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Differential sensitivity to platinum-based chemotherapy in primary uterine serous papillary carcinoma cell lines with high versus low HER-2/neu expression in vitro

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Abstract

Objective—To identify effective chemotherapy regimens against uterine-serous-papillary-carcinoma (USPC).

Study Design—Six USPC, half of which overexpress HER-2/neu at 3+ level, were evaluated for growth-rate and *in-vitro*-sensitivity to fourteen single-agent chemotherapies and five combinations by ChemoFx (Precision Therapeutics, Pittsburgh, PA).

Results—Cell lines overexpressing HER-2/neu showed higher proliferation when compared to low HER-2/neu-expressing cell lines and a lower half-maximum inhibitory concentration (IC₅₀) when exposed to the majority of single-agent chemotherapies. High HER-2/neu-expressors were more sensitive to platinum compounds, manifesting a 5.22-fold decrease in carboplatin-IC₅₀ ($P=0.005$) and a 5.37-fold decrease in cisplatin-IC₅₀ ($P=0.02$). When all cell lines were analyzed as a group, chemotherapy agents tested demonstrated lower IC₅₀s when used in combination than as individual agents.

Conclusions—USPC overexpressing HER-2/neu display greater *in-vitro*-sensitivity to platinum-compounds when compared to low HER-2/neu-expressors. Higher proliferative capability rather than increased drug resistance may be responsible for the adverse prognosis associated with HER-2/neu overexpression in USPC.

Keywords

Endometrial neoplasms; uterine serous tumors; HER2/neu; chemosensitivity; chemoresistance

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Introduction

Endometrial cancer is the most common female genital tract malignancy in the United States, with an incidence of 42,160 new cases and 7,780 deaths annually¹. Two types of endometrial carcinoma, namely Type I and Type II tumors, have been described, based on both clinical and histopathological variables². Type I endometrial cancers, which account for the majority (about 80%) of cases, are usually well or moderately-differentiated and endometrioid in histology. These neoplasms are frequently diagnosed in younger women, are associated with a history of hyperestrogenism as the main risk factor, and typically have a favorable prognosis with appropriate therapy. In contrast, Type II endometrial cancers are poorly differentiated tumors, or tumors with serous papillary or clear cell histology. Although Type II tumors account for only a minority of endometrial cancers, most recurrences and deaths occur in this group of patients.

Uterine serous papillary adenocarcinoma (USPC), which accounts for about 10% of endometrial cancers, has a propensity for early intraabdominal and lymphatic spread even at presentation and is characterized by a highly aggressive biologic behavior³. Unlike the histologically similar high-grade ovarian cancer, USPC is a chemoresistant disease from onset, with *in vivo* responses to combined cisplatin-based chemotherapy in the order of 20% and of short duration^{4–6}. Our group has recently reported HER-2/neu overexpression by IHC and amplification of the c-erbB2 gene by FISH in a large percentage of patients harboring USPC^{7–9}. These findings, recently confirmed by other groups¹⁰ including the Gynecologic Oncology Group (GOG) in a cooperative multicentric study¹¹, have identified HER-2/neu overexpression in USPC as an independent variable associated with poor outcome, and one that occurs more frequently in African-American women than in Caucasian women^{7–9}.

Although HER-2/neu overexpression has been previously associated with resistance to chemotherapy and poor survival in multiple human malignancies including breast¹², ovarian¹³ and endometrial carcinoma¹⁴, to our knowledge, no study has carefully evaluated the *in vitro* chemo-sensitivity/resistance of this highly aggressive variant of endometrial cancer. To fill this gap in knowledge, we used the ChemoFx (Precision Therapeutics, Inc., Pittsburgh, PA) to analyze the *in vitro* sensitivity of six primary UPSC cell lines recently established and characterized in our laboratory¹⁵ to fourteen standard single-agent chemotherapies and five combination chemotherapies¹⁶. Half the cell lines selected overexpress HER-2/neu at 3+ levels and harbor amplification of the c-erbB2 oncogene by FISH. Surprisingly, although *in vivo* HER-2/neu overexpression is correlated with a more aggressive disease in patients with UPSC^{8,9}, growth-inhibition data suggest that these tumors display greater *in vitro* chemosensitivity to platinum compounds and higher proliferative capability when compared to HER-2/neu-negative tumors.

