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Differential *in vitro* sensitivity to patupilone versus paclitaxel in uterine and ovarian carcinosarcomas cell lines is linked to tubulin-beta-III expression

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Abstract

Objective—To compare the *in vitro* sensitivity/resistance to patupilone versus paclitaxel in uterine and ovarian carcinosarcomas (CS).

Methods—Five primary carcinosarcoma cell lines, two from uterine and three of ovarian origin, were evaluated for growth rate and tested for their *in vitro* sensitivity/resistance to patupilone versus paclitaxel by MTS assays. To identify potential mechanisms underlying the differential sensitivity/resistance to patupilone, expression levels of β -tubulin III (TUBB3) were determined with quantitative-real-time-polymerase-chain-reaction (q-RT-PCR) in primary uterine and ovarian CS cell lines and in 26 uterine and 9 ovarian CS fresh-frozen-tissues.

Results—No appreciable difference in sensitivity to patupilone versus paclitaxel was noted in ovarian CS cell lines, or when uterine and ovarian CS cell lines were compared in their response to paclitaxel. In contrast, uterine CS cell lines were found to be significantly more sensitive to patupilone than to paclitaxel ($P < 0.002$) and demonstrated lower IC₅₀s to patupilone (range 0.76–0.93 nM) when compared to ovarian CS (range 1.9–3.4 nM, $P < 0.05$). Higher levels of TUBB3 were detected in uterine CS cell lines and fresh frozen tissues when compared to ovarian CS ($P < 0.05$).

Conclusions—Uterine CS cell lines are significantly more sensitive than ovarian CS cell lines to patupilone versus paclitaxel. High expression of TUBB3 is associated with sensitivity to patupilone in primary CS cell lines and may act as a genetic marker to predict chemotherapy efficacy. Patupilone may represent a promising drug in the treatment of this subset of rare but highly aggressive gynecological tumors.

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Conflict of interest statement

The authors have no conflict of interest to declare.

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Keywords

Endometrial neoplasms; uterine serous tumors; HER-2/neu; Patupilone; Paclitaxel

Introduction

Gynecological carcinosarcomas (CS) are rare but highly aggressive neoplasms comprised of malignant epithelial and stromal components that are classified based on the homologous (tissues physiologically native to the primary site) or heterologous (mesenchymal components that are physiologically foreign to the primary site) nature of their stromal elements [1]. The pathogenesis of CS remains under debate but most research supports the origin of both elements from a common epithelial cell line, rather than two independent progenitors. While CS comprise only 2% to 3% of ovarian and uterine cancers, the overall 5 year survival for ovarian and uterine CS patient remains a dismal 24% and 36%, respectively [1]. The discovery of novel diagnostic and therapeutic markers against this aggressive subset of gynecological tumors remains a high priority.

Presently, standard management of CS consists of surgical tumor cytoreduction and adjuvant systemic chemotherapy [2]. The challenge, however, lies in determining the most effective systemic treatment regimen for these women with this uncommon, yet devastating malignancy. A commonly used systemic treatment for uterine CS has been the inpatient regimen of ifosfamide and cis-diamminedichloroplatinum (II) (CDDP/cisplatin) chemotherapy. Response rates (RRs) with this combination have been disappointing, however, and adverse effects have been often severe [3]. In the last few years, microtubule-stabilizing-targeting agents (MTAs) such as taxanes have been explored in combination therapies for the treatment ovarian and uterine carcinosarcomas [4–7]. While these taxane-based regimens have yielded discordant results in terms of response rate and tolerability, ifosfamide plus paclitaxel is currently considered the gold-standard regimen supported by randomized phase III trials through the Gynecologic Oncology Group (GOG). However, the toxicity, multi-day schedule, and limited activity of this regimen indicate that development of other regimens is still needed.

