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<b>14. ABSTRACT</b> We have developed a mouse model of recurrent epithelial ovarian cancer for which we can monitor tumor formation, response and recurrence in vivo using live imaging. We are using this model to test UCN-01 and Olipraz in the consolidation setting. Despite demonstration of response of CAO V2-GFP/LUC cells in vivo to carboplatin, there remains variability between experiments, which we believe has to do with the age of the mice and/or their size when first injected with tumor cells, as well as the number of cells injected. We are also making progress toward developing the primary cells for use in xenograft formation that can be monitored through live imaging. To our knowledge, there are no reported animal models of recurrent ovarian cancer, yet recurrent disease is usually the lethal event for humans. Finding drugs that can target residual cells during remission is perhaps the best hope for delaying or preventing recurrence. Development of this mouse model will open new opportunities for testing a wide variety of other drugs in this setting.					
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## INTRODUCTION

One of the major impediments to improving survival of women diagnosed with epithelial ovarian cancer is the frequent recurrence of chemoresistant disease. Multiple research groups have provided evidence supporting ovarian cancer stem cells as etiologic agents of this disease. Cancer stem cells inherently resist commonly used chemotherapeutic agents which may enable the ability to remain in the body for long periods of time, during which they are thought to enter into a state of slow proliferation or cellular dormancy. This proposal seeks to test the ability of UCN-01 and Oltipraz to inhibit the tumorigenic capacity of such cells *in vivo* using a mouse xenograft model of human epithelial ovarian cancer with an aim of translating positive findings to clinical trials. To do this, we **will** determine if UCN-01 or Oltipraz reduces intraperitoneal xenograft tumor formation following IP carboplatin treatment of established ovarian cancer cell lines and cells from ascitic fluid of ovarian cancer patients. There are five study arms for each analysis. We inject cells engineered to express luciferase into the peritoneal cavity of nude mice, treat the mice with IP carboplatin and follow this with further IP treatment with carboplatin (*Arm 1*), UCN-01 (*Arm 2*), Oltipraz *per os* (*Arm 3*) or no additional treatment (control; *Arm 4*). *Arm 5* will comprise mice injected with tumor cells but without treatment, as a control to monitor tumorigenicity of the injected cells. Tumor formation, response to treatment and recurrence are monitored through use of live *in vivo* imaging following intraperitoneal administration of luciferin. The primary experimental endpoint is survival, with secondary endpoints of weight, size of tumor(s), ascites volume and tumor proliferation index.

## BODY

The following details the progress made toward fulfilling each of the tasks, as relevant, in the Statement of Work.

### **Task 1.** Obtain human subjects approvals for 10 subjects, months 1-6.

Human subjects approvals were obtained from both Duke University and the US Army Materiel Research Command as of 16 March 2011.

### **Task 2.** Obtain approval for use of 240 athymic NCr-nu/nu mice, months 1-4.

Animal protocols were approved by both the Duke Institutional Animal Care and Use Committee and the US Army Materiel Research Command's ACURO as of February 10, 2011. Research personnel underwent training for handling and restraint techniques, intraperitoneal injections, oral gavage and euthanasia on October 26, 2011.

### **Task 3.** Determine time to IP tumor take in 15 mice each for HEYA8 and OVCA420 cells, months 4-6.

We have completed this task, but with modifications due to new findings. In light of reviewer's comments, we revised our strategy to implement use of cells engineered to express luciferase in order to enable live, non-invasive imaging to monitor tumor formation, regression and recurrence. We therefore used a lentiviral system to transduce ovarian cancer cells with replication incompetent virus particles that delivered a construct (pLENTI-Fire) that contains the genes encoding luciferase as well as green fluorescent protein. This also allowed us to positively select GFP<sup>+</sup> cells that contain the construct using fluorescent microscopy combined with flow activated cell sorting. This required submission of recombinant DNA protocols and viral vector registration with the Duke Biosafety Committee, protocols that were approved on January 26, 2011. We had also made this change prior to submission of the animal use and human subjects protocols, so these protocols were approved such that they included thus use of live imaging to monitor tumors in them mice. We successfully stably transduced two cell lines with this construct (after these cell lines were confirmed to be free of murine pathogens) and began testing tumor formation using the HEYA8 cells in nude mice while simultaneously working out the protocols and precautions required for using live *in vivo* imaging.

We received 12 5-week old female Ncr-nu/nu mice on 11/1/11 to conduct a preliminary study. The mice were acclimated, and two were injected IP with 7.5X10<sup>5</sup> HEYA8-GFP/LUC cells. The cells were grown

under standard culture conditions but we exploited the presence of the GFP expressed from the pLENTI-Fire construct to use FACS sorting to select a highly enriched GFP-expressing population of cells prior to injection. On day 8 post-injection, the mice underwent imaging using the IVIS 200 Optical Imaging System. Tumor growth was evident in both mice.

