available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Phase II trial of the histone deacetylase inhibitor belinostat in women with platinum resistant epithelial ovarian cancer and micropapillary (LMP) ovarian tumours ☆,☆☆

Helen J. Mackay [a,], Hal Hirte [a], Terrence Colgan [b], Al Covens [a], Katrina MacAlpine [a], Pamela Grenci [a], Lisa Wang [a], Jaqueline Mason [a], Pnu-An Pham [a], Ming-S. Tsao [a], James Pan [a], James Zwiebel [c], Amit M. Oza [a]*

[a] *Princess Margaret Phase II Consortium, Toronto, Canada*
[b] *Sunnybrook Regional Cancer Centre, Toronto, Canada*
[c] *National Cancer Institute, Rockville, MD, USA*

ARTICLE INFO

Article history:
Received 9 February 2010
Received in revised form 16 February 2010
Accepted 24 February 2010
Available online 19 March 2010

Keywords:
Micropapillary (LMP) ovarian tumours
Platinum resistant ovarian cancer
Belinostat
Phase II

ABSTRACT

Aim: Micropapillary/borderline (LMP) ovarian tumours are rarely included in clinical trials and are intrinsically resistant to radiation and chemotherapy. Platinum resistant epithelial ovarian cancer (EOC) has a poor prognosis. The histone deacetylase inhibitor belinostat demonstrated antitumour activity in pre-clinical ovarian cancer models.

Methods: A phase II study was performed to evaluate the activity of belinostat in two patient populations: women with metastatic or recurrent platinum resistant (progression within 6 months) EOC and LMP ovarian tumours, both groups had received no more than 3 prior lines of chemotherapy. Belinostat 1000 mg/m^2/d was administered iv days 1–5 of a 21 d cycle. Peripheral blood mononuclear cells (PBMCs) and tumour biopsies, where possible, for correlative studies were obtained prior to and following treatment.

Results: Eighteen patients with EOC and 14 patients with LMP tumours were enrolled on study. Belinostat was well tolerated with no grade four toxicity (179 cycles). Grade 3 toxicity consisted of thrombosis (3 patients), hypersensitivity (1) and elevated ALP (1). One patient with LMP tumour had a partial response (unconfirmed) and 10 had stable disease (SD), 3 were non-evaluable. Median progression-free survival (PFS) was 13.4 months (95% confidence interval (CI), 5.6 – not reached). Best response in patients with EOC was SD (nine patients) and median PFS was 2.3 months (95% CI, 1.2–5.7 months). An accumulation of acetylated histones H3 and H4 was noted in PBMCs and in tumour tissue.

Conclusions: Belinostat is well tolerated in both patient groups and shows some activity in patients with micropapillary (LMP) disease.

☆ *Support*: This trial was supported by funding from N01-CM-62203 and drug was supplied by the Cancer Therapeutics Evaluation Program, US National Cancer Institute and the Campbell Family Institute.
☆☆ *Presented:* Poster discussion ASCO 2008.

* *Corresponding author:* Address: Princess Margaret Hospital, 610 University Ave, Toronto, ON, Canada M5G 2M9. Tel.: +1 416 946 2253; fax: +1 416 946 6546.
E-mail address: helen.mackay@uhn.on.ca (H.J. Mackay).

0959-8049/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2010.02.047

1. Introduction

Epithelial ovarian cancer (EOC) is the fifth leading cause of death in women in North America.[1] Patients whose disease does not respond or in whom the duration of response is short to initial platinum-based chemotherapy have a median survival of only 6–9 months.[2] Borderline ovarian tumours or tumours of low malignant potential (LMP) account for up to 20% of all ovarian tumours.[3] Although survival is excellent in early stage disease between 10% and 20% of women will die of their disease.[3–5] If surgery is not feasible there is no established standard of care. Chemotherapy and radiation are ineffective and these patients are rarely included in clinical trials.[6–8] New therapeutic approaches are urgently required for both of these diseases.