The epothilones are a novel class of microtubule interfering agents, a promising strategy for anti-cancer therapy. Like taxanes, the epothilones create an interference with microtubule function that leads to defective mitotic spindle formation and to G2/M phase cell cycle arrest and consequent apoptosis [8] [9]. The epothilones, however, are water soluble, can be produced in large quantities through bacterial fermentation, and are less likely to lead to the development of multi-drug resistance (MDR) [10]. Patupilone is a notable member of this drug class [11]. Of interest, recent evidence suggests that differential expression of specific β -tubulin isotypes may predict tumor sensitivity/resistance to microtubule-stabilizing agents such as paclitaxel and patupilone in mammalian cells. Consistent with this view, while eight β -tubulin isotypes have been discovered in normal and neoplastic tissues (i.e., classes I, II, III, IVa, IVb, V, VI, and VII) [12,13], overexpression of tubulin β III (TUBB3) has been recently reported in colon [14], breast [15], prostate [16], non-small cell lung [17,18] and ovarian cancers [19,20]. Importantly, in studies in breast and ovarian cancer a significant correlation has been described between higher concentrations of TUBB3 mRNAs and resistance to paclitaxel/docetaxel and sensitivity to patupilone [19–22].

In this study we have carefully analyzed the sensitivity/resistance to patupilone of five primary CS cell lines from uterine and ovarian origin, and compared such response to that of paclitaxel *in vitro*. In addition, we have investigated the value of microtubule-associated expression of TUBB3 as a potential biomarker for chemotherapy sensitivity/resistance in the

five cell lines and in a larger number of uterine and ovarian CS fresh frozen specimens. We report the first evidence that primary uterine CS cell lines may display higher levels of the patupilone molecular target -tubulin β III and greater *in vitro* sensitivity to patupilone when compared to ovarian CS. Patupilone may represent a promising novel drug to combine to platinum compounds in patients harboring this rare but highly aggressive variant of endometrial cancer.

Materials and Methods

Primary Cell cultures and tumor specimens

Five different primary CS cell lines were used. The cell lines were established from uterine (UMMT-ARK-1 and UMMT-ARK-2) and ovarian (OMMT-ARK-1, OMMT-ARK-2 and OMMT-ARK-3) CS specimens obtained at the time of surgery. UMMT cell lines were established from biopsies of the uterus of chemotherapy naïve patients at the time of the primary staging surgery, while two out of three OMMT cell lines were established from the biopsy of metastatic sites of disease in patients harboring recurrent disease. Source-patient characteristics of these five CS cell lines are described in Table 1. The cells were maintained as a monolayer in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) (Gemini, Woodland, CA), 1% antibiotic (penicillin, streptomycin) and 0.3% antimycotic amphotericin B (Invitrogen, Carlsbad, CA). Cells were incubated at 37°C in a humidified atmosphere of 95% air/5% CO₂. Tumor specimens were obtained from biopsies collected at the time of primary debulking, under approval of an institutional review board.

Primary CS growth rate analysis

The growth rate for each of the CS 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 (Gemini, Woodland, CA), 1% antibiotic (penicillin, streptomycin) and 0.3% antimycotic amphotericin B. When cultures had grown to approximately 80% confluence, cells were harvested from the flask using 0.05% Trypsin EDTA (Invitrogen, Carlsbad, CA), then counted on a hemocytometer chamber and assessed for viability via trypan blue exclusion. Cell density was adjusted to a concentration of 50,000 cells/mL, then 1×10^5 cells/well were seeded into a 6-well microtiter plate (Corning Life Sciences, Lowell, MA). Duplicate 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, harvested from the wells using 0.05% Trypsin EDTA, counted on a hemocytometer chamber and assessed for viability via trypan blue exclusion.

Drugs

Patupilone – provided by Novartis Pharma, Basel, Switzerland – was dissolved in dimethyl sulfoxide (Sigma-Aldrich, St. Louis, Mo., USA) as a 10-mM stock solution, divided into 5- μ l aliquots, and stored at –20 °C until use. Paclitaxel was purchased from Bristol-Myers Squibb, Princeton, N.J., USA. Both drugs were diluted in culture medium immediately before addition to cell lines.

MTS assay

Cells at log phase of growth were seeded in 96-well culture plates at optimum density and allowed to attach for 24 h. Culture media were removed after 24 h and replaced with 200 μ l of fresh medium or the same volume of medium containing various concentrations of patupilone or paclitaxel (i.e., from 1 nM to 20,000 nM). After 72-hour incubation, 20 μ l of CellTiter 96[®] AQueous One Solution Reagent containing a tetrazolium compound [3-(4,5-

dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium, inner salt; MTS] (Promega Corporation, Madison, WI) was added, incubated and removed according to manufacturer's instructions. Absorbance was measured by a 96-well VERSA max plate reader (Molecular Devices, Sunnyvale, CA, USA) at a reference wavelength of 490 nm. Each drug concentration was obtained in 3 replicate wells, and each experiment was repeated a minimum of 3 times. The effect of paclitaxel and patupilone on cell growth inhibition was assessed as percent cell viability where vehicle-treated cells were taken as 100% viable. The results are expressed as mean cytotoxic concentrations (IC₅₀).

Beta-Tubulin class III expression by Real-Time PCR

Total RNA from the five primary CS cell lines and 26 uterine and 9 ovarian fresh frozen CS and 6 normal uterine and ovarian epithelia (i.e., controls) was extracted and purified using the RNeasy Mini Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's protocol. The median age of the uterine CS patients was 68 years (range 46–89); 54% (14/26) of patients had disease confined to the uterus (stage I/II) and 46% (12/26) had extra-uterine disease (stage III/IV). Among ovarian CS the median age was 70 years (range 50–77) and all of those patients had advanced disease (Stage III/IV). Since *TUBB3* is an intronless gene, all RNA samples were treated with TURBO DNase enzyme (TURBO DNA-free kit; Ambion, Inc., Austin, TX) to remove any contaminating genomic DNA that was present. The purity of total RNA was evaluated by absorbance readings (ratio A260/A230 and A260/A280) using the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). First-strand cDNA was synthesized from 5µg total RNA using Superscript III reverse transcriptase (Invitrogen, San Diego, CA) according to the manufacturer's instructions.

Quantitative real-time polymerase chain reaction (q-RT-PCR) was performed with a 7500 Real Time PCR System using the manufacturer's recommended protocol (Applied Biosystems, Foster City, CA) to evaluate expression of *TUBB3* gene in samples from all specimens. Each reaction was run in triplicate. The comparative threshold cycle (CT) method (Applied Biosystems) was used to determine gene expression in each sample relative to the value observed in the nonmalignant endometrial epithelial cells, using GAPDH (Assay-on-Demand Hs99999905_m1) RNA as an internal control. Five microliters of reverse transcribed RNA samples (from 500 µl of total volume) was amplified by using the TaqMan Universal PCR Master Mix (Applied Biosystems) to produce PCR products specific for *TUBB3*. Primers were obtained from Applied Biosystems: Assay ID: Hs00964962_g1 (*TUBB3*).

Statistical Analysis

Statistical analysis was conducted using GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA). For each independent MTS experiment of a given drug on a given cell line, the measures of growth under different dose levels were normalized to the mean of the control group receiving no drug, so that all data were expressed as a proportion of the control. Normalized data then were fit via nonlinear regression to a three-parameter logistic response curve against the base-10 logarithms of dose in nM, and the resulting parameter estimates were used to calculate the value of the IC₅₀ (in log₁₀ units) for that experiment. The collection of log₁₀(IC₅₀) values were then compared within each cell line for the difference between patupilone versus paclitaxel using mixed-models ANOVA with drug as the fixed effect and experiment as the random effect. For qRT-PCR experiments, the relative expressions were converted to log₂ units to facilitate hypothesis testing on ratios, and analyzed via mixed-models ANOVA with *TUBB3* status as the fixed effect, and cell line and RT-PCR replication number as the random effects. Differences in all comparisons were considered significant at P values < 0.05.

Results

Growth curves

The doubling time of all 5 primary CS cell lines was evaluated by counting the number of live cells 24, 48, 72 and 96 h after plating as described. As shown in Table 2, population doubling times for UMMT-ARK-1 and UMMT-ARK-2 were 26 hr and 23 hr, respectively, while OMMT-ARK-1, OMMT-ARK-2 and OMMT-ARK-3 doubling times were 22 hr 26 hr and 46 hr, respectively.

