# Histone Deacetylase Inhibitors Enhance Paclitaxel-induced Cell Death in Ovarian Cancer Cell Lines Independent of p53 Status

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**Abstract.** *Background: Recurrence of drug-resistant disease contributes to the high mortality of ovarian cancer patients, which necessitates the identification of additional chemotherapeutic drugs. Histone deacetylase inhibitors (HDAIs) induce apoptosis in a number of malignant cell types and may represent a new class of drugs clinically relevant in the treatment of ovarian cancer. Materials and Methods: Ovarian cancer cells were treated with various combinations of a HDAI and paclitaxel (PTX). Cell death was measured using annexin V / propidium iodide exclusion. Results: The PTX/HDAI drug combination was as efficient in inducing cell death as continuous PTX treatment and superior to continuous HDAI treatment. Reversing the sequence of drug exposure reduced the cytotoxic efficacy of the drug combination. The p53 status of the cell lines did not alter the cytotoxic efficacy of the treatment protocols. Conclusion: These results suggest that HDAIs possess possible clinical applications as an adjuvant therapy in the treatment of ovarian cancer.*

Ovarian cancer is one of the most common female gynecologic cancers in the United States. The American Cancer Society estimates that over 25,000 new cases will be diagnosed in the year 2003 alone (1). Due to the absence of definitive presenting symptoms, many patients will have advanced stage disease at the time of diagnosis. Although the initial response to chemotherapy is 70-80%, prognosis is poor, with only a small percentage of patients (20-30%)

surviving five years after diagnosis. The high mortality rate within this group is largely due to recurrence of drug-resistant disease and the poor response rates to salvage chemotherapeutic regimens (2). In order to improve the outcome for patients with advanced and/or refractory disease, it is imperative to identify novel agents that, alone or in combination with current therapeutic regimens, enhance tumor cell killing.

Paclitaxel (PTX) is presently employed as a first-line chemotherapeutic agent in the treatment of breast and ovarian cancer (3, 4). PTX (Taxol™) is a taxane, which binds to the N-terminal region of β-tubulin, preventing the depolymerization of microtubules (5). The stabilization of microtubules by PTX leads to G2/M-phase arrest and promotes apoptosis. The advent of PTX-induced cytotoxicity has been correlated with the activation of multiple protein kinases including Raf-1, cAMP-dependent protein kinase (PKA) and mitogen-activated protein (MAP) kinases, with the hyperphosphorylation of the anti-apoptotic protein, Bcl-2 and with the up-regulation of the pro-apoptotic proteins, Bax and Bak, the tumor suppressor protein, p53 and the cyclin-dependent kinase (cdk) inhibitor, p21/WAF1 (6-10).

Histone deacetylase inhibitors (HDAIs) have gained attention as possible anti-neoplastic agents in recent years and are being employed in a number of clinical trials (11, 12). HDAIs exhibit growth inhibitory and pro-apoptotic activity in a number of different cell types by altering the expression of genes that regulate these processes. HDAIs are thought to alter gene expression by decreasing the activity of HDAC, which in turn, maintains chromatin structure in a decompressed or relaxed state, increasing the accessibility of transcription factors to their DNA targets (13, 14). The inhibition of growth and the induction of apoptosis by HDAIs correlate with G1 and G2/M cell cycle arrest, with the up-regulation of the cdk inhibitor, p21 and pro-apoptotic proteins, Bax and Bak, with the downregulation of Bcl-2 and p53 and with the activation of the caspase cascade (15-20).

Studies in our laboratory utilizing the HDAIs, sodium butyrate (NaB), a short-chain fatty acid produced by the bacterial fermentation of dietary fiber within the colon and trichostatin A (TSA), a hydroxamic acid-based drug (21), demonstrated that HDAIs arrest anaplastic thyroid cells in both the G1- and G2/M-phases of the cell cycle and induce apoptosis in a p53-independent manner through the activation of the caspase cascade. Cell cycle arrest was associated with the up-regulation of p21 and the cdk inhibitor, p27/Kip1, the down-regulation of cyclin A and cyclin B and the reduced activity of cdk1 and cdk2-associated kinases (22, 23).

Although both drugs induce similar molecular events (*i.e.* G2 arrest and apoptosis), their mode of action is quite different. Based on this information, we theorized that combining PTX with a HDAI would enhance cell killing by activating distinct signaling pathways that promote cell death.