The ten remaining mice were injected with  $2.5 \times 10^5$  (2 mice),  $5.0 \times 10^5$  (4 mice) or  $7.5 \times 10^5$  HEYA8-GFP/LUC cells on 12/5/11 and had tumor formation visible by 12/12/11 (7 days post-injection). In a subsequent experiment with  $2.5 \times 10^5$  injected cells (described below), tumor formation was evident 6 days post-injection.

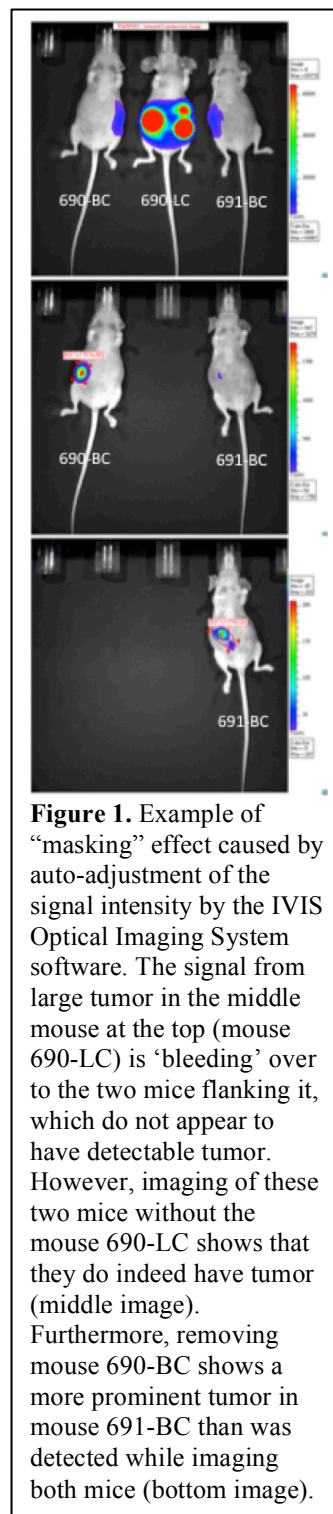
We conclude that time to detectable tumor formation is 6-8 days, using  $2.5$ - $7.5 \times 10^5$  injected HEYA8-GFP/LUC cells.

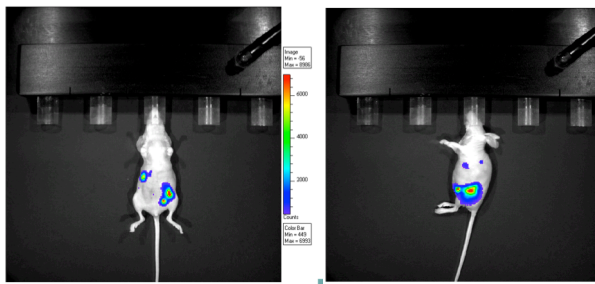
**Task 4.** Optimize duration of carboplatin treatment in 15 mice each for HEYA8 and OVCA420 derived tumors, months 6-8.

Following tumor formation in the mice described above, we initiated carboplatin treatment shortly after tumors were detected. In the first two mice, we were encouraged to see that, following IP carboplatin treatment (3 doses of 30 mg/kg in one mouse and 2 doses of 60 mg/kg in the other mouse) the tumor in the mouse treated with 30 mg/kg was undetectable while the other mouse showed a decrease in the tumor signal measured, although had a larger tumor to begin with. We terminated carboplatin treatment to determine time to recurrence, which was 10 days by as assessed using live imaging. We then planned to test injection of UCN-01 in these mice to assess the tolerability as the mice were still of good weight, active and eating normally, and injected 7.5 mg/kg in DMSO. Unfortunately, we did not realize that UCN-01 was soluble in diluted DMSO and it had already been solubilized in 100% DMSO. The mice succumbed within two hours. An adverse report was immediately filed with the Duke IACUC, and we worked with the Duke veterinarians to resolve how to deliver the UCN-01 safely. As mentioned above, we later found that the UCN-01 was actually soluble in dilute DMSO, and thus we ordered fresh UCN-01 to prepare more concentrated stock from which we could dilute to a level that was safe for the mice.

Most of the ten remaining mice from this first group, described above, had visible tumors and were thus started on a carboplatin regimen of 60 mg/kg every other day for three consecutive treatments. Imaging results showed increases in the tumor signal over the course of treatment, and we initially thought the carboplatin was somehow ineffective or that we had not treated long enough. We repeated the same dosing regimen beginning three days after completion of the first round of treatment. One of the mice was euthanized during this time due to  $>15\%$  loss in body weight.

We discovered that during the *in vivo* imaging procedure, where five mice can be simultaneously anesthetized and imaged side-by-side using the standard protocol, there was a masking effect in which a large tumor in one mouse would mask smaller tumors in other mice as a result of the image signal intensity being auto-adjusted by the software. Please see images in **Figure 1** for an example of this effect. Upon this discovery, we altered our imaging strategy such that only one mouse is imaged at a time.





**Figure 2.** Representative imaging of mice immediately post-tumor cell injection shows the injected cells are present within the peritoneal cavity.

than the peