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Additive effects of vorinostat and MLN8237 in pediatric leukemia, medulloblastoma, and neuroblastoma cell lines

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Summary

Purpose—Histone deacetylase (HDAC) inhibitors, such as vorinostat, decrease Aurora kinase activity by a variety of mechanisms. Vorinostat and MLN8237, a selective Aurora A kinase inhibitor, disrupt the spindle assembly and the mitotic checkpoint at different points, suggesting that the combination could have increased antitumor activity. The purpose of this study was to determine the cytotoxicity of vorinostat and MLN8237 in pediatric tumor cell lines.

Methods—Cell survival was measured after 72 h of drug treatment using a modified methyl tetrazolium assay. For drug combination experiments, cells were exposed to medium alone (controls), single drug alone, or to different concentrations of the combination of the two drugs, for a total of 36 concentration pairs per plate. The interaction of the drug combination was analyzed using the universal response surface approach.

Results—The cells express the target of MLN8237, Aurora A. For each cell line, the single agent IC₅₀ for MLN8237 and for vorinostat was in the clinically relevant range. Both drugs inhibited cell survival in a concentration-dependent fashion. At concentrations of MLN8237 exceeding approximately 1 μ M, there was a paradoxical increase in viability signal in all three lines that may be explained by inhibition of Aurora B kinase. The combination of MLN8237 and vorinostat showed additive cytotoxicity in all three cell lines and nearly abrogated the paradoxical increase in survival noted at high single-agent MLN8237 concentrations.

Conclusion—MLN8237 and vorinostat are active in vitro against cancer cell lines. These results provide important preclinical support for the development of future clinical studies of MLN8237 and vorinostat.

Keywords

Vorinostat; Histone deacetylase inhibitor; MLN8237; Aurora kinase inhibitor; Cytotoxicity

Introduction

Despite major progress in the survival of children with pediatric malignancies, refractory and relapsed cancer remains a therapeutic challenge. Development of agents with new mechanisms of action is a critical strategy to overcome chemoresistance. Vorinostat (suberoylanilide hydroxamic acid, SAHA, Zolinza®) is a pan-histone deacetylase (HDAC)

inhibitor that inhibits class I and class II enzymes [1]. HDAC inhibitors induce cell differentiation, cell cycle arrest, and apoptosis, and inhibit migration, invasion and angiogenesis in many cancer models [2, 3]. Treatment with HDAC inhibitors results in an acetylated form of chromatin that is associated with active gene expression [4, 5]. HDAC inhibitors also directly interfere with mitotic progression, probably through inhibition of HDAC3 activity [6, 7]. Numerous transcription factors involved in controlling oncogenic processes show increased acetylation in response to HDAC inhibitors [1, 5]. The mechanism involved in the antineoplastic effect of vorinostat and other HDAC inhibitors is not well understood.

In the Pediatric Preclinical Testing Program (PPTP), vorinostat inhibited growth of all tested cell lines including leukemia, lymphoma, neuroblastoma, rhabdomyosarcoma, rhabdoid tumor, Ewing sarcoma, and glioblastoma [8]. However, the drug concentrations that were active in vitro were generally beyond the clinically achievable levels (1–2 μ M) [8]. Similarly, in vivo, vorinostat induced differences in event free survival (EFS) in pediatric solid tumor xeno-grafts, but no objective responses were observed [8]. Although vorinostat may not be effective as a single agent in pediatric solid tumors, HDAC inhibitors do appear to have the potential to exert additive or synergistic effects if other pathways are also inhibited. Additionally, vorinostat has been well-tolerated in children, with a maximum tolerated dose of 230 mg/m²/day for 4 days every 3 weeks as a single agent [9].

MLN8237 is a reversible small molecule inhibitor of the Aurora A serine/threonine kinase [10]. The Aurora family of kinases includes Aurora A, B, and C. Aurora A and B are expressed in all actively dividing cells, while Aurora C appears to be restricted to testicular tissue [11]. Aurora A kinase is critical in centrosome duplication, bipolar spindle assembly, and cell entry into mitosis [11]. The gene encoding Aurora A is located on chromosome 20q13.2, which is frequently amplified in tumors, and overexpression of Aurora A kinase results in the transformation of normal cells, supporting the role of Aurora A as an oncogene [12–14]. Treatment of tumor cells with Aurora A kinase inhibitors results in centrosome maturation and separation, G2-M transition, formation of mitotic spindle poles and spindles, and alignment of chromosomes to the metaphase plate [15–19]. Aurora B kinase inhibition is associated with an accumulation of tetraploid cells as cytokinesis, but not cell cycle progression, is inhibited [12]. In the PPTP, MLN8237 showed the highest in vitro activity in leukemia cell lines [20]. In vivo, MLN8237 induced significant differences in EFS in 80 % of solid tumor models and all six leukemia models. The in vivo activity observed against the neuroblastoma panel exceeded that observed for conventional chemotherapy [20]. MLN8237 is currently being studied in a phase I/II trial by the Children's Oncology Group; the recommended phase 2 dose in children has been established as 80 mg/m²/dose once daily for 7 of 21 days.

HDAC inhibitors interact with Aurora kinases on a number of levels. They have been shown to decrease expression of Aurora A and Aurora B kinases in lung cancer cells [21, 22]. Additionally, by inducing acetylation of the chaperone heat shock protein 90 (hsp90), HDAC inhibitors disrupt the association of hsp90 with Aurora kinases and deplete Aurora kinase activity [23]. HDAC inhibitors inactivate the spindle assembly checkpoint (SAC) in a transcription-independent fashion. This inhibition permits cells to enter anaphase prematurely, resulting in accumulation of aneuploid cells that eventually undergo apoptosis [24]. It is unclear whether Aurora A has a direct or indirect role in maintaining the SAC. If the intersection of HDAC inhibitors and Aurora A kinase inhibitors occurs at mitosis, then the combination of HDAC inhibitors with Aurora kinase inhibitors might disrupt the SAC at two different points, resulting in mitotic catastrophe and synergistic activity.

Methods

Drugs

Vorinostat (SAHA, suberoylanilide hydroxamic acid) was obtained from Cayman Chemical (Ann Arbor, MI) and MLN8237 was obtained from Millennium Pharmaceuticals, Inc. (Cambridge, MA). Vorinostat was dissolved in dimethyl sulfoxide (DMSO) to an initial concentration of 75.7 mM. MLN8237 was dissolved in DMSO to an initial concentration of 10 mM. Each drug was then diluted in phosphate buffered saline to the final concentrations used in each experiment.

Cell line and culture

Three human pediatric cell lines were used in the experiments: IMR-32, a neuroblastoma line; MOLT-4, a T-cell leukemia line; and Daoy, a medulloblastoma line. MOLT-4 and Daoy were cultured in RPMI-1640 medium with L-glutamine (Invitrogen Corp., Grand Island, NY) with heat inactivated 10 % fetal bovine serum (Invitrogen Corp.) in 5 % CO₂ at 37°C. IMR-32 was cultured using alpha-MEM medium (Invitrogen Corp.) with heat inactivated 10 % fetal bovine serum (Invitrogen Corp.) in 5 % CO₂ at 37°C.

Cytotoxicity assays

A modified methyl tetrazolium assay (MTT; 3-(4,5 dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide) was used to determine the sensitivity of the cell lines to vorinostat and MLN8237 [25, 26]. For single drug experiments, 135 µl of 1×10^5 cells/ml of each cell line was plated into 96-well microtiter plates using an automated drug delivery system (Biomek Automated Laboratory Workstation, Beckman Coulter, Inc., Fuller, CA). All cells were growing exponentially at the time of drug addition. The effect of evaporation in the wells was minimized by using only the inner wells for analyses. Twenty-four hours after plating, a single drug at specified concentrations was added to each well. Replicates of six wells were used at each concentration. Two replicate plates were used for each experiment. After 72 h of drug exposure, 15 µl of MTT (5 mg/ml) was added to each well, the plates were incubated for 4 h at 37°C, the medium was replaced with 150 µl of DMSO to solubilize the formazan precipitate and the optical density (OD) was measured at 550 nm using a microplate spectro-photometer (Anthos Labtec Instruments, Salzburg, Austria). Cell survival was calculated by subtracting the background OD of media alone and then dividing the OD of test wells by the OD of the control (untreated) wells.

For drug combinations, similar experiments were performed except that in each 96-well plate, cells were exposed to medium alone (controls), single drugs alone (vorinostat only, MLN8237 only) or varying concentrations of the combination of two drugs (vorinostat and MLN8237), for a total of 36 concentration pairs per plate. All combination drug concentrations were tested in four replicate plates.