Materials and Methods

Establishment and HER-2/neu expression of USPC Cell Lines

Primary USPC tumor cell lines from six patients with invasive USPC were obtained from fresh tumor biopsies collected at the time of surgery, under approval of the Institutional Review Board. Tumors were staged according to the International Federation of Gynecologists and Obstetricians operative staging system. Six primary USPC cell lines (USPC ARK-1, USPC ARK-2, USPC ARK-3, USPC ARK-4, USPC ARK-5, and USPC ARK-6) were established after sterile processing of the tumor samples from surgical biopsies as described previously¹⁵. Source-patient characteristics of these six USPC cell lines are described in Table 1. The amplification of the c-erbB2 gene by FISH, expression

levels of HER-2/neu receptor by immunohistochemistry (IHC) and mRNA expression levels by quantitative RT-PCR for these primary USPC cell lines have been recently reported¹⁵ and are presented in Table 2.

Primary UPSC growth rate analysis

The growth rate of each of the UPSC cell lines was determined by counting the number of live cells 24, 48, 72 and 96 hours after plating. In brief, cell lines were cultured in RPMI 1640 (Mediatech, Manassas, VA) supplemented with 10% fetal bovine serum (Hyclone, Logan, Utah). When cultures had grown to approximately 80% confluence, cells were harvested from the flask using 0.25% Trypsin EDTA (Invitrogen, Carlsbad, CA), then counted on a hemacytometer chamber and assessed for viability via trypan blue exclusion. Cell density was adjusted to a concentration of 8,000 cells/mL, then cells were seeded into a 384-well microtiter plate (Corning Life Sciences, Lowell, MA) at a density of 320 cells per well. Twenty-two replicate wells were plated per cell line per time point. Cell plates were incubated at 37°C with 5% CO₂ for 24, 48, 72, and 96 hours, at which time they were removed from the incubator, fixed with 95% ethanol (FisherScientific, Pittsburgh, PA) then stained with DAPI (Molecular Probes). When DAPI staining was complete, cell plates were scanned on an automated fluorescent imaging system, and the number of cells in each well was counted.

Chemoresponse assay

The chemoresponse assay was performed as previously described^{16,17}. The chemotherapy agents, concentrations and combination used in the assays are described in Table 3. Briefly, cell lines were cultured in RPMI 1640 (Mediatech, Manassas, VA) supplemented with 10% fetal bovine serum (Hyclone, Logan, Utah). When cultures had grown to approximately 80% confluence, cells were harvested from the flask using 0.25% Trypsin EDTA (Invitrogen, Carlsbad, CA), then counted on a hemacytometer chamber and assessed for viability via trypan blue exclusion. Cell density was adjusted to a concentration of 8,000 cells/mL. Then cells were seeded into a 384-well microtiter plate (Corning Life Sciences, Lowell, MA) at a density of 320 cells per well. Cell plates were incubated at 37°C with 5% CO₂ overnight. On the next day, serial dilutions of each anticancer agent or combination of two agents were prepared in growth medium to create 8, 9 or 10 distinct testing concentrations. In total, fourteen single agents were tested as well as five combinations of two agents. Due to the limited amounts of cells available for testing for USPC-ARK-5 primary cell line, six of the fourteen single agents were not tested (i.e., cyclophosphamide, fluorouracil, ifosfamide, vinblastine, vincristine, and vinorelbine) nor any of the combinations. Three replicates of each dose of agent or combination were applied to each cell line. The cell plates were incubated for an additional 72 hours after treatment, at which time they were removed from the incubator, fixed with 95% ethanol (FisherScientific, Pittsburgh, PA) then stained with DAPI (Molecular Probes). When DAPI staining was complete, cell plates were scanned on an automated fluorescent imaging system, and the number of surviving cells in each well was counted, and compared to a control population not exposed to any agent.

Statistical Analysis

For each cell line, the IC₅₀ for a given drug was determined from the dose-response curve fit to the cell-count data using XLFit program (ID Business Solutions, Parsippany, NJ, USA). The Lavenburg-Marquardt algorithm was used to obtain and calculate the IC₅₀ and IC₅₀ curves for each drug and drug combination in each cell line using a non-linear regression-curve fit. To facilitate statistical inference on IC₅₀ fold changes, IC₅₀s were transformed to log₁₀ units. Differences in log₁₀(IC₅₀)s between high and low HER-2/neu expressors were assessed for significance via unequal-variance t-test, while cell-line differences in log₁₀(IC₅₀)s for agents used singly versus in combination were assessed for significance via

paired t-test. Fold changes in IC_{50} s were calculated as 10 raised to the power of the differences in $\log_{10}(IC_{50})$ s. Differences were considered significant at p values < 0.05 . All statistical analysis was conducted using Excel 2007 (Microsoft corporation, Redmond, WA).