Histone deacetylases (HDACs) are involved in post-translational acetylation of core nucleosomal histones thus playing a key role in the epigenetic regulation of gene expression.[9] Inhibition of HDACs has emerged as a promising therapeutic strategy, restoring expression of silenced genes leading to cell differentiation and subsequent cell cycle arrest or apoptosis in transformed cells,[10] including in ovarian cancer cell lines.[11–13]

Belinostat (formally known as PXD101) is a low molecular weight, hydroxamic acid inhibitor of class I and II HDACs.[14,15] Pre-clinical studies suggest that belinostat may have activity in women with EOC.[16]

The aim of this 2-stage, multicentre, phase II study was to evaluate the efficacy and safety of belinostat in two patient populations: metastatic or recurrent platinum resistant (progression within 6 months) EOC and micropapllilary/LMP ovarian tumours. Secondary aims were survival and pharmacodynamic effects of belinostat in peripheral blood mononuclear cells (PBMCs) and tumour tissue. The dose and scheduling for this study were established by a phase I study with belinostat in patients with solid tumours.[17]

2. Materials and methods

This study was conducted by the Princess Margaret Phase II Consortium according to Good Clinical Practice guidelines and with full research ethics board approval. All patients signed written informed consent before study entry.

2.1. Eligibility

Patients with histologically or cytologically confirmed platinum resistant (defined as progression within 6 months of platinum therapy) recurrent EOC or micropapillary/borderline (LMP) ovarian tumours who had received $\leqslant 3$ prior lines of chemotherapy for advanced disease were eligible for this trial. Eligibility criteria included: life expectancy $\geqslant 12$ weeks, Eastern Cooperative Group (ECOG) performance status $\leqslant 2$, adequate haematological, hepatic and renal function and Response Evaluation Criteria In Solid Tumours (RECIST) measurable disease[18]; prior treatment had to be completed >4 weeks before study entry. Exclusion criteria included: bowel obstruction, significant cardiovascular disease, prolongation of QT/QTc interval, uncontrolled hypertension or a history of/or known brain metastases.

2.2. Study design

Belinostat 1000 mg/m^2 was administered intravenously on days 1–5 of a 21 d cycle. No pre-medication was required. Treatment was discontinued for disease progression, patient choice or toxicity.

2.3. Patient evaluation

Baseline evaluations included medical history, physical examination, laboratory tests; CA125 and ECG and were performed within 7 d of starting protocol therapy and repeated day 1 of each cycle. Tumour response was evaluated every two cycles (6 weeks) by RECIST.[18] CA125 was measured every 3 weeks according to Gynecologic Cancer InterGroup (GCIG) criteria for response and progression.[19] Toxicity was evaluated according to NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 resolution to baseline or grade 1 was required prior to re treatment.

2.4. Correlative studies

Representative HE sections of the tumours were reviewed by an expert gynecopathologist (TC).

Blood samples, for peripheral blood mononuclear cells (PBMCs), and tumour biopsies were collected $\leqslant 7$ d prior to and post belinostat on day 4 or 5 of cycle 1. Histone acetylation was evaluated using Western Blotting for histone H3 and H4 isolated from PBMCs. Acetylated histones were detected using anti-Acetyl-Histone H3 or H4 (Lys9, Lys8) antibodies (Cell Signalling Technology, Inc.).[20]

Paraffin sections of pre- and post-treatment biopsies from two patients were processed using standard operating procedures at the Applied Molecular Profiling Laboratory (University Health Network, Toronto, ON, Canada). Microwave antigen retrieval was followed by detection of acetylated histone H3 using anti-Acetyl–histone H3 antibody (1:400 dilution) labelled on the Ventana Benchmark XT using the uVIEW DAB (Tucson, AZ, United States of America). Positive staining was visually evaluated (M-S T) and image analysis was performed to obtain positive average staining intensity in regions of interest as previously described.[21]

2.5. Statistical methods and end-points

The primary end-point of this study was objective response according to RECIST and Rustin criteria.[18,19] A two-stage design was used within each cohort.[22] Using response hypotheses of H0 $\leqslant$ 5% and Ha $\geqslant$ 20%, 15 evaluable patients with LMP/borderline tumours were required for the first stage, with $\geqslant 1$ response required to continue into second stage accrual (significance level, $\alpha = 0.05$ and $\beta = 0.20$).

Using response hypotheses of H0 $\leqslant$ 10% and Ha $\geqslant$ 30%, 12 evaluable patients with platinum resistant EOC were required for the first stage requiring $\geqslant 1$ response to proceed to the second stage (significance level, $\alpha = 0.10$ and $\beta = 0.10$).

PFS and OS were summarised using the Kaplan–Meier method.

3. Results

Eighteen patients with platinum resistant EOC and 14 with micropapillary/LMP tumours were enrolled at three centres across Canada.

At the time of this analysis all the 18 patients with EOC were off study with progression (14 patients), bowel obstruction (1), patient choice (2) and other (1). Thirteen patients with micropapillary/LMP tumours were off study: progression (5), bowel obstruction (1), toxicity (2) patient choice (5). One patient remains on study.

3.1. *Patient characteristics*

Table 1 summarises the pretreatment patient and disease characteristics in both groups of eligible patients. All patients had a diagnosis of micropapillary/LMP tumour confirmed on expert pathology review (TC) and were actively progressing on the basis of CT scan prior to entering the study (confirmed by expert radiological review).

3.2. *Treatment administration*

Thirty-two patients received 179 treatment cycles (115 cycles, LMP: 64 cycles EOC). Median two cycles/patient EOC (range 1–14 cycles) and eight cycles/patient micropapillary/LMP (range 1–20 cycles). Sixteen patients had missed or delayed doses due to patient request (4), adverse events (3) and other (3). Three patients had dose delays. There were no dose reductions. Two patients with LMP tumours were taken off study for possibly drug-related adverse events hypersensitivity (one patient) and grade 3 elevation of ALP (1).

3.3. *Toxicity*

All patients were evaluable for toxicity. Table 2 displays the adverse events considered to be at least possibly drug related for both patient groups The most common events (all grade 1 or 2) were fatigue (69% of patients) and nausea (59%). No patients died during study. Four patients developed disease-related bowel obstruction.

3.4. *Efficacy*

Fifteen patients with platinum resistant EOC were evaluable for response: nine had SD and six progressive disease (PD) (Fig. 1A). Median progression-free survival (PFS) was 2.3 months (95% confidence interval (CI), 1.2–5.7 months), 6-month progression-free proportion (PFP) was estimated at 13% (95% CI, 4–49%).

Twelve patients with micropapillary/LMP tumours were evaluable for response: one had an unconfirmed partial response (uPR), reduction in target lesions of 32% post cycle eight increasing by 4% post cycle 10, 10 had SD and one PD (Fig. 1A). The patient with uPR had received prior chemotherapy (carboplatin/paclitaxel). An additional patient had a CA125 response (Fig. 1B). Median PFS was 13.4 months (95% CI, 5.6 – not reached), 6 months PFP was estimated at 56% (95% CI, 32–96%). Median duration of SD was 5.8 months (range 2.8–13.6 months).

Table 1 – Patient characteristics.

Characteristic	Micropapillary/borderline ovarian tumours N = 14	Platinum resistant EOC N = 18
Age (years)		
Median	50	59.5
Range	28–82	31–78
Performance status		
0	11	11
1	2	6
2	1	1
Primary site		
Ovarian	14	16
Peritoneal		1
Unknown		1
Target: Non-target sites	4.5	5
Median (range)	1–9	1–10
Prior radiotherapy		
Yes	1	0
No	13	18
No. of prior chemotherapy regimens		
0	3	0
1	6	4
2	5	5
3+	0	9

Table 2 – Adverse events on treatment (n = 32 evaluable patients).

Belinostat related toxicity CTCAE version 3.	Grade 1/2	Grade 3/4	Patients (total no. %)
Fatigue	22	–	22 (69%)
Nausea	22	–	19 (59%)
Vomiting	11	–	11 (34%)
Diarrhoea	9	–	9 (28%)
Pneumonitis	2	–	2 (6%)
Sinus bradycardia	6	–	6 (19%)
Sensory peripheral neuropathy	5	–	5 (16%)
Headache	5	–	5 (16%)
Hypersensitivity	2	1	3 (9%)
Thrombosis	0	3	3 (9%)
Leukopenia	2	–	2 (6%)
Anaemia	4	–	4 (13%)
Elevated alkaline phosphatase	1	1	2 (6%)