Analysis of drug effects

The average percent cell survivals for each drug alone were calculated and plotted against the drug concentrations. The estimated concentrations at which cell growth was inhibited by 10 %, 25 %, 75 %, and 90 % (i.e., the IC₁₀, IC₂₅, etc.) from the single drug experiments were used to choose the range of concentrations of each drug for the combination experiments. The universal response surface approach (URSA) [27] as implemented in ADAPT II [28] was used to analyze the cytotoxic effects of the combination of vorinostat and MLN8237. The following equation was fitted to experimental data using nonlinear regression:

$$1 = \frac{D_a}{IC_{50a} \left(\frac{E}{100-E} \right)^{\frac{1}{m_a}}} + \frac{D_b}{IC_{50b} \left(\frac{E}{100-E} \right)^{\frac{1}{m_b}}} + \frac{\alpha \cdot D_a \cdot D_b}{IC_{50a} \cdot IC_{50b} \cdot \left(\frac{E}{100-E} \right)^{\frac{1}{2m_a} + \frac{1}{2m_b}}}$$

where D_a is the concentration of drug a, D_b is the concentration of drug b, IC_{50} is the median effective drug concentration, E is the measured effect (fraction of cells surviving), and m_a or m_b is the slope parameter of the individual drug's concentration-effect curve [29]. When α is positive, Loewe synergy is indicated, whereas a negative α reflects Loewe antagonism. The interaction is considered additive if the 95 % confidence interval (CI) for α includes zero [27, 29].

Immunoblotting

Pediatric cancer cells (IMR-32, MOLT-4, Daoy) were exposed to MLN8237 (0.5 μ M) or vorinostat (1 μ M) for 6 h. Cells treated with vorinostat were lysed in M-PER Mammalian Protein Extraction Reagent (Thermo Scientific, Rockford, IL), rinsed with phosphate buffered saline three times, and core histones were extracted (Supplementary Material 1). Cells treated with MLN8237 were lysed with Aurora A lysis buffer (see Supplemental Material 2). Proteins or core histones were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred onto a nitrocellulose membrane (Invitrogen, Carlsbad, CA), and blocked in Odyssey blocking buffer (Li-cor, Lincoln, NE) for 1 h at room temperature. Immunoblots were incubated overnight (4°C) with monoclonal anti-Aurora A (1:500; Cell Signaling, Danvers, MA), anti-phospho-histone H3 (Ser10) (1:1,000; Cell Signaling), β -actin (1:10,000; Sigma-Aldrich, St. Louis, MO), anti-acetylH4 (1:1,000; EMD Millipore, Billerica, MA), anti-acetylH3 (1:1,000; Millipore) or anti-histone H3 (1:2,000; Abcam, Cambridge, MA) antibodies diluted in Odyssey blocking buffer with 0.1 % Tween-20. Bound primary antibodies were incubated for 1 h at room temperature with IRDye800 conjugated affinity purified anti-rabbit or anti-mouse secondary antibody (1:2,000; Rockland, Gilbertsville, PA), and the signal was visualized on an Odyssey infrared imaging system. Relative band density was analyzed using ImageQuant software (GE Healthcare, Uppsala, Sweden). Aurora A and phospho-histone H3 levels were normalized to β -actin levels. AcetylH4 and acetylH3 levels were normalized to total histone H3 levels.

Results

Expression of target proteins

All three cell lines express Aurora A kinase protein (Fig. 1). MLN8237 does not inhibit histone H3 phosphorylation at ser10 in Daoy, IMR-32, or MOLT-4 cells, indicating active Aurora B kinase in the MLN8237-treated cells (Fig. 2). Histone acetylation increased after treatment with vorinostat (Fig. 3a and b).