Results

Growth Curve

The doubling time of all 6 primary USPC cell lines was evaluated by counting the number of live cells 24, 48, 72, and 96 hours after plating as described in the methods section. As shown in Table 4, population doubling times for UPSC ARK-1, UPSC ARK-2, UPSC ARK-3 (all high HER-2/neu expressors), and UPSC ARK-4 (a low HER-2/neu expressor) were similar, with the USPC cell lines exhibiting doubling times of 22.3 hours, 21.9 hrs, 22.1 hours and 21.1 hours, respectively. In contrast, a lower growth rate was detected in UPSC ARK-5 and UPSC ARK-6 cells (both low HER-2/neu expressors), with doubling times of 72 hours and 45.7 hours, respectively (Table 4).

Chemoresponse assay

Single agent therapy—We compared by ChemoFx the effects of 14 single-agent chemotherapy drugs against 6 primary USPC cell lines expressing different levels of HER-2/neu using serial dilutions of each anticancer agent in growth medium to create 8, 9 or 10 distinct testing concentrations, as described in the methods section. A significant difference was observed in the IC_{50} of the high versus low HER-2/neu expressor USPC cell lines when treated with both carboplatin and cisplatin (Table 5, Figure 1a and 1b). Carboplatin $\log_{10}(IC_{50})$ s showed an average $\pm$ standard deviation (SD) of 0.738 ± 0.173 among the HER-2/neu overexpressors (UPSC ARK-1, UPSC ARK-2 and UPSC ARK-3) versus 1.456 ± 0.120 among the low expressors (UPSC ARK-4, UPSC ARK-5 and UPSC ARK-6); the difference was significant ($P=0.0059$) and equivalent to a 5.22-fold decrease in carboplatin IC_{50} among high compared to low expressors. Similarly, cisplatin $\log_{10}(IC_{50})$ s showed an average $\pm$ SD of only -0.345 ± 0.238 among overexpressors, compared to 0.385 ± 0.068 among low expressors, equivalent to a 5.37-fold decrease in cisplatin IC_{50} with overexpression ($P=0.026$). Importantly, the low HER-2/neu expressors, regardless of their fast or slow doubling times, were found to be highly resistant to both carboplatin and cisplatin (Figure 1a and 1b). When the sensitivity to other single agent drug was analyzed in the two groups of primary USPC cell lines, we found the average IC_{50} s for the HER-2/neu overexpressors to be consistently less than those of the low HER-2/neu expressors for the majority of tested drugs, although this difference did not reach statistical significance (Table 5). Of interest, when paclitaxel was tested as single agent against the high and low HER-2/neu expressors, UPSC-ARK-1 stood out as a highly resistant primary tumor cell line to the exposure to this agent (Figure 1c) as well as to multiple other microtubular inhibitor drug tested including docetaxel, vincristine and vinorelbine (data not shown).

Drug combinations—Combinations of carboplatin with paclitaxel, doxorubicin, gemcitabine, topotecan or docetaxel were evaluated in five USPC cell lines. With all USPC cell lines considered as a group, the chemotherapy agents demonstrated lower IC_{50} s when used in combination than as individual agents (Table 6 a,b,c,d,e). The change in IC_{50} reached statistical significance for each of these agents when combined with carboplatin (Table 6 a,b,c,d,e). In all combinations used, the IC_{50} of carboplatin changed less when compared to carboplatin as a single agent, suggesting that carboplatin's effect on the combination IC_{50} was greater than that of each of the other agents with which it was combined (Table 6 a,b,c,d,e). Importantly, as representatively shown for UPSC ARK-1 cell line (Figure 2), a tumor overexpressing HER-2/neu found sensitive to platinum compounds but highly resistant to paclitaxel (Figure 1c) as well as to multiple microtubular inhibitors

when used as single agents, the combination of carboplatin with paclitaxel, docetaxel, gemcitabine or topotecan, yielded a significantly lower IC₅₀ for each of these drugs when used in combination with carboplatin (Figure 2).