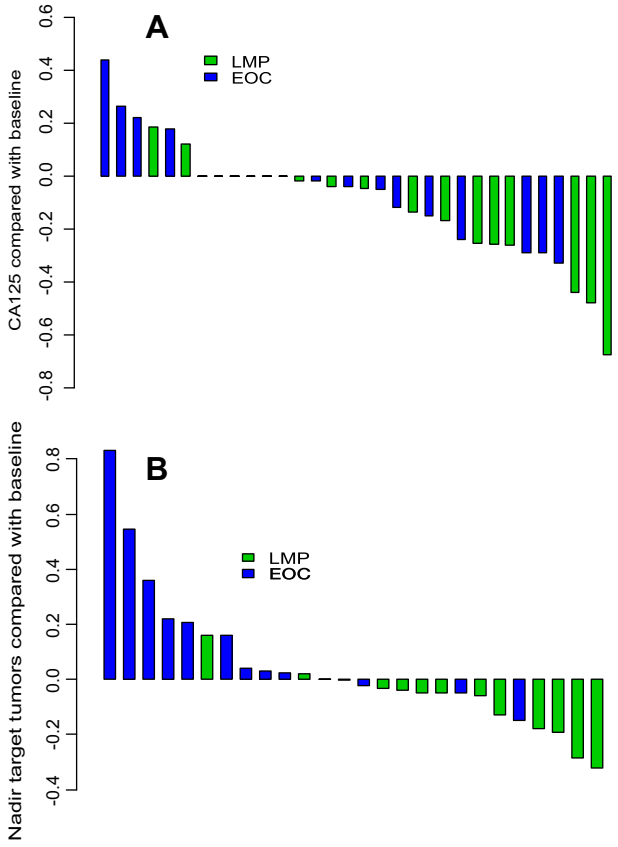

Fig. 1 – Waterfall plots for response to treatment (A) CA125 and (B) by RECIST criteria.

Twelve patients have died (10 in the EOC group and two LMP patients) Median overall survival (OS) for patients with platinum resistant EOC was 7.5 months (95% CI: 3.7 – not reached) and median OS for the LMP patients has not been reached.

3.5. *Pharmacodynamic analyses*

Paired samples of PBMCs prior to and following belinostat were available for nine patients with micropapillary/LMP tumours. A marked increase in acetylated histones H3 and H4 was demonstrated for all patients, Fig. 2. Paired tumour samples were available for two patients, patient nos. 6 and 7. A marked increase in acetylation was observed following treatment with belinostat in both patients, Fig. 3.

4. Discussion

When surgery is no longer an option, there is no standard of care for women with micropapillary (LMP) tumours.[7] To the best of the authors knowledge, this represents the first prospective study conducted in this group of women. Belinostat shows modest activity (Fig. 1A/B) in this patient population. Although the study did not meet criteria to proceed to the second stage, we feel that the PFS and waterfall plots of both tumour size and CA125 suggest activity that may warrant further investigation. Defining activity by objective response, however, may not be the best approach for patients with this, indolent, disease. Future trials incorporating a randomised discontinuation design to allow assessment of prolonged SD may be preferable.

Interpreting and designing studies for women with micropapillary/borderline LMP tumours are challenging. The histological diagnosis and correct classification of these tumours require expert pathological review (TC in this study).[6,23] Prognosis for these women differs depending on the presence of non-invasive (mortality rate 6%) versus invasive peritoneal (25%) implants.[24] Eight patients had invasive implants in this study. A further confounding factor is the histology of the recurrence one series suggesting 73% may recur as low grade serous EOC.[7] Data on outcome for non-surgical therapies are scarce and retrospective.[7] One study suggesting chemotherapy and radiation may even be detrimental for those with non-invasive implants.[6] The highest reported response rate to first line chemotherapy (retrospect review) is 26%. No response was observed >1 non-surgical therapy.[7] In our study 11 patients had received prior chemotherapy. Although only one uPR and one CA125 response were seen 10 patients had a fall in CA125 and 10 a reduction in tumour size, having previously demonstrated actively progressing disease. Changes in the patterns of calcification observed on CT scanning also suggest a biological effect. Again the lack of comparative data makes interpreting survival problematic. In a retrospective

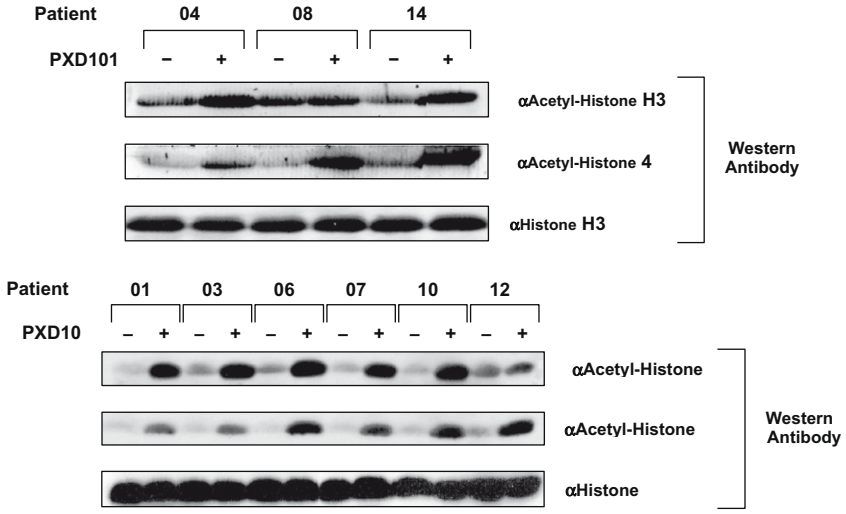

Fig. 2 – Histone acetylation evaluated using Western Blotting for histone H3 and H4 isolated from PBMCs Acetylated histones detected using anti-Acetyl-Histone H3 or H4 (Lys9, Lys8) antibodies (Cell Signalling Technology, Inc.) in PBMCs taken prior to and following belinostat in patients with micropapillary/LMP tumours.

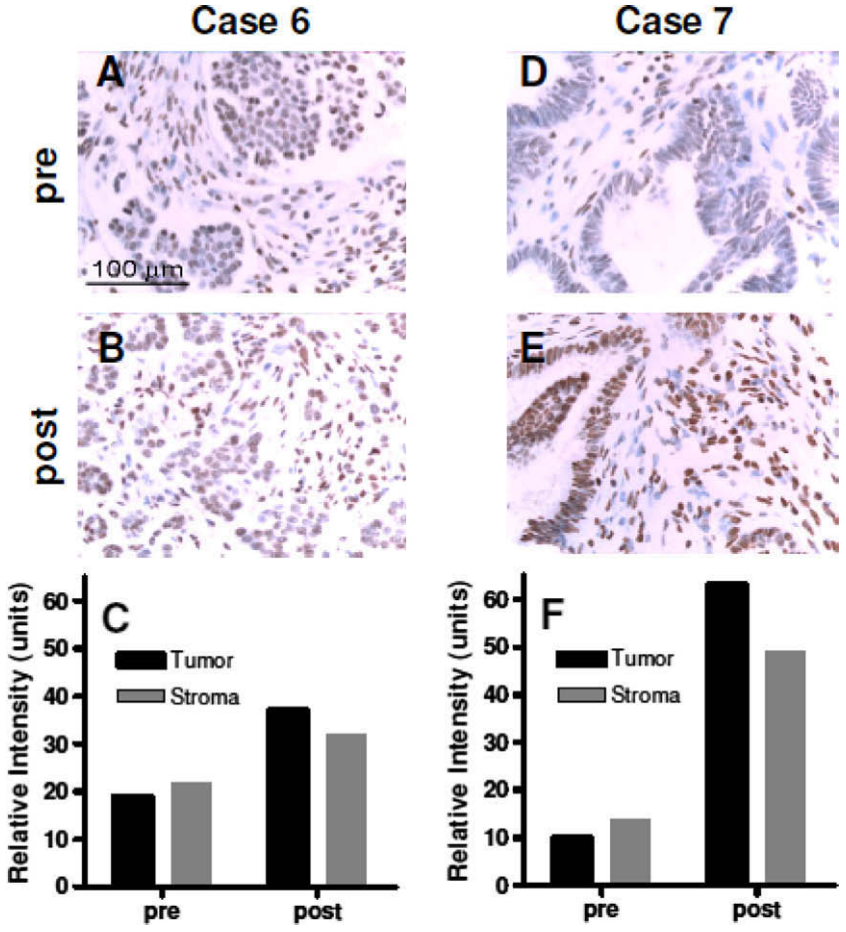

Fig. 3 – Acetylated histone H3 measured prior to and following belinostat (cycle 1). For patient 6 the average mean intensity in tumour was pre 19 units and post 37 units, for stroma pre 22 units and post 32 units. For patient 7 in tumour pre 10 units and post 63 units and stroma pre 14 units and post 49 units.

series of 21 patients receiving first line chemotherapy median duration of SD was 12 months (range 3–77.5 months).[7] Based on this a median PFS of 13.4 months in a largely, pre-treated population appears promising.

An increase in H3 and H4 histone acetylation was observed in all nine LMP patients who had paired PBMC samples Fig. 2. In pre-clinical studies with belinostat acetylated histones measured in mice showed a similar dose response.[15] In two patients (patient nos. 6 and 7) additional paired tumour samples showed an increase in acetylated histone H3 in tumour cells and stroma (Fig. 3). Both patients had SD, patient 6 receiving eight and patient 7 four treatment cycles. These data would suggest that in these patients belinostat was having a pharmacodynamic effect in tumour tissue.

The outlook for women with platinum resistant EOC is very poor. Despite promising activity in pre-clinical ovarian cancer models single agent belinostat did not display sufficient efficacy to warrant further investigation results similar to those seen with Vorinostat, another HDAC inhibitor.[25] Belinostat in combination with conventional cytotoxics may be a more promising approach in women with EOC.[15,16,26]

Belinostat was very well tolerated in both study populations with fatigue the most common adverse event. Three patients developed thrombosis on study although not reported previously for belinostat this is a recognised adverse event in other HDAC inhibitor studies.[27,28]

Epigenetic changes at an intermediate level between normal ovarian tissue and EOC are well recognised for micropapillary (LMP) tumours.[29] It is possible that Belinostat in this low grade, indolent tumour is inducing differentiation and disease stabilisation similar to that which is seen in another indolent condition, myelodysplastic syndrome following administration of demethylating agents.[30] Whilst the lack of single agent activity in platinum resistant EOC is disappointing, the future for epigenetic therapy in this disease may lie in combination with other agents as a response modifier.

Further investigation into the biology and treatment of micropapillary/borderline (LMP) tumours are urgently needed. Belinostat is the first biological agent to show promising activity in this group of women and this warrants further investigation.

Conflict of interest statement

None declared.