Single drug activity

The single toxicity curves for vorinostat and MLN8237 for the leukemia, medulloblastoma, and neuroblastoma cell lines are illustrated in Figs. 4 and 5. The IC_{50} s of vorinostat for the various cell lines were 0.5 μ M for MOLT-4, 0.7 μ M for IMR-32, and 2.7 μ M for Daoy. MLN8237 demonstrated a narrow range of IC_{50} s; 0.02 μ M for MOLT-4, 0.03 μ M for IMR-32, and 0.04 μ M for Daoy. There was no precipitation of vorinostat or MLN8237 at the highest concentration. Both vorinostat and MLN8237 inhibited cell survival in a concentration-dependent manner. Treatment with vorinostat resulted in near complete inhibition of cell growth in MOLT-4 and IMR-32 cells at concentrations >1 μ M. In the

Daoy medulloblastoma cell line, treatment with vorinostat concentrations of 10 μM were needed to completely inhibit cell growth. Maximum inhibition of cell growth in all three lines occurred with 0.05–0.5 μM MLN8237. However, at concentrations of MLN8237 exceeding approximately 1 μM , there was a paradoxical increase in apparent survival in all three lines, most pronounced for Daoy.

Cytotoxicity of drug combinations

The concentration-effect curves for leukemia (Fig. 6), medulloblastoma (Fig. 7), and neuroblastoma (Fig. 8) cell lines are illustrated with the different concentrations of vorinostat and MLN8237. Table 1 shows the results of the synergy analysis. Vorinostat and MLN8237 are additive ($\alpha = 0$) in the leukemia cell line MOLT-4, medulloblastoma cell line Daoy, and neuroblastoma cell line IMR-32. Antagonism was not observed in any cell line. The paradoxical increase in cell survival seen with MLN8237 was not seen with the combination.

Discussion

Although the PPTP published preclinical data on vorinostat and MLN8237 as single agents [8, 20], no information is available about the combination of vorinostat and MLN8237 in pediatric tumor models. Our experiments demonstrate that Aurora A kinase, the target of MLN8237, is expressed in pediatric medulloblastoma, neuroblastoma, and leukemia cells. MLN8237 is a selective inhibitor of Aurora A kinase. Aurora B kinase activity remains intact, as shown by the increased phosphorylation of histone H3 at Ser10 in treated cell lines. Additionally, treatment of the cells with vorinostat resulted in the expected increase in histone acetylation, which is the chromatin conformation associated with active gene transcription. Our experiments show that the cytotoxic effects of the combination of vorinostat and MLN8237 are additive for MOLT-4, Daoy, and IMR-32. For MLN8237, the single agent IC_{50}s in medulloblastoma, neuroblastoma, and leukemia ($<0.04 \mu\text{M}$) are well below the average steady state concentrations achieved in adults ($\sim 1.7 \mu\text{M}$) [30] and children ($3.7 \mu\text{M}$) [31] at the recommended phase 2 dose of MLN8237. For vorinostat, the single-agent IC_{50}s were in or near the clinically achievable range ($1\text{--}2 \mu\text{M}$) [32].

MLN8237 at high concentrations loses selectivity for Aurora A kinase and inhibits Aurora B kinase as well [10]. Therefore, the apparent paradoxical increase in cell survival at high MLN8237 concentrations in our studies may actually result from “off target” Aurora B inhibition [33]. Aurora B inhibition leads to a defective spindle assembly checkpoint, resulting in accelerated mitotic slippage. The G1 tetraploids that derive subsequent to mitotic slippage can remain viable, and depending on various underlying genetic factors, may undergo additional rounds of DNA synthesis leading to endoreduplication. These tetraploid cells could remain metabolically active during the period of time assayed in the experiments performed here and, therefore, could appear viable using the MTT assay. Interestingly, the addition of vorinostat abrogated this effect suggesting that vorinostat may be a beneficial combination partner for either Aurora A or Aurora B kinase inhibitors.

The dose limiting toxicities of vorinostat in children were neutropenia, thrombocytopenia, and hypokalemia [9], while the dose limiting toxicities of MLN8237 included mucositis, mood alteration, neutropenia, and thrombocytopenia [31]. Thus the combination of vorinostat and MLN8237 may result in additive myelosuppression. The data from this study will be helpful in designing the dose escalation plan for future clinical trials.