## Materials and Methods

*Cell culture.* The human epithelial ovarian cancer cell lines, Caov3, A2780 and SKOV3 were kindly provided by Dr Michael Birrer, NCI, Bethesda, Maryland, USA. The cell lines were cultured in complete RPMI medium 1640 (Gibco/BRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO, USA) and 100 U/ml of penicillin G and 100 mg/L of streptomycin sulfate (Gibco/BRL) at 37°C/5% $CO_2$/100% humidity.

*Cell cycle analysis.* Cells were plated at a density of $5 \times 10^5$ per 12 ml of RPMI in 100-mm tissue culture dishes in duplicate and treated with increasing concentrations of PTX immediately after plating for analysis. Twenty-four hours after treatment, the cells were harvested. The culture medium and trypsinized monolayer were combined and centrifuged. The cell pellets were washed in cold phosphate-buffered saline (PBS), resuspended in PBS and fixed in a 70% solution of ethanol (>15 minutes). The samples were centrifuged and resuspended in one milliliter of a solution consisting of a 1/50 volume of 0.5 mg/ml of propidium iodide (Sigma) in $3.8 \times 10^{-2}$ M sodium citrate and a 1/40 volume of 10 mg/ml of RNase A (Sigma) in 10 mM Tris-HCl pH 7.5, 15 mM sodium chloride in PBS. The samples were incubated for 30 minutes at 37°C and DNA content was analyzed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA).

*Analysis of apoptosis.* Confluent cell cultures were plated at a density of $5 \times 10^5$ per 12 ml of RPMI in 100-mm tissue culture dishes in triplicate. Cells were treated with a combination of vehicle (DMSO), PTX (Sigma), 5 mM NaB (Sigma) or 0.5 μM of TSA (Sigma) at the times stated in the figure legends. Seventy-two hours after initial treatment, the culture medium and trypsinized monolayer were collected, combined and pelleted by centrifugation. Apoptosis was measured in duplicate samples using the ApoAlert Annexin V Apoptosis Kit (CLONTECH Laboratories, Palo Alto, CA, USA) as instructed by the manufacturer.

Cell pellets were washed with binding buffer, centrifuged, resuspended in binding buffer and incubated with annexin V-FITC and propidium iodide for 15 minutes at room temperature in the dark. Cell samples were analyzed by two-color flow cytometry using a FACScan flow cytometer (Becton Dickinson). The appearance of phosphatidyl serine on the cell surface is indicative of early apoptosis, allowing the quantitation of this population by flow cytometry through the specific binding of fluorochrome-labeled annexin V. The nonviable population, including cells in the later stages of apoptosis and necrotic cells, stain positive for both annexin V and propidium iodide (PI) due to a breach in membrane integrity, also allowing the quantitation of this segment of the cell population. Thus, the percentage of viable cells was calculated as the percentage of the cell population that was annexin V- and PI-negative. A protein extract was prepared in parallel from each group as described below.

*Western blot analysis.* Cells were treated and collected as described above. The expression of p53 was determined in cells harvested at 48 hours after initial treatment and PARP cleavage was examined 72 hours after initial treatment. Cells were washed with phosphate-buffered saline (PBS), counted and pelleted by centrifugation. Protein extracts were prepared by the addition of a 2X lysis solution (250mM Tris, pH 6.8, 4% sodium dodecyl sulfate [SDS], 10% glycerol, 2% B-mercaptoethanol, 0.006% bromophenol blue) to the cell pellets, resulting in a final concentration of 5000 cells per microliter. An equal volume of each protein extract was separated by electrophoresis through 4% to 20% gradient Tris glycine gels (Invitrogen, Carlsbad, CA, USA). The proteins were transferred to polyvinyl difluoride (PVDF) membranes, blocked and incubated overnight at 4°C with primary antibody. The membranes were then washed (5 times) in Tris-buffered saline-Tween 20 (TBS-T) and incubated for 30 minutes to 1 hour at room temperature with a sheep anti-mouse horseradish peroxidase-linked antibody (Amersham Life Sciences, Arlington Heights, IL, USA. Cat. #NA931) or a goat anti-rabbit horseradish peroxidase-linked antibody (Amersham Life Sciences, Cat. #NA934). Protein bands were visualized using a chemiluminesent ECL kit (Amersham) as instructed by the manufacturer and exposed to autoradiography film to visualize the protein bands (5 seconds to 15 minutes). Anti-β-actin antibody (Cat.# A-5441) was purchased from Sigma, anti-p53 antibody (Cat.# 610184) was purchased from PharMingen, San Diego, CA, USA and anti-PARP antibody (Cat.# sc-7150) was purchased from Santa Cruz Biotechnology, Santa Cruz, CA, USA. Protein extracts from induced HL60 cells (BioMol, Plymouth Meeting, PA, USA) were used as a positive control for PARP.

## Results

*Cytotoxic effectiveness of PTX and HDAI combinations.* Recent studies have indicated that enhancing G2/M arrest increases the sensitivity of cancer cells to the cytotoxic effects of chemotherapeutic drugs (24-26). Therefore, we determined the concentration of PTX that results in a G2/M accumulation of >50% by 24 hours after treatment. A concentration of 10 nM of PTX resulted in a >85% G2/M accumulation in the A2780 and Caov3 cell lines and a 25 nM concentration of PTX resulted in a 60-80% G2/M accumulation in the SKOV3 cell line (data not shown). Based on our previous studies, a concentration of 5 mM
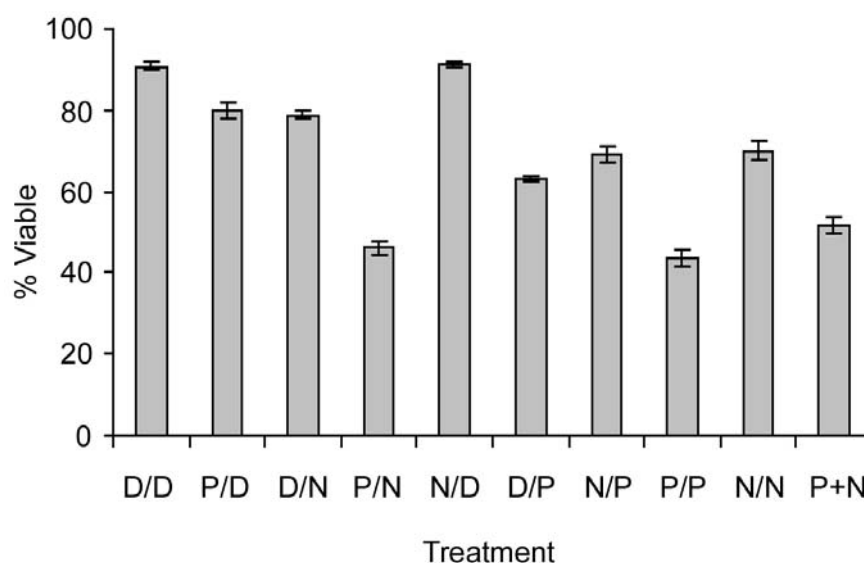
Figure 1. *Cytotoxic effectiveness of PTX and HDAI combinations. Ovarian cancer cells (A2780) were treated with vehicle (DMSO), 10 nM paclitaxel (PTX), or 5 mM sodium butyrate (NaB) immediately after plating. The medium was replaced 24 hours later and the cells were treated with DMSO (D), 10 nM PTX (P), or 5 mM NaB (N) as indicated (i.e. D/D denotes cells received DMSO at time zero and time 24 hours, etc. and P + N denotes cells received PTX and NaB at time zero and time 24 hours). Cell viability was measured 72 hours after initial treatment using annexin V/ propidium iodide exclusion as described in Materials and Methods. Results represent the mean±SEM of viable cells calculated from three independent experiments, each performed in duplicate.*

NaB and 0.5 μM TSA are sufficient to induce differential cell cycle arrest (G1 and G2/M) and apoptosis (22).

To test the hypothesis that combining PTX with a HDAI would increase the advent of cell death, the ovarian cancer cell line, A2780, was treated with 5 mM NaB, 10 nM PTX or DMSO (vehicle) for 24 hours. The culture media was replaced and cells were retreated with 5 mM NaB, 10 nM PTX or DMSO in the combinations indicated and cell viability was measured 72 hours after initial treatment using annexin V/ propidium iodide exclusion. As shown in Figure 1, cell viability was dramatically reduced in comparison to the controls (91±1.0%) in cells treated with the combinations of PTX/NaB (46±1.7%), PTX/PTX (44±2.1%) and PTX + NaB (52±2.2%). In contrast, continuous exposure to NaB (70±2.3%) and the combination NaB/PTX (69±1.8%) was less efficient in inducing cell death. These results demonstrate that a limited exposure to PTX sensitizes the cells to the cytotoxic effects of NaB and is sufficient to induce cell killing as efficiently as continuous exposure to PTX. However, reversing the sequence of drug exposure reduced the cytotoxic effectiveness of this combination.

*PTX-induced p53 expression is decreased by HDAIs.* The tumor suppressor protein, p53, regulates both cell proliferation and apoptosis through the regulation of specific genes (27). While the expression of p53 is positively regulated by PTX (6, 8), the expression of p53

Table I. *Enhanced cell killing with the combination of paclitaxel and histone deacetylase inhibitors.*

| | Viable cell population (%) | | |
|---|---|---|---|
| Treatment | Caov3 | A2780 | SKOV3 |
| Control | 93.8 ± 0.6 | 93.7 ± 0.4 | 88.4 ± 2.0 |
| PTX | 71.0 ± 1.3 | 75.5 ± 4.4 | 68.1 ± 2.5 |
| NaB | 78.8 ± 1.2 | 79.0 ± 1.2 | 73.8 ± 2.3 |
| PTX/NaB | 46.8 ± 2.3 | 48.6 ± 5.1 | 49.6 ± 2.9 |
| Control | 90.5 ± 0.7 | 92.0 ± 0.8 | ND |
| PTX | 65.5 ± 4.8 | 74.3 ± 4.9 | ND |
| TSA | 53.7 ± 2.4 | 30.7 ± 0.9 | ND |
| PTX/TSA | 23.5 ± 3.1 | 24.3 ± 2.0 | ND |

Ovarian cancer cell lines were treated with 10 nM (Caov3 and A2780) or 25 nM (SKOV3) paclitaxel (PTX) or vehicle (DMSO) for 24 hours, the medium was replaced and the appropriate dishes were treated with 5 mM sodium butyrate (NaB) or 0.5 μM trichostatin A (TSA). Cells were harvested 72 hours after initial treatment. Apoptosis was measured using the ApoAlert Annexin V Kit as described in Materials and Methods. Results represent the mean ± SEM of viable cells (annexin V- and propidium iodide-negative) calculated from at least three independent experiments, each performed in duplicate.
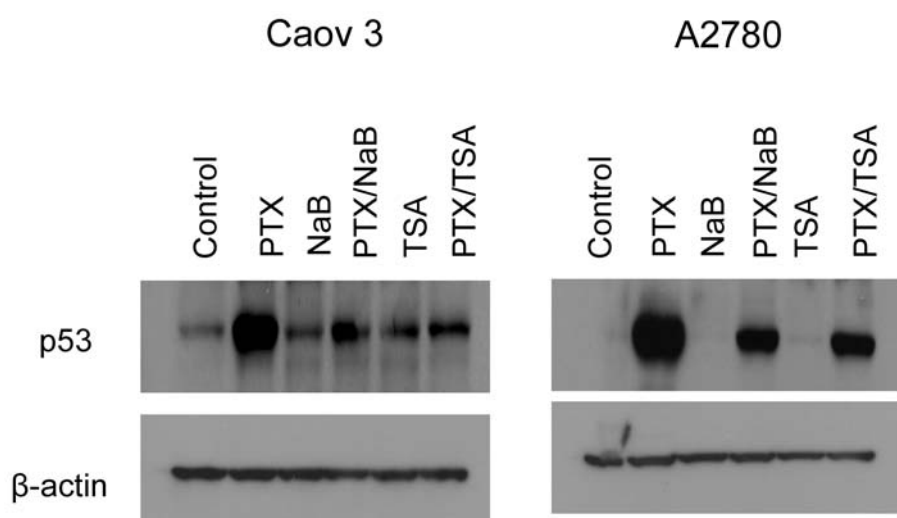
Figure 2. *HDAI treatment decreases PTX-induced p53 expression. Ovarian cancer cells were treated with 10 nM paclitaxel (PTX) or vehicle (DMSO) for 24 hours. The medium was replaced and cells were treated with 5 mM sodium butyrate (NaB) or 0.5 µM trichostatin A (TSA) for an additional 24 hours. The cells were harvested and protein extracts were prepared and analyzed by Western blot using a p53 specific antibody as described in Materials and Methods. Membranes were reprobed with a β-actin specific antibody to verify equal loading and transfer.*

is negatively regulated by HDAIs (18,20). Interestingly, studies have indicated that the loss of p53 sensitizes ovarian cells to PTX (28, 29). In order to investigate whether cell killing induced by the PTX/HDAI combination is influenced by the expression of wild-type p53, we verified that PTX increases the expression of p53 and HDAIs reduce the expression of p53 in ovarian cancer cell lines. As shown in Figure 2, the expression of p53 increased in the PTX-treated cells as compared to the controls in the A2780 and Caov3 cell lines. In contrast, the addition of a HDAI reduced the expression of PTX-induced p53. We then compared the response of ovarian cancer cell lines that express wild-type p53 (A2780), mutant p53 (Caov3), or are p53 null (SKOV3) to the PTX/HDAI drug combination. As shown in Table I, the percentage of viable cells decreased dramatically in cells treated with the drug combination as compared to the controls. Cell viability was reduced from 93.8% to 46.8% in the Caov3 cell line, from 93.7% to 48.6% in the A2780 cell line and from 88.4% to 49.6% in the SKOV3 cell line. A more pronounced response was obtained when PTX was combined with the TSA, decreasing cell viability from 90.5% to 23.5% in the Caov3 cell line and from 92% to 24.3% in the A2780 cell line. These data suggest that the molecular mechanism by which the PTX/HDAI combination decreases cell viability is not specifically determined by p53 status. It is unclear whether the HDAI-induced reduction in p53 levels is

necessary for the cytotoxic activity of the PTX/HDAI combination.

*PTX and HDAIs activate the caspase cascade*. Under certain conditions, apoptosis is promoted and executed by caspases, a family of cysteine proteases that cleave and inactivate proteins necessary for cell survival (30). The DNA repair enzyme, PARP (116-kDa), is cleaved by caspases during the execution phase of apoptosis, resulting in the appearance of an 85-kDa fragment (31). In order to determine whether PTX and/or HDAI treatment activates the caspase cascade in our model system, we examined whether drug treatment resulted in the appearance of the cleaved form of PARP. Protein extracts were prepared from samples treated in parallel with those shown in Table I and PARP cleavage was visualized by Western blot. As shown in Figure 3, PTX and HDAI treatment, alone or in combination, results in the activation of the caspase cascade as indicated by the appearance of the cleaved form of PARP (85-kDa).

**Discussion**

The present study examined the response of ovarian cancer cells to treatment with HDAIs, alone and in combination with PTX. We found that the PTX/NaB combination induced cell death as efficiently as continuous treatment with PTX. These results could have a significant clinical impact. In clinical trials,
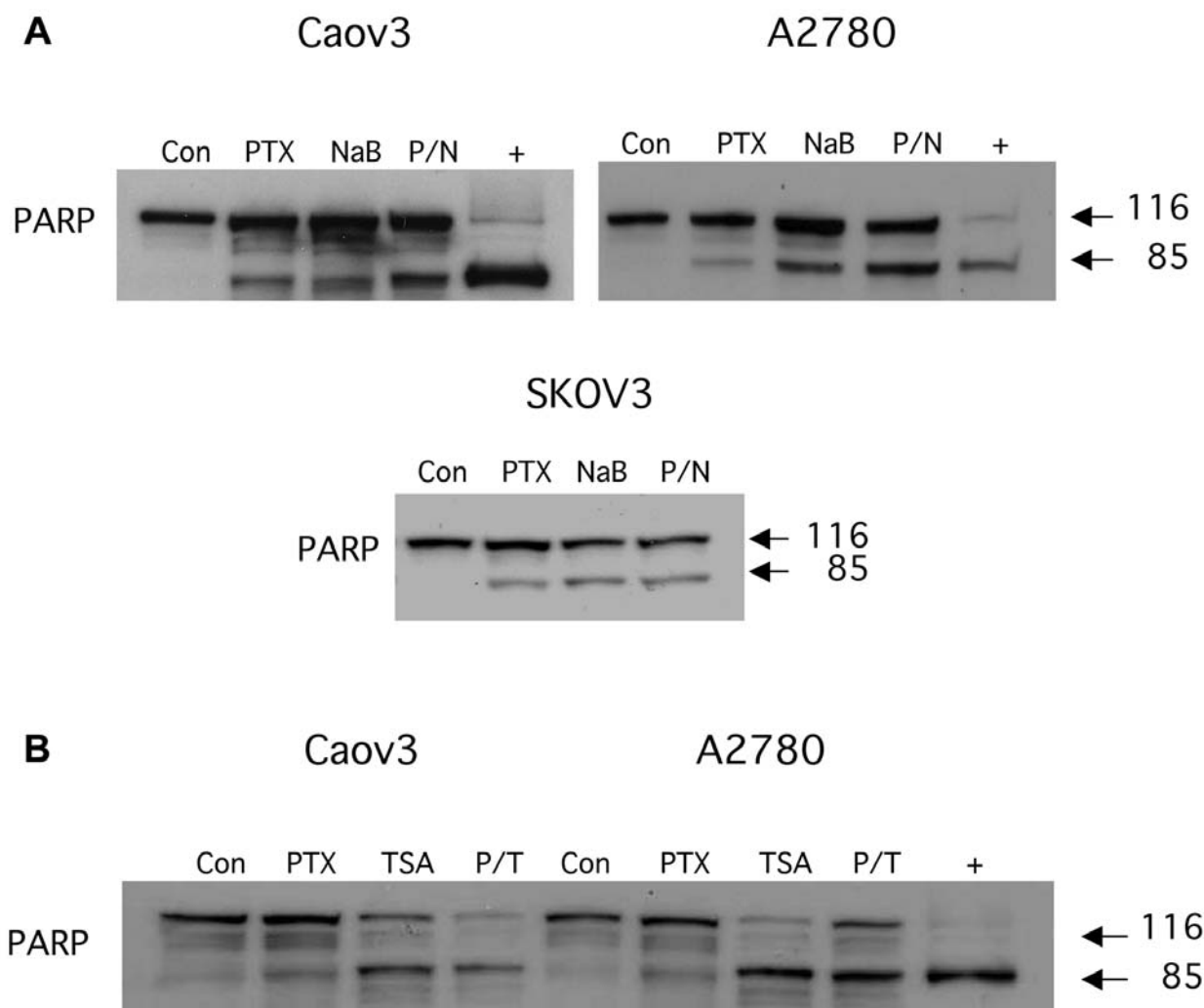
Figure 3. *PTX and HDAI treatment results in cleavage of the caspase substrate, poly (ADP-ribose) polymerase (PARP). Ovarian cancer cells were treated with 10 nM (Caov3 and A2780) paclitaxel (PTX), 25 nM (SKOV3) PTX or vehicle (DMSO) for 24 hours. The medium was replaced and cells were treated with 5 mM sodium butyrate (NaB) or 0.5 μM trichostatin A (TSA) for an additional 48 hours. Cells were harvested 72 hours after initial treatment. Protein extracts were prepared and analyzed by Western blot using a PARP specific antibody as described in Materials and Methods. Protein extracts from induced HL60 cells were used as a positive control (+) for PARP cleavage. Con (DMSO), PTX (paclitaxel), NaB (sodium butyrate), TSA (trichostatin A), P/N (PTX/NaB) and P/T (PTX/TSA).*

HDAIs are reportedly well tolerated by patients (11,12). In contrast, the current therapeutic regimen of PTX can induce severe side-effects such as, neurotoxicity (4). In our study, low concentrations of PTX and a limited exposure time (24 hours), combined with a HDAI, produced significant cell death *in vitro*. Thus, combining PTX with a HDAI might allow the clinician to use lower doses of PTX for a shorter period of time, thereby reducing PTX-induced side-effects. However, the dose and schedule of drug administration must be carefully evaluated to ensure that initial drug treatment results in G2/M arrest.

In response to DNA damaging events such as hypoxia, ionizing radiation and chemotherapeutic drugs, the level and DNA binding activity of p53 increases which, in turn, promotes cell cycle arrest and apoptosis (32). However, the contribution of p53 in the induction of cell death by PTX is unclear.

Several studies support the hypothesis that the loss of functional p53 increases the sensitivity of cells to the cytotoxic effects of PTX. The depletion of p53 in normal fibroblasts increased the cytotoxic efficacy of PTX (28) and the exogenous expression of functional p53, in a p53-deficient cell line, resulted in a reduction in the cytotoxic effectiveness of

PTX treatment (29). On the other hand, low doses of PTX increased apoptosis in a nasopharyngeal carcinoma (NPC) cell line expressing wild-type p53 over that induced by PTX in a NPC cell line expressing mutant p53 (33).

We found that the p53 status of the cell line did not alter its sensitivity to any of the drug treatments. The amount of cell death induced by drug treatment in the A2780 cell line (p53 wild-type) was not significantly different from that induced in the Caov3 cell line (p53 mutant) and the SKOV3 cell line (p53 null). Our results indicate that cell death induced by PTX and/or a HDAI does not require functional p53. However, we are unable to rule out that the reduction in cell viability by the PTX/HDAI combination does not involve the HDAI-induced reduction in p53 expression or alterations in factors downstream of p53.

In addition, we found that treatment with PTX and/or HDAI led to the cleavage of the caspase substrate PARP. These results are in agreement with a number of studies, which demonstrate that treatment with PTX and HDAIs induce caspase-dependent apoptosis (7,20,34). Conversely, PTX has been reported to induce caspase-independent apoptosis also (35,36).