MTS Chemoresponse assay

We examined the effects of paclitaxel and patupilone on 2 endometrial CS cell lines (i.e., UMMT-ARK-1 and UMMT-ARK-2), and 3 ovarian CS cell lines (i.e., OMMT-ARK-1, OMMT-ARK-2 and OMMT-ARK-3) *in vitro*, using MTS assays with 72 hour drug exposure. The linear quadratic dose-response curves and IC₅₀ values for each cell line after treatment with paclitaxel and patupilone are representatively shown in Figures 1 and 2. Uterine and ovarian CS cell lines were found to be equally responsive to paclitaxel with IC₅₀s ranging from 2.47 nM to 5.04 nM (Figure 1). In contrast, uterine CS cell lines showed significantly lower IC₅₀ for patupilone compared to paclitaxel ($p=0.0002$ and $p=0.002$, respectively), whereas the three ovarian CS cell lines did not ($p=0.0650$, $p=0.8413$ and $p=0.0571$). As presented in Figure 2, UMMT-ARK-1 and UMMT-ARK-2 demonstrated higher sensitivity to patupilone with IC₅₀ value of 0.76 nM and 0.93 nM, respectively. In contrast, ovarian cell lines OMMT-ARK-1, OMMT-ARK-2 and OMMT-ARK-3 showed lower sensitivity to patupilone with IC₅₀ values of 2.27 nM, 3.41 nM and 1.9 nM, respectively (Figure 1 and 2). The difference in sensitivity to patupilone between uterine and ovarian CS cell lines was significant ($P<0.05$). As represented in Figure 2, in all primary CS cell lines, the linear quadratic IC₅₀ values for paclitaxel were higher than the corresponding values for patupilone. The exception was the OMMT-ARK-2 cell line, which had an IC₅₀ value of 2.47 nM for paclitaxel and 3.15 nM for patupilone.

TUBB3 mRNA expression levels in primary uterine and ovarian CS cell lines and fresh-frozen CS tissue specimens

To evaluate TUBB3 as a potential marker of chemosensitivity to patupilone, its expression was measured by q-RT-PCR in all 5 established CS cell lines tested in our *in vitro* experiments. As representatively demonstrated in Figure 3 (i.e., upper panel), TUBB3 expression levels were significantly higher in primary uterine CS cell lines (mean copy number $\pm$ SEM = 625 ± 107.9) when compared to ovarian CS cell lines (320.1 ± 29.6) ($p=0.006$). Consistent with these results, the primary ovarian CS showing the lower levels of TUBB3 expression by RT-PCR (i.e., OMMT-ARK-1 and OMMT-ARK-2 cell lines) were found to be the less sensitive to patupilone (Figure 1). To confirm the TUBB3 expression results obtained with the primary CS cell lines in a larger set of CS specimens, TUBB3 expression was quantified by qRT-PCR in 26 additional samples of primary uterine fresh-frozen CS and 9 additional samples of primary ovarian fresh-frozen CS. As demonstrated in the lower panel of Figure 3, TUBB3 copy numbers were again found to be significantly over-expressed in uterine versus ovarian fresh frozen CS specimens (mean $\pm$ SEM = 1014 ± 188.5 in uterine vs 363.4 ± 78.57 in ovarian CS, $P=0.04$).

Discussion

Uterine and ovarian carcinosarcomas are extremely aggressive neoplasms. A high proportion of patients present with advanced stage disease at diagnosis and the average 5-year survival across all stages is only 34% [1,2]. Although surgical debulking is the mainstay of treatment, there continues to be a need to identify effective adjuvant therapies

given the high rate of tumor recurrence and poor survival in these groups of patients [1,2] Ifosfamide plus paclitaxel is currently considered the gold-standard regimen supported by randomized phase III trials through the GOG [23]. However, the significant toxicity, multi-day schedule, and limited activity of this regimen mandate that development of other novel regimens. Consistent with this view, the antitumor activity and toxicity of paclitaxel plus carboplatin in patients with uterine carcinosarcomas is currently studied by the GOG in prospective studies. Nevertheless, there is a clear need to understand the molecular basis of carcinosarcomas better and to develop more effective treatment modalities against these rare but highly aggressive tumor variants.

Among the various classes of anticancer agents developed in the past two decades, few have achieved the same degree of preclinical and clinical validation as the microtubule stabilizers like the taxanes. Unfortunately despite excellent initial responses a majority of cancer patients eventually develop resistance to taxanes. Epothilones, another class of microtubule-stabilizing agents, appear to offer distinct advantages over taxane therapy. Indeed, early human phase I–II trials have noted significant clinical responses in taxane-resistant patients [24–27]. Consistent with these data, results published by Chen et al [25] demonstrated that 25% of patients (8 of 32) with refractory/resistant ovarian cancer, who had received prior paclitaxel therapy, had a complete or partial response after being treated with patupilone every 3 weeks. A phase II study conducted by the GOG reported a 14.3% complete or partial response to ixabepilone in patients with recurrent platinum and taxane-resistant ovarian or primary peritoneal carcinoma [26]. Finally, a phase II study to determine the response rate of ixabepilone in patients with persistent or recurrent endometrial cancer who have progressed despite standard therapy has recently been reported [27]. The overall response rate was 12%; one patient achieved a complete remission (2%), and five achieved partial remission (10%). Stable disease for at least 8 weeks was noted in 30 patients (60%). In this study, major grade 3 toxicities were neutropenia (52%), leukopenia (48%), gastrointestinal (24%), neurologic (18%), constitutional (20%), infection (16%), and anemia (14%).