In summary, we have shown that the combination of vorinostat and MLN8237 are additive *in vitro* against a variety of pediatric malignancies at concentrations that are readily obtained after single agent administration in the clinical setting. In light of the high activity that was

seen for MLN8237 by the PPTP, these results provide important preclinical support for the development of future clinical studies combining vorinostat and MLN8237, and we are currently developing a phase 1 trial of this combination in children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Glaser KB. HDAC inhibitors: clinical update and mechanism-based potential. *Biochem Pharmacol.* 2007; 74(5):659–671. doi:10.1016/j.bcp.2007.04.007. [PubMed: 17498667]
2. Johnstone RW. Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nat Rev Drug Discov.* 2002; 1(4):287–299. [PubMed: 12120280]
3. Lane A, Chabner BA. Histone deacetylase inhibitors in cancer therapy. *J Clin Oncol.* 2009; 27(32):5459–5468. doi:10.1200/JCO.2009.22.1291. [PubMed: 19826124]
4. Richon VM, Sandhoff TW, Rifkind RA, Marks PA. Histone deacetylase inhibitor selectively induces p21WAF1 expression and gene-associated histone acetylation. *Proc Natl Acad Sci USA.* 2000; 97(18):10014–10019. doi:10.1073/pnas.180316197. [PubMed: 10954755]
5. Richon V, O'Brien JP. Histone deacetylase inhibitors: a new class of potential therapeutic agents for cancer treatment. *Clin Cancer Res.* 2002; 8(3):662–664. [PubMed: 11895892]
6. Li Y, Kao GD, Garcia BA, Shabanowitz J, Hunt DF, Qin J, Phelan C, Lazar MA. A novel histone deacetylase pathway regulates mitosis by modulating Aurora B kinase activity. *Genes Dev.* 2006; 20(18):2566–2579. doi:10.1101/gad.1455006. [PubMed: 16980585]
7. Ishii S, Kurasawa Y, Wong J, Yu-Lee LY. Histone deacetylase 3 localizes to the mitotic spindle and is required for kinetochore-microtubule attachment. *Proc Natl Acad Sci USA.* 2008; 105(11):4179–4184. doi:10.1073/pnas.0710140105. [PubMed: 18326024]
8. Keshelava N, Houghton PJ, Morton CL, Lock RB, Carol H, Keir ST, Maris JM, Reynolds CP, Gorlick R, Kolb EA, Wu J, Smith MA. Initial testing (stage 1) of vorinostat (SAHA) by the pediatric preclinical testing program. *Pediatr Blood Cancer.* 2009; 53(3):505–508. doi:10.1002/pbc.21988. [PubMed: 19418547]
9. Fouladi M, Park JR, Stewart CF, Gilbertson RJ, Schaiquevich P, Sun J, Reid JM, Ames MM, Speights R, Ingle AM, Zwiebel J, Blaney SM, Adamson PC. Pediatric Phase I trial and pharmacokinetic study of vorinostat: a children's oncology group Phase I consortium report. *J Clin Oncol.* 2010; 28(22):3623–3629. doi:10.1200/JCO.2009.25.9119. [PubMed: 20606092]
10. Sells, T.; Ecsedy, J.; Stroud, S.; Janowick, D.; Hoar, K.; LeRoy, P.; Wysong, D.; Zhang, M.; Huck, J.; Silverman, L.; Chen, W.; Bembenek, M.; Claiborne, C.; Manfredi, M. 2008, abstract #237) MLN8237: an orally active small molecule inhibitor of Aurora A kinase in phase I clinical trials [abstract]. *Proceedings of the 99th Annual Meeting of the American Association for Cancer Research*; 2008 Apr 12–16; San Diego, CA Philadelphia (PA): AACR;
11. Gautschi O, Heighway J, Mack PC, Purnell PR, Lara PN Jr, Gandara DR. Aurora kinases as anticancer drug targets. *Clin Cancer Res.* 2008; 14(6):1639–1648. doi:10.1158/1078-0432.CCR-07-2179. [PubMed: 18347165]
12. Keen N, Taylor S. Aurora-kinase inhibitors as anticancer agents. *Nat Rev Cancer.* 2004; 4(12):927–936. doi:10.1038/nrc1502. [PubMed: 15573114]
13. Marumoto T, Zhang D, Saya H. Aurora-A—a guardian of poles. *Nat Rev Cancer.* 2005; 5(1):42–50. doi:10.1038/nrc1526. [PubMed: 15630414]