Discussion

The treatment of advanced, metastatic and/or recurrent USPC no longer amenable to control with surgery or radiation therapy has not improved significantly with the advent of modern chemotherapy. Although the majority of USPC patients, similar to high grade ovarian cancer patients, are treated with multiple chemotherapy agents, the clinical responses to combined cisplatin-based regimens remain low and of short duration^{4–6}. A greater understanding of the chemosensitivity/chemoresistance profile of USPC and a better comprehension of the relationship between activation of an oncogene like c-erbB2 and drug sensitivity remains paramount.

The erbB2 gene encodes for HER-2/neu, a member of the erbB receptor tyrosine kinase family. This is a family of 4 transmembrane glycoproteins (erbB1, erbB2, erbB3, and erbB4) that are expressed on epithelial, mesenchymal, and neuronal cells¹⁸. ErbB receptors are activated in response to binding with multiple ligands produced in an autocrine fashion in the individual cells, or a paracrine fashion in the surrounding tissue. Ligand binding results in dimerization of the receptor, either with a twin receptor (homodimerization), or with one of its siblings (heterodimerization)¹⁸. This leads to phosphorylation of the intracellular tyrosine kinase residues, which serve as docking sites for various effectors and transcription factors that ultimately modulate various biological responses such as proliferation, survival, migration and differentiation¹⁸.

In a recent exploratory immunohistochemical analysis of HER-2/neu expression in advanced endometrial carcinoma performed by the GOG, HER-2/neu overexpression (either 2+ (moderate) or 3+ (strong)) was detected in 23 of 38 (61%) of the USPC tested¹¹. These results are consistent with the HER-2/neu positivity previously reported by our own research group who found moderate-to-strong expression of HER-2/neu protein in 16 (62%) of 26 USPC samples evaluated⁷. High HER-2/neu expression levels in USPC have also been reported by Diane-Montez et al¹⁰, with 12 of the 25 (48%) USPC cases in the John Hopkins' series demonstrating HER-2/neu overexpression. Of interest, in this study, a significant association of HER-2/neu overexpression with surgical staging was detected, with 81.8% advanced stage disease vs. 28.6% early stage disease showing HER-2/neu overexpression¹⁰. Importantly, while other studies have reported lower percentages of HER-2/neu overexpression in USPC patients^{19–20}, in the majority, overexpression of the HER-2/neu oncogene has been associated with a shorter patient survival when compared to patients harboring tumors showing a normal amount of gene product^{7–10,19,20}. While these reports suggest that overexpression of the HER-2/neu oncogene in USPC patients may be a marker for intrinsic multidrug resistance, as in other human cancers^{12–14}, to our knowledge, no studies have yet analyzed the *in vitro* sensitivity/resistance of primary USPC cell lines to multiple cytotoxic drugs and whether overexpression of the HER-2/neu gene in these tumors may directly confer higher chemotherapy resistance.

We found USPC cell lines overexpressing HER-2/neu to have higher proliferation when compared to low HER-2/neu-expressing cell lines, and lower half maximum inhibitory concentration (IC₅₀) when exposed to the majority of single-agent chemotherapies. Importantly, high HER-2/neu expressors were found to be significantly more sensitive than low HER-2/neu expressors to platinum compounds *in vitro*. With no exception, USPC cell lines showing low HER-2/neu expression, regardless of their fast or slow growth rate, were found to be significantly more resistant to platinum compounds when compared to high

HER2/neu-expressor cell lines. Taken together, these results exclude the possibility that the different sensitivity to platinum compounds is secondary to a different growth rate in the two groups of USPC cell line studied, and also suggest that HER-2/neu overexpression results in increase in cell proliferation *in vitro*, potentially leading to a more rapid recovery from the cytotoxic effects of chemotherapeutic drugs *in vivo*.