We are currently investigating possible mechanisms that could account for the cell death induced by the PTX/HDAI combination. For example, changes in the expression of IAPs (inhibitors of apoptosis) such as survivin may play a role. Inhibiting the cdk1-dependent phosphorylation of survivin reduces endogenous survivin levels (37). This possibility is a viable mechanism due to the fact that our previous studies have shown that HDAIs inhibit cdk1-associated kinase activity which correlated with an increase in p21 expression and a down-regulation of cyclin B expression (22) and that PTX and/or HDAI treatment up-regulates the expression of p21 in the ovarian cancer cell line, A2780 (unpublished data). Therefore, the combination of PTX/HDAI could alter the expression of IAPs beyond that induced by single drug therapy, resulting in an increase in cell death. Potential mechanisms such as these are currently the subject of on-going studies in our laboratory. In summary, this study demonstrates that HDAIs possess possible clinical applications as an adjuvant therapy in the treatment of ovarian cancer.

## Acknowledgements

## References

1  Jemal A, Murray T, Samuels A, Ghafoor A, Ward E and Thun MJ: Cancer Statistics, 2003. CA Cancer J Clin *53*: 5-26, 2003.

2  Kaye SB: Future directions for the management of ovarian cancer. Eur J Cancer 37 Suppl *9*: S19-23, 2001.

3  Vogel CL and Nabholtz JM: Monotherapy of metastatic breast cancer: A review of newer agents. The Oncologist *4*: 17-33, 1999.

4  du Bois A: Treatment of advanced ovarian cancer. Eur J Cancer *37 Suppl 9*: S1-7, 2001.

5  Downing KH: Structural basis for the interaction of tubulin with proteins and drugs that affect microtubule dynamics. Annu Rev Cell Dev Biol *16*: 89-111, 2000.

6  Wang LG, Liu XM, Kreis W and Budman DR: The effect of antimicrotubule agents on signal transduction pathways of apoptosis: a review. Cancer Chemother Pharmacol *44(5)*: 355-61, 1999.

7  Srivastava RK, Srivastava AR, Korsmeyer SJ *et al*: Involvement of microtubules in the regulation of Bcl2 phosphorylation and apoptosis through cyclic AMP-dependent protein kinase. Mol Cell Biol *18(6)*: 3509-17, 1998.

8  Bacus SS, Gudkov AV, Lowe M *et al*: Taxol-induced apoptosis depends on MAP kinase pathways (ERK and p38) and is independent of p53. Oncogene *20(2)*: 147-55, 2001.

9  Wang TH, Wang HS and Soong YK: Paclitaxel-induced cell death: where the cell cycle and apoptosis come together. Cancer *88(11)*: 2619-28, 2000.

10 Blagosklonny MV and Fojo T: Molecular effects of paclitaxel: myths and reality (a critical review). Int J Cancer *83(2)*: 151-6, 1999.

11 Marks P, Rifkind RA, Richon VM *et al*: Histone deacetylases and cancer: causes and therapies. Nat Rev Cancer *1(3)*: 194-202, 2001.

12 Johnstone RW: Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. Nat Rev Drug Discov *1(4)*: 287-99, 2002.

13 Narlikar GJ, Fan HY and Kingston RE: Cooperation between complexes that regulate chromatin structure and transcription. Cell *108(4)*: 475-87, 2002.

14 Richon VM and O'Brien JP: Histone deacetylase inhibitors: a new class of potential therapeutic agents for cancer treatment. Clin Cancer Res *8(3)*: 662-4, 2002.

15 Munster PN, Troso-Sandoval T, Rosen N, Rifkind R, Marks PA, Richon VM: The histone deacetylase inhibitor suberoylanilide hydroxamic acid induces differentiation of human breast cancer cells. Cancer Res *61*: 8492-7, 2001.

16 Blagosklonny MV, Robey R, Sackett DL, Du L, Traganos F, Darzynkiewicz Z, Fojo T and Bates SE: Histone deacetylase inhibitors all induce p21 but differentially cause tubulin acetylation, mitotic arrest, and cytotoxicity. Mol Cancer Ther *1*: 937-41, 2002.

17 Terao Y, Nishida J, Horiuchi S *et al*: Sodium butyrate induces growth arrest and senescence-like phenotypes in gynecologic cancer cells. Int J Cancer *94(2)*: 257-67, 2001.

18 Suzuki T, Yokozaki H, Kuniyasu H *et al*: Effect of trichostatin A on cell growth and expression of cell cycle- and apoptosis-related molecules in human gastric and oral carcinoma cell lines. Int J Cancer *88(6)*: 992-7, 2000.

