panobinostat comparison. (B) Venn diagram of differentially expressed transcript clusters (1% FDR) showing shared and unique transcript clusters in THP-1 cells. 1736, 2456 and 4767 transcript clusters were differentially expressed when comparing (1) vehicle and azacitidine treatments, (2) vehicle and panobinostat treatments and (3) vehicle and azacitidine-panobinostat treatments, respectively. 2295 transcript clusters were unique to the vehicle vs azacitidine-panobinostat comparison. (C) Venn diagram of transcript clusters that were differentially expressed when comparing vehicle and azacitidine-panobinostat treatments

and unique to this comparison in MV4;11, THP-1 and AML-193 cells. 1577, 1623 and 2295 transcript clusters met these criteria in MV4;11, AML-193 and THP1 cells, respectively.

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Fig. 6.

(A) Representative immunoblot showing the level of p53 in MV4;11 and MV4;11 cells overexpressing p53 R248W. The antibody recognizes both forms of p53. β-tubulin was used as a loading control. (B) Cell viability of MV4;11 and MV4;11 cells with mutant p53 expressed as a percentage of the viability of the respective cell line when treated with vehicle. Average values from three independent experiments were plotted. *p-value < 0.05, **p-value < 0.001.

Azacitidine and panobinostat synergistically induce cytotoxicity in pediatric AML cells. Chart showing IC50s and combination indices of these two drugs

Table 1

Cell line	Fusion protein	p53 status	Azacitidine IC50 (μM)	Panobinostat IC50 (nM)	Combination index
MV4;11	MLL-AF4	Wild-type	3.11	3.35	0.633
THP-1	MLL-AF9	Mutated	17.98	43.04	0.890
AML-193	None	Mutated	10.61	10.76	0.937