These results were somewhat unexpected considering the abundant *in vivo* data showing worse prognosis in USPC patients harboring tumors overexpressing the HER-2/neu oncogene^{8–10} and the experimental evidence indicating a correlation between HER-2 overexpression and increased resistance to chemotherapy in other human tumors^{21,22}. On the other hand, similarly to our experimental results, other studies have previously shown increasing sensitivity to chemotherapy with increasing HER-2 expression levels^{23–25}. While it is difficult to compare our *in vitro* experimental results in USPC with those obtained with human tumors from different organs and genetic backgrounds^{21,22}, it is likely, as previously suggested²⁵, that alterations in chemosensitivity in tumors are dependent on the combined effects of c-erbB2 overexpression and the intrinsic genetic alterations associated with a particular cell line studied. Consistent with this hypothesis, USPC-ARK-1, a primary cell line overexpressing HER-2/neu and sensitive to platinum compounds, was found in our assays to be highly resistant to paclitaxel, docetaxel and other microtubule inhibitors.

While the reason for the differences in resistance/sensitivity to chemotherapy between the high and low HER-2/neu expressors remains poorly understood, we hypothesized that USPC with low/negligible HER-2/neu expression may differ from their HER-2/neu-overexpressor counterparts not only in cell-cycle patterns but also in the activation of multiple molecular pathways and oncogene(s). To validate/exclude this hypothesis, gene expression profiling experiments to evaluate genomic differences between the two groups of primary USPC cell lines are underway in our laboratory.

Finally, when all USPC cell lines were considered as a group, all chemotherapy agents tested demonstrated lower IC₅₀s when used in combination than as individual agents. These data confirm that chemotherapy combinations may better modulate drug responsiveness *in vitro* and likely *in vivo*. These findings may be particularly important for the treatment of USPC because even tumors found unresponsive to a large class of cytotoxic drugs (i.e., microtubule inhibitors) when used as single agents, (i.e., USPC-ARK-1 in our experiments), may dramatically increase their response to therapy when exposed to drug combinations.

In summary, USPC cell lines overexpressing HER-2/neu showed higher proliferation when compared to low HER-2/neu expressing cell lines and lower half maximum inhibitory concentration (IC₅₀) when exposed to the majority of single-agent chemotherapies. While a greater understanding of the molecular/genetic differences between USPC with high and low HER-2/neu expression will be necessary before tailoring of chemotherapy for improved clinical outcomes, these data suggest that the apparent lack of response to chemotherapy among patients with HER-2/neu positive tumors seen in clinical trials may be due to rapid tumor regrowth of surviving tumor cells following initial response to chemotherapy rather than intrinsic chemotherapeutic drug resistance at the time of chemotherapy treatment.

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References

1. Thun M, Xu J, Hao Y, Ward E, Siegel R, Jemal A. Cancer statistics, 2009. *CA* 2009;59:225. [PubMed: 19474385]
2. Bokhman JV. Two pathogenetic types of endometrial carcinoma. *Gynecol.Oncol* 1983;15:10. [PubMed: 6822361]
3. Hendrickson M, Ross J, Eifel P, Martinez A, Kempson R. Uterine papillary serous carcinoma: a highly malignant form of endometrial adenocarcinoma. *Am J Surg Pathol* 1982;6:93–108. [PubMed: 7102898]
4. Levenback C, Burke TW, Silva E, et al. Uterine papillary serous carcinoma (USPC) treated with cisplatin, doxorubicin, and cyclophosphamide (PAC). *Gynecol. Oncol* 1992;46:317–321. [PubMed: 1526508]
5. Nicklin L, Copeland LJ. Endometrial papillary serous carcinoma: pattern of spread and treatment. *Clin. Obstet. Gynecol* 1996;39:686–695. [PubMed: 8862892]
6. Schwartz PE. The management of serous papillary uterine cancer. Schwartz PE. *Current Opinion in Oncology* 2006;18(5):494–499. [PubMed: 16894299]
7. Santin AD, Bellone S, Van Stedum S, et al. Determination of HER-2/neu Status in Uterine Serous Papillary Carcinoma: Comparative Analysis of Immunohistochemistry and Fluorescence in situ Hybridization. *Gynecol. Oncol* 2005;98:24–30. [PubMed: 15894362]
8. Santin AD, Bellone S, Van Stedum S, et al. Amplification of c-erbB2 Oncogene: a Major Prognostic Indicator in Uterine Serous Papillary Carcinoma. *Cancer* 2005;104(7):1391–1397. [PubMed: 16116605]
9. Santin AD, Siegel ER, Bellone S, et al. Racial differences in overexpression of epidermal growth factor type II receptor (HER2/neu): a major prognostic indicator in Uterine Serous Papillary Cancer. *Am. J. Obstet. Gynecol* 2005;192:813–818. [PubMed: 15746676]
10. Diaz-Montes TP, Ji H, Smith Sehdev AE, et al. Clinical significance of HER-2/neu overexpression in uterine serous carcinoma. *Gynecol Oncol* 2006;100:139–144. [PubMed: 16182348]
11. Grushko TA, Filiaci VL, Mundt AJ, Ridderstrale K, Olopade OI, Fleming GF. Gynecologic Oncology Group. An exploratory analysis of HER-2 amplification and overexpression in advanced endometrial carcinoma: a Gynecologic Oncology Group study. *Gynecologic Oncology* 2008;108(1):3–9. [PubMed: 17945336]
12. Wright C, Angus B, Nicholson S, et al. Expression of c-erbB-2 oncoprotein: a prognostic indicator in human breast cancer. *Cancer Res* 1989;49:2087–2090. [PubMed: 2564806]
13. Berchuck A, Kamel A, Whitaker R, et al. Overexpression of HER-2/neu is associated with poor survival in advanced epithelial ovarian cancer. *Cancer Res* 1990;50:4087–4091. [PubMed: 1972347]
14. Hetzel DJ, Wilson TO, Keeney GL, Roche PC, Cha SS, Podratz KC. HER-2/neu expression: a major prognostic factor in endometrial cancer. *Gynecol Oncol* 1992;47:179–185. [PubMed: 1361478]
15. El-Sahwi K, Bellone S, Cocco E, Cargnel, et al. In vitro Activity of Pertuzumab in Combination with Trastuzumab in Uterine Serous Papillary Adenocarcinoma. *Brit. J. Cancer*. (in press).
16. Brower, SL.; Fensterer, JE.; Bush, JE. The ChemoFx® assay: an ex vivo chemosensitivity and resistance assay for predicting patient response to cancer chemotherapy. In: Mor, G.; Alvero, AF., editors. *Methods in Molecular Biology; Apoptosis and Cancer: Methods and Protocols*. Totowa NJ: Humana Press; 2008. p. 57-78.
17. Gallion H, Christopherson WA, Coleman RL, et al. Progression-free interval in ovarian cancer and predictive value of an ex vivo chemoresponse assay. *Int J Gynecol Cancer* 2006;16(1):194–201. [PubMed: 16445633]
18. Yarden Y, Sliwkowski MX. Untangling the erbB signaling network. *Nat Rev Mol Cell Biol* 2001;2:127–137. [PubMed: 11252954]
19. Slomovitz BM, Broaddus RR, Burke TW. HER-2/neu overexpression and amplification in uterine papillary serous carcinoma. *J Clin Oncol* 2004;22:2136.

20. Morrison C, Zanagnolo V, Ramirez N, et al. HER-2 is an independent prognostic factor in endometrial cancer: association with outcome in a large cohort of surgically staged patients. *J Clin Oncology* 2006;24(15):2376–2385.
21. Tsai CM, Chang KT, Perng RP, et al. Correlation of intrinsic chemoresistance of non-small-cell lung cancer cell lines with HER-2/neu gene expression but not with ras gene mutations. *Journal of the National Cancer Institute* 1993;85(11):897–901. [PubMed: 8098377]
22. Tsai CM, Yu D, Chang KT, et al. Enhanced chemoresistance by elevation of p185neu levels in HER-2/neu-transfected human lung cancer cells. *Journal of the National Cancer Institute* 1995;87(9):682–684. [PubMed: 7538595]
23. Hasegawa Y, Goto M, Hanai N, et al. Prediction of chemosensitivity using multigene analysis in head and neck squamous cell carcinoma. *Oncology* 2007;73(1–2):104–111. [PubMed: 18337622]
24. Orr MS, O'Connor PM, Kohn KW. Effects of c-erbB2 overexpression on the drug sensitivities of normal human mammary epithelial cells. *J Natl Cancer Inst* 2000;92:987–994. [PubMed: 10861310]
25. Pegram MD, Finn RS, Arzoo K, Beryt M, Pietras RJ, Slamon DJ. The effect of HER-2/neu overexpression on chemotherapeutic drug sensitivity in human breast and ovarian cancer cells. *Oncogene* 1997;15:537–547. [PubMed: 9247307]

Figure 1a

Figure 1b

Figure 1c

Figure 1. Representative dose-response curves following exposure to carboplatin (a), cisplatin (b) and paclitaxel (c) of USPC primary cells with High versus Low HER-2/neu expression
Survival was assessed by ChemoFx. Each point on the cell line graph represents the mean of 8 to 10 estimations $\pm$ SE. Average IC_{50} values, for each the cell lines are reported in Table 5.

Figure 2. Representative dose-response curves following exposure to docetaxel and carboplatin + docetaxel (upper panel) versus paclitaxel and carboplatin + paclitaxel (lower panel) in USPC-ARK1 primary cell line

Survival was assessed by ChemoFx. Each point on the cell line graph represents the mean of 8 to 10 estimations $\pm$ SE. Average IC_{50} values, for each of the cell lines when treated with the single agent or the combination are reported in Table 6.

Table 1

Patient characteristics from which the six USPC cell lines were established

Patient	Age (years)	Race [*]	FIGO [^] Stage	USPC Histopathology	Year of Diagnosis
USPC ARK-1	62	AA	IVA	Pure	1997
USPC ARK-2	63	AA	IVB	Pure	1998
USPC ARK-3	59	AA	IVB	Mixed	2006
USPC ARK-4	73	C	IVB	Pure	2004
USPC ARK-5	73	AA	IIIC	Pure	2006
USPC ARK-6	62	C	IB	Mixed	2005

^{*} AA, African American; C, Caucasian;

[^] FIGO, International Federation of Gynecology and Obstetrics

Table 2

HER-2/neu expression in primary USPC cell lines

<i>Sample</i>			RT-PCR[°]
	IHC[*]	FISH[^]	mRNA Copy Number
Control			1
USPC ARK-1	3+	2.5	373
USPC ARK-2	3+	5.2	607
USPC ARK-3	3+	4.7	677
USPC ARK-4	0	1.6	7
USPC ARK-5	0	1.4	13
USPC ARK-6	1+	0.9	6

* IHC, Immunohistochemistry;

[^] FISH, Fluorescent In-situ Hybridization;

[°] RT-PCR, Real-time Polymerase Chain Reaction.

Table 3

Doses of chemotherapy agents

Chemotherapy	Lowest Dose	Highest Dose	Units	Number of Concentrations
Carboplatin	0.98	500	μM	10
Cisplatin	0.2	50	μM	8
Cyclophosphamide	0.33	14	μM	9
Docetaxel	0.1	25	nM	9
Doxorubicin	2.34	1200	nM	9
Etoposide	2.867	200	μM	9
Fluorouracil	0.2	50	μM	8
Gemcitabine	0.73	50	nM	9
Ifosfamide	0.2	100	μM	9
Paclitaxel	0.2	100	nM	9
Topotecan	0.78	200	nM	8
Vinblastine	0.15	10	nM	9
Vincristine	0.98	125	nM	8
Vinorelbine	0.42	29.41	nM	9
Carboplatin & Docetaxel	0.98	500	μM	9
	0.1	25	nM	
Carboplatin & Doxorubicin	0.98	500	μM	9
	2.34	1200	nM	
Carboplatin & Gemcitabine	0.98	125	μM	8
	0.73	19.53	nM	
Carboplatin & Paclitaxel	0.98	250	μM	9
	0.2	50	nM	
Carboplatin & Topotecan	0.98	250	μM	9
	0.39	100	nM	

Table 4

Growth rate of primary USPC cell lines

Cell Line	Mean Cell Count				
	Time 0	24 Hours	48 Hours	72 Hours	96 Hours
UPSC ARK-1	320	402.64	1357.14	3735.36	6269.05
UPSC ARK-2	320	490.14	1802.86	4038.86	6561.27
UPSC ARK-3	320	635.09	1968.32	3956.36	6330.41
UPSC ARK-4	320	594.41	2072.14	5200.27	7399.00
UPSC ARK-5	320	359.61	398.33	485.62	812.45
UPSC ARK-6	320	337.14	520.23	564.64	1367.23

Table 5

Log₁₀(IC₅₀) values for HER-2/neu overexpressors versus low expressors treated with single-agent chemotherapies.