19 Strait KA, Dabbas B, Hammond EH, Warnick CT, Ilstrup SJ and Fork CD: Cell cycle blockade and differentiation of ovarian cancer cells by the histone deacetylase inhibitor trichostatin A are associated with changes in p21, Rb, and Id proteins. Mol Cancer Ther *1*: 1181-90, 2002.

20 Giuliano M, Lauricella M, Calvaruso G *et al*: The apoptotic effects and synergistic interaction of sodium butyrate and MG132 in human retinoblastoma Y79 cells. Cancer Res *59(21)*: 5586-95, 1999.

21 Vigushin DM and Coombes RC: Histone deacetylase inhibitors in cancer treatment. Anticancer Drugs *13(1)*: 1-13, 2002.

22 Greenberg VL, Williams JM, Cogswell JP, Mendenhall M and Zimmer SG: Histone deacetylase inhibitors promote apoptosis and differential cell cycle arrest in anaplastic thyroid cancer cells. Thyroid *11(4)*: 315-25, 2001.

23 Greenberg VL, Williams JM, Boghaert E, Mendenhall M, Ain KB and Zimmer SG: Butyrate alters the expression and activity of cell cycle components in anaplastic thyroid carcinoma cells. Thyroid *11(1)*: 21-9, 2001.

24 Bible KC and Kaufmann SH: Cytotoxic synergy between flavopiridol (NSC 649890, L86-8275) and various antineoplastic agents: the importance of sequence of administration. Cancer Res *57(16)*: 3375-80, 1997.

25 Tyagi AK, Singh RP, Agarwal C, Chan DCF and Agarwal R: Silibinin strongly synergizes human prostate carcinoma DU145 cells to doxorubicin-induced growth inhibition, G2-M arrest, and apoptosis. Clin Cancer Res *8*: 3512-19, 2002.

26 DiPaola RS: To arrest or not to G(2)-M cell-cycle arrest: commentary *re*: Tyagi *et al.*: Silibinin strongly synergizes human prostate carcinoma DU145 cells to doxorubicin-induced growth inhibition, G(2)-M arrest, and apoptosis. Clin Cancer Res *8*: 3512-3519, 2002. Clin Cancer Res *8(11)*: 3311-4, 2002.

27 Vousden KH: Activation of the p53 tumor suppressor protein. Biochim Biophys Acta *1602(1)*: 47-59, 2002.

28 Wahl AF, Donaldson KL, Fairchild C *et al*: Loss of normal p53 function confers sensitization to Taxol by increasing G2/M arrest and apoptosis. Nat Med *2(1)*: 72-9, 1996.

29 Cassinelli G, Supino R, Perego P *et al*: A role for loss of p53 function in sensitivity of ovarian carcinoma cells to taxanes. Int J Cancer *92(5)*: 738-47, 2001.

30 Chang HY and Yang X: Proteases for cell suicide: Functions and regulation of caspases. Micro Mol Bio Rev *64(4)*: 821-46, 2000.

31 Wood DE and Newcomb EW: Caspase-dependent activation of calpain during drug-induced apoptosis. J Biol Chem *274(12)*: 8309-15, 1999.

32 Blagosklonny MV: P53: an ubiquitous target of anticancer drugs. Int J Cancer *98(2)*: 161-6, 2002.

33 Tan G, Heqing L, Jiangbo C *et al*: Apoptosis induced by low-dose paclitaxel is associated with p53 upregulation in nasopharyngeal carcinoma cells. Int J Cancer *97(2)*: 168-72, 2002.

34 Henderson C, Mizzau M, Paroni G, Maestro R, Schneider C and Brancolini C: Role of caspases, Bid and p53 in the apoptotic response triggered by histone deacetylase inhibitors TSA and SAHA. J Biol Chem *278(14)*: 12579-89, 2003.

35 Ofir R, Seidman R, Rabinski T *et al*: Taxol-induced apoptosis in human SKOV3 ovarian and MCF7 breast carcinoma cells is caspase-3 and caspase-9 independent. Cell Death Differ *9(6)*: 636-42, 2002.

36 Huisman C, Ferreira CG, Broker LE *et al*: Paclitaxel triggers cell death primarily *via* caspase-independent routes in the non-small cell lung cancer cell line NCI-H460. Clin Cancer Res *8(2)*: 596-606, 2002.

37 Wall NR, O'Connor DS, Plescia J *et al*: Suppression of survivin phosphorylation on Thr34 by flavopiridol enhances tumor cell apoptosis. Cancer Res *63(1)*: 230-5, 2003.