14. Katayama H, Brinkley WR, Sen S. The Aurora kinases: role in cell transformation and tumorigenesis. *Cancer Metastasis Rev.* 2003; 22(4):451–464. [PubMed: 12884918]
15. Glover DM, Leibowitz MH, McLean DA, Parry H. Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles. *Cell.* 1995; 81(1):95–105. [PubMed: 7720077]
16. Hoar K, Chakravarty A, Rabino C, Wysong D, Bowman D, Roy N, Ecsedy JA. MLN8054, a small-molecule inhibitor of Aurora A, causes spindle pole and chromosome congression defects leading to aneuploidy. *Mol Cell Biol.* 2007; 27(12):4513–4525. doi:10.1128/MCB.02364-06. [PubMed: 17438137]
17. Katayama H, Zhou H, Li Q, Tatsuka M, Sen S. Interaction and feedback regulation between STK15/BTAK/Aurora-A kinase and protein phosphatase 1 through mitotic cell division cycle. *J Biol Chem.* 2001; 276(49):46219–46224. doi:10.1074/jbc.M107540200. [PubMed: 11551964]
18. Marumoto T, Honda S, Hara T, Nitta M, Hirota T, Kohmura E, Saya H. Aurora-A kinase maintains the fidelity of early and late mitotic events in HeLa cells. *J Biol Chem.* 2003; 278(51):51786–51795. doi:10.1074/jbc.M306275200. [PubMed: 14523000]
19. Sasai K, Parant JM, Brandt ME, Carter J, Adams HP, Stass SA, Killary AM, Katayama H, Sen S. Targeted disruption of Aurora A causes abnormal mitotic spindle assembly, chromosome misalignment and embryonic lethality. *Oncogene.* 2008; 27(29):4122–4127. doi:10.1038/onc.2008.47. [PubMed: 18345035]
20. Maris JM, Morton CL, Gorlick R, Kolb EA, Lock R, Carol H, Keir ST, Reynolds CP, Kang MH, Wu J, Smith MA, Houghton PJ. Initial testing of the aurora Kinase A inhibitor MLN8237 by the Pediatric Preclinical Testing Program (PPTP). *Pediatr Blood Cancer.* 2010; 55(1):26–34. doi: 10.1002/pbc.22430. [PubMed: 20108338]
21. Zhang XH, Rao M, Loprieato JA, Hong JA, Zhao M, Chen GZ, Humphries AE, Nguyen DM, Trepel JB, Yu X, Schrupp DS. Aurora A, Aurora B and survivin are novel targets of transcriptional regulation by histone deacetylase inhibitors in non-small cell lung cancer. *Cancer Biol Ther.* 2008; 7(9):1388–1397. [PubMed: 18708766]
22. Park JH, Jong HS, Kim SG, Jung Y, Lee KW, Lee JH, Kim DK, Bang YJ, Kim TY. Inhibitors of histone deacetylases induce tumor-selective cytotoxicity through modulating Aurora-A kinase. *J Mol Med.* 2008; 86(1):117–128. doi:10.1007/s00109-007-0260-8. [PubMed: 17851643]
23. Bali P, Pranpat M, Bradner J, Balasis M, Fiskus W, Guo F, Rocha K, Kumaraswamy S, Boyapalle S, Atadja P, Seto E, Bhalla K. Inhibition of histone deacetylase 6 acetylates and disrupts the chaperone function of heat shock protein 90: a novel basis for antileukemia activity of histone deacetylase inhibitors. *J Biol Chem.* 2005; 280(29):26729–26734. doi:10.1074/jbc.C500186200. [PubMed: 15937340]
24. Magnaghi-Jaulin L, Eot-Houllier G, Fulcrand G, Jaulin C. Histone deacetylase inhibitors induce premature sister chromatid separation and override the mitotic spindle assembly checkpoint. *Cancer Res.* 2007; 67(13):6360–6367. doi:10.1158/0008-5472.CAN-06-3012. [PubMed: 17616695]
25. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods.* 1983; 65(1–2):55–63. [PubMed: 6606682]
26. Twentyman PR, Luscombe M. A study of some variables in a tetrazolium dye (MTT) based assay for cell growth and chemo-sensitivity. *Br J Cancer.* 1987; 56(3):279–285. [PubMed: 3663476]
27. Greco WR, Park HS, Rustum YM. Application of a new approach for the quantitation of drug synergism to the combination of cis-diamminedichloroplatinum and 1-beta-D-arabinofuranosylcytosine. *Cancer Res.* 1990; 50(17):5318–5327. [PubMed: 2386940]
28. D'Argenio, D.; Schumitzky, A. ADAPT II user's guide: pharmacokinetic/pharmacodynamic systems analysis software. Biomedical Simulations Resource; Los Angeles: 1997.
29. Horton TM, Gannavarapu A, Blaney SM, D'Argenio DZ, Plon SE, Berg SL. Bortezomib interactions with chemotherapy agents in acute leukemia in vitro. *Cancer Chemother Pharmacol.* 2006; 58(1):13–23. doi:10.1007/s00280-005-0135-z. [PubMed: 16292537]
30. Cervantes-Rupierez A, Burris HA, Cohen RB, Dees EC, Infante JR, Fingert HJ, Shinde V, Venkatakrishnan K, Chakravarty A, Tabernero J. Pharmacokinetics and pharmacodynamic results from two phase I studies of the investigational selective Aurora A kinase (AAK) inhibitor