Chemotherapy	mean±SD, log ₁₀ (IC ₅₀) values		<i>P</i> [†]	Fold decrease [‡] in IC ₅₀
	HER-2/neu negative	HER-2/neu positive		
Carboplatin (μM)	1.456±0.120	0.738±0.173	0.0059	5.22
Cisplatin (μM)	0.385±0.068	-0.345±0.238	0.0262	5.37
Cyclophosphamide (μM)	0.852±0.417	0.257±0.041	0.2912	3.94
Docetaxel (nM)	0.631±0.384	0.460±0.093	0.5240	1.48
Doxorubicin (nM)	2.064±0.752	1.132±0.069	0.1639	8.55
Etoposide (μM)	0.025±0.478	-0.479±0.098	0.2053	3.19
Fluorouracil (μM)	1.428±0.384	0.853±0.412	0.2309	3.76
Gemcitabine (nM)	1.291±0.490	0.602±0.062	0.1330	4.89
Ifosfamide (μM)	1.229±0.395	0.962±0.210	0.5061	1.85
Paclitaxel (nM)	0.741±0.209	0.721±0.464	0.9506	1.05
Topotecan (nM)	1.917±0.547	1.025±0.017	0.1056	7.80
Vinblastine (nM)	0.353±0.481	0.224±0.064	0.7692	1.35
Vincristine (nM)	1.191±1.281	0.699±0.333	0.6826	3.10
Vinorelbine (nM)	0.816±0.367	0.810±0.236	0.9858	1.01

[†] *P* value, unequal-variance t-test.

[‡] Fold decrease (positive compared to negative) in IC₅₀ (original units as shown), calculated as 10 to the power (difference in mean log₁₀(IC₅₀)s between groups).

Table 6

Log₁₀(IC₅₀) values for USPC primary cell lines treated with drug combinations.

A: Decreases in Log ₁₀ IC ₅₀ for Carboplatin and Docetaxel when used in combination					
	Carboplatin	Docetaxel	Combination	Difference	Fold Decrease in IC ₅₀ [†]
mean±SD *	1.048±0.444	0.493±0.263	0.923± 0.405 -0.178±0.359	0.125±0.128 0.672±0.346	1.33 4.70
B: Decreases in Log ₁₀ IC ₅₀ for Carboplatin and Doxorubicin when used in combination					
	Carboplatin	Doxorubicin	Combination	Difference	Fold Decrease in IC ₅₀ [†]
mean±SD *	1.048±0.444	1.439±0.649	0.675± 0.516 1.055±0.516	0.373±0.371 0.384±0.138	2.36 2.42
C: Decreases in Log ₁₀ IC ₅₀ for Carboplatin and Gemcitabine when used in combination					
	Carboplatin	Gemcitabine	Combination	Difference	Fold Decrease in IC ₅₀ [†]
mean±SD *	1.048±0.444	0.851±0.480	0.842± 0.491 0.440±0.333	0.206±0.166 0.411±0.210	1.61 2.58
D: Decreases in Log ₁₀ IC ₅₀ for Carboplatin and Paclitaxel when used in combination					
	Carboplatin	Paclitaxel	Combination	Difference	Fold Decrease in IC ₅₀ [†]
mean±SD *	1.048±0.444	0.688±0.340	0.771± 0.201 0.072±0.201	0.277±0.270 0.616±0.455	1.89 4.13
E: Decreases in Log ₁₀ IC ₅₀ for Carboplatin and Topotecan when used in combination					
	Carboplatin	Topotecan	Combination	Difference	Fold Decrease in IC ₅₀ [†]

A: Decreases in Log ₁₀ IC ₅₀ for Carboplatin and Docetaxel when used in combination					
	Carboplatin	Docetaxel	Combination	Difference	P [†]
mean±SD [*]	1.048±0.444	0.334±0.553	0.770±0.359 0.372±0.359	0.278±0.179 0.961±0.251	0.0254 0.0010
					1.90 9.15

^{*} mean±SD of log₁₀(IC₅₀)s of agents used singly or in combination as shown, calculated for five USPC cell lines (ARK1, ARK2, ARK3, ARK4, and ARK6). Cell line ARK5 was excluded because it was not exposed to combinations.

[†] P value, paired t-test.

[‡] Fold decrease in IC₅₀ of agents in combination compared to when used as single agents. Fold decrease is calculated as 10 to the power of the mean difference in log₁₀(IC₅₀)s.