Selection of the optimal therapeutic strategy requires the availability of predictive biomarkers that help predict responses to specific therapies [28]. Recent evidence suggests that overexpression of β -tubulin III contributes to taxane resistance and that the antitumor activity of epothilones may be related to its ability to suppress this specific tubulin subtype. Ferrandina et al [19] investigated the clinical role of β -tubulin III as a potential predictor of response to neoadjuvant platinum/paclitaxel treatment in advanced ovarian cancer patients with unresectable disease. They demonstrated that tumors overexpressing β -tubulin III levels experienced an overall shorter survival compared to cases with relatively lower β -tubulin III content (median overall survival 25 vs. 46 months, $P=0.002$). Concomitant with these findings, Umezu et al [20] was able to show through immunohistochemical staining, that the expression of β -tubulin III was higher in taxol resistant ovarian cancer cells compared to wild-type. Of 19 patients treated with paclitaxel, 10 patients with high levels of β -tubulin III had no response to chemotherapy compared to 9 patients with low levels of β -tubulin III who had overall response rate of 56% [20]. Taken together, these findings suggest that therapeutic agents able to target β -tubulin III such as patupilone may be specifically active against tumors endowed with an inborn or acquired resistance to paclitaxel.

In this study we first tested the chemosensitivity/chemoresistance of 2 uterine and 3 ovarian CS cell lines to paclitaxel and patupilone. To our knowledge, no studies have carefully analyzed and compared the in vitro sensitivity/resistance of primary CS cell lines from uterine and ovarian source to microtubular-targeting agents such as paclitaxel and patupilone

in vitro and/or whether overexpression of TUBB3 in these rare but highly aggressive tumors may confer higher sensitivity to patupilone.

We found primary uterine CS to be significantly more sensitive to patupilone when compared to paclitaxel. In contrast, no significant differences were found in the sensitivity to paclitaxel versus patupilone in the three ovarian CS cell lines available to our experiments. Importantly, while no appreciable differences were detected in the response to paclitaxel between the two groups of CS, uterine CS cell lines consistently demonstrated significant lower IC₅₀ to patupilone when compared to ovarian CS. Taken together, these results suggest that while paclitaxel may be equally cytotoxic against primary ovarian and uterine CS cell lines *in vitro*, patupilone may represent a significantly more effective agent against primary uterine CS. While the reason for the differences in resistance/sensitivity to the exposure to patupilone between the ovarian and uterine CS remains poorly understood, on the basis of the recent literature suggesting TUBB3 overexpression as a factor of patupilone sensitivity [22] and the results of our own studies in primary uterine carcinoma cell lines (USC) challenged with paclitaxel and patupilone [29], we hypothesized that uterine CS may differ from ovarian CS in the expression of genes that are potential targets for patupilone and paclitaxel. To validate/exclude this hypothesis, the expression of TUBB3 was measured and compared by RT-PCR in all primary CS cell lines available and in additional thirty-five fresh frozen CS with uterine and ovarian origin. Consistent with our hypothesis, we found significantly higher levels of TUBB3 in uterine primary CS cell lines and fresh frozen tissues when compared to ovarian CS ($P < 0.05$). Furthermore, we showed that tumors expressing higher TUBB3 levels were associated with an overall lower IC₅₀ for patupilone when compared to the cell lines with lower TUBB3 expression. Indeed, analysis of each individual cell line reveals that the cell line more sensitive to patupilone (UMMT-ARK-1), exhibited the highest expression β -tubulin III, while the primary CS showing the lower levels of TUBB3 expression by RT-PCR (i.e., OMMT-ARK-1 and OMMT-ARK-2 cell lines), were the least sensitive to patupilone. Our results are consistent with those of Mozzetti et al. [22] who also demonstrated increased levels of TUBB3 expression in patupilone sensitive ovarian cancer cell lines. Interestingly, in this study they found that when tumor cells were selected with patupilone, TUBB3 expression diminished by up to 30-fold, demonstrating that cells with elevated levels of TUBB3 are more sensitive to patupilone [22]. Taken together, these findings combined with our experimental results in uterine CS suggest that the higher sensitivity of these tumors to patupilone when compared to ovarian CS may be at least partially mediated by its interaction with β -tubulin III.