- MLN8237: exposure-dependent AAK inhibition in human tumors. *J Clin Oncol.* 2010; 28(7S): 3031A.
31. Mosse Y, Lipsitz EG, Maris JM, Weigel B, Adamson PC, Ingle M, Ahern CH, Blaney S. A pediatric phase I trial and pharmacokinetic study of MLN8237, an oral selective small molecule inhibitor of Aurora A kinase: a children's oncology group Phase I consortium study. *J Clin Oncol.* 2010; 28(7S):9529A.
 32. Kelly WK, O'Connor OA, Krug LM, Chiao JH, Heaney M, Curley T, MacGregore-Cortelli B, Tong W, Secrist JP, Schwartz L, Richardson S, Chu E, Olgac S, Marks PA, Scher H, Richon VM. Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. *J Clin Oncol.* 2005; 23(17):3923–3931. doi:10.1200/JCO.2005.14.167. [PubMed: 15897550]
 33. Kallio MJ, McClelland ML, Stukenberg PT, Gorbsky GJ. Inhibition of aurora B kinase blocks chromosome segregation, overrides the spindle checkpoint, and perturbs microtubule dynamics in mitosis. *Curr Biol.* 2002; 12(11):900–905. [PubMed: 12062053]

Fig. 1.

Expression of Aurora A kinase protein in pediatric cancer cells. Total protein extracts (30 μ g) from untreated Daoy, IMR-32, and MOLT-4 cells were analyzed by Western immunoblot. β -actin was used as loading control

Fig. 2.
Effect of MLN8237 on Aurora B kinase. Cells were treated with 0.5 μ M MLN8237 for 24 h, and total protein extracts (30 μ g) were analyzed by Western immunoblot. β -actin was used as loading control

Fig. 3. Vorinostat increases histone acetylation. a Cells were treated with 1 μ M vorinostat and core histone extracts were analyzed by Western immunoblot. Total H3 was used as loading control. b Quantification of histone H3 and H4 acetylation levels compared to total H3 loading control

Fig. 4.
Cytotoxicity of vorinostat against Daoy (Black diamond), IMR-32 (Black square) and MOLT-4 (Black circle) cell lines

Fig. 5. Cytotoxicity of MLN8237 against Daoy (Black diamond), IMR-32 (Black square) and MOLT-4 (Black circle) cell lines

Fig. 6.
Cytotoxicity of MLN8237+Vorinostat on MOLT-4 Leukemia Cells

Fig. 7.
Cytotoxicity of MLN8237+Vorinostat on Daoy Medulloblastoma Cells

Fig. 8.
Cytotoxicity of MLN8237+Vorinostat on IMR-32 Neuroblastoma Cells

Table 1

Cytotoxic effects of the combination of vorinostat and MLN8237

Cell line	Drug	IC ₅₀ (μM)	m	alpha	95 % CI
MOLT-4	Vorinostat	0.59	1.59	0.001	(-0.26, 0.26)
	MLN8237	0.02	5.48		
Daoy	Vorinostat	1.45	1.75	0.002	(-0.52, 0.53)
	MLN8237	<0.01	0.18		
IMR-32	Vorinostat	0.62	1.96	0.003	(-0.24, 0.25)
	MLN8237	0.02	3.28		

IC₅₀: the concentration at which cell survival is inhibited by 50 %; m: the slope parameter that indicates sigmoidicity of dose-response curve; 95 % CI: the 95 % confidence interval around estimate of alpha