REFERENCES

1. Cannistra SA. Cancer of the ovary. *N Engl J Med* 2004;**351**:2519–29.
2. Cannistra SA. Is there a "Best" choice of second-line agent in the treatment of recurrent, potentially platinum-sensitive ovarian cancer? *J Clin Oncol* 2002;**20**:1158–60.
3. Tinelli R, Tinelli A, Tinelli FG, et al. Conservative surgery for borderline ovarian tumors: a review. *Gynecol Oncol* 2006;**100**:185–91.
4. Leake JF, Currie JL, Rosenshein NB, et al. Longterm follow up of serous ovarian tumors of low malignant potential. *Gynaecol Oncol* 1992;**47**:150–8.
5. Longacre TA, Mckenney JK, Tazelaar HD, et al. Ovarian serous tumors of low malignant potential (borderline tumors): outcome based study of 276 patients with longterm (>5 year) follow up. *Am J Surg Path* 2005;**29**:707–23.
6. Seidman J, Kurman RJ. Subclassification of serous borderline tumors of the ovary into benign malignant types: a clinicopathologic study of 65 advanced stage cases. *Am J Surg Pathol* 1996;**20**:1331–45.
7. Crispens MA, Bodurka D, Deavers M, et al. Response and Survival in Patients With Progressive or Recurrent Serous Ovarian Tumors of Low Malignant Potential. *Obstet Gynecol* 2002;**99**:3–10.
8. Trope C, Kaern J, Vergote IB, et al. Are borderline tumors of the ovary overtreated both surgically and systemically? A review of four prospective randomized trials including 253 patients with borderline tumors. *Gynecol Oncol* 1993;**51**:236–43.
9. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. *Nat Rev Drug Discov* 2006;**5**:769–84.
10. Caslini C, Capo-Chici CD, Roland IH, et al. Histone modification silences the GATA transcription factor genes in ovarian cancer. *Oncogene* 2006;**25**:5446–61.
11. Terasawa K, Sagae S, Toyota T, et al. Epigenetic Inactivation of *TMS1/ASC* in Ovarian Cancer. *Clin Cancer Res* 2004;**10**:2000–6.
12. Yarden I, Brody LC. BRCA1 interacts with components of the histone deacetylase complex. *Proc Natl Acad Sci USA* 1996;**96**:4983–8.
13. Finn P, Watkins C, Romero R. Discovery and structure-activity relationships of novel classes of histone deacetylase inhibitors. In: *Proceedings of the American association of cancer research*, vol. 43. p. A3672.
14. Plumb JA, Finn PW, Williams RJ, et al. Pharmacodynamic Response and Inhibition of Growth of Human Tumor Xenografts by the Novel Histone Deacetylase Inhibitor PXD101. *Mol Cancer Ther* 2003;**2**:721–8.
15. Gian X, LaRochelle WJ, Guishan A, et al. Activity of PXD101, a histone deacetylase inhibitor, in preclinical ovarian cancer studies. *Mol Cancer Ther* 2006;**5**:2086–95.
16. Steele N, Plumb J, Vidal L, et al. A phase 1 pharmacokinetic and pharmacodynamic study of the histone deacetylase inhibitor Belinostat in patients with advanced solid tumour. Clin. *Cancer Res* 2008;**14**:804–10.
17. Therasse P, Arbuck S, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors; European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;**92**:205–16.
18. Rustin GJ, Quinn M, Thigpen T, et al. Re: New guidelines to evaluate the response to treatment in solid tumors (ovarian cancer). *J Natl Cancer Inst* 2004;**96**:487–8.
19. Shechter D, Dormann HL, Allis CD, et al. Extraction, purification and analysis of histones. *Nat Protoc* 2007;**2**:1445–57.
20. Pham NA, Morrison A, Schwock J, et al. Quantitative image analysis of immunohistochemical stains using a CMYK color model. *Diagn Pathol* 2007;**2**:8.
21. Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials* 1989;**10**:1–10.
22. Bell K, Smith Sehdev AE, Kurman R. Refined diagnostic criteria for implants associated with ovarian atypical proliferative serous tumors (borderline) and micropapillary serous carcinomas. *Am J Surg Pathol* 2001;**25**:419–32.
23. Gershenson DM. Clinical management potential tumours of low malignancy. *Best Pract Res Clin Obstet Gynaecol* 2002;**16**:513–27.
24. Cusido M, Balaguero L, Hernandez G, et al. Results of the national survey of borderline ovarian tumors in Spain. *Gynecol Oncol* 2007;**104**:617–22.
25. Modesitt S, Sill MB, Hoffman JS, et al. A phase II study of vorinostat in the treatment of persistent or recurrent epithelial ovarian or primary peritoneal carcinoma: A

Gynecologic Oncology Group study. *Gynecol Oncol* 2008;**109**:182–6.
26. Finkler N, Dizon DS, Braly P, et al. Phase II multicentre trial of the histone deacetylase inhibitor (HDACi) Belinostat, carboplatin and paclitaxel (BelCAP) in patients with relapsed epithelial ovarian cancer. *J Clin Oncol* 2008;**26**(Suppl.) [abstr 5519].
27. Woyach JA, Kloos RT, Ringel MD, et al. Lack of therapeutic effect of the histone deacetylase inhibitor Vorinostat in patients with metastatic radioiodine-refractory thyroid carcinoma. *J Clin Endocrinol Metab* 2009;**94**:164–70.
28. Ramalingam SS, Belani CP, Reul C, et al. Phase II study of belinostat (PXD101), a histone deacetylase inhibitor, for second line therapy of advanced malignant pleural mesothelioma. *J Thorac Oncol* 2009;**4**:97–101.
29. Wiley A, Katsaros D, Chen H, et al. Aberrant promoter methylation of multiple genes in malignant ovarian tumors and ovarian tumors with low malignant potential. *Cancer* 2006;**107**:299–308.
30. Jabbour E, Issa J-P, Garcia-Manero G, et al. Evolution of decitabine development: accomplishments, ongoing investigations, and future strategies. *Cancer* 2008;**112**:2341–51.