In summary, our study showed for the first time that uterine CS overexpress β -tubulin III and demonstrate significantly higher sensitivity to patupilone when compared to ovarian CS. While a greater understanding of the molecular/genetic differences between CS with uterine and ovarian origin will be necessary before tailoring chemotherapy to improve clinical outcomes, these data suggest that uterine CS overexpressing TUBB3 may be significantly more sensitive to patupilone than paclitaxel when used alone or in combination with a platinum compound. The future design and implementation of clinical trials in this regard will ultimately determine the validity of this novel therapeutic combination in patients harboring these aggressive gynecological tumors.

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Structured Highlights

- Uterine carcinosarcomas (CS) are more sensitive to patupilone than paclitaxel.
- Uterine CS show enhanced sensitivity to patupilone relative to ovarian CS.
- Tubulin- β -3 expression correlates with sensitivity to patupilone in CS.

Figure 1. Representative dose-response curves following exposure to patupilone versus paclitaxel of primary cell lines with Uterine versus Ovarian origin

Survival was assessed by MTS assay as described in the Methods section. Each point on the cell line graph represents the mean of 3 estimations + SE. Uterine CS cell lines (upper panel) demonstrated significantly lower IC_{50} for patupilone compared to paclitaxel ($p=0.0002$ and $p=0.002$, respectively), whereas the three ovarian CS cell lines (lower panel) did not ($p=0.0650$, $p=0.8413$ and $p=0.0571$).

Figure 2. IC₅₀s of Patupilone and Paclitaxel in five Uterine and Ovarian CS cell lines
 Symbols (error bars) denote means (standard deviations) of IC₅₀ for paclitaxel and patupilone. Text to the left of the symbols denotes the cell line. Note that the individual ratios are significant (at $P < 0.05$) for the uterine CS cell lines, but not significant in the ovarian CS cell lines.

Figure 3A

Figure 3B

Figure 3. β -tubulin class III (TUBB3) expression by RT-PCR in CS

Upper panel: TUBB3 expression in Uterine vs Ovarian primary CS cell lines. Lower Panel: TUBB3 expression in Uterine vs Ovarian CS fresh-frozen tissues. Symbols (error bars) denote the geometric means (SDs) of relative expression of TUBB3 mRNA copy numbers. TUBB3 expression difference between primary uterine CS cell lines vs ovarian CS cell lines was significant at a p value = 0.006). TUBB3 expression difference between primary uterine CS fresh frozen tissues (i.e., 26 specimens) vs ovarian CS fresh frozen tissues (i.e., 9 specimens) was significant at a p value = 0.04).

Table 1

Patient characteristics from which the five CS cell lines were established

PATIENT	Age (years)	Race	FIGO stage [^]	GRADE	HISTOLOGY	CKAE1/AE3	Vimentin
UMMT-ARK-1	70	AA [*]	IC	G3	Homologous	90%	100%
UMMT-ARK-2	46	C ^{**}	IIB	G3	Homologous	0%	95%
OMMT-ARK-1	55	AA	IV	G3	Heterologous	30%	100%
OMMT-ARK-2	78	C	IV	G3	Homologous	100%	0%
OMMT-ARK-3	74	C	IIIC	G3	Heterologous	ND [§]	ND

^{*} AA, African American;

^{**} C, Caucasian;

[^] FIGO, International Federation of Gynecology and Obstetrics (1988 staging system).

[§] ND, not done.

Table 2

Growth rate of CS cell lines

Cell line	Mean cell count				
	Time 0	24 hours	48 hours	72 hours	96 hours
UMMT-ARK-1	100,000	180,000	340,000	600,000	1,140,000
UMMT-ARK-2	100,000	140,000	250,000	520,000	610,000
OMMT-ARK-1	100,000	186,666	224,000	362,666	773,333
OMMT-ARK-2	100,000	146,666	186,666	200,000	453,000
OMMT-ARK-3	100,000	133,333	160,000	220,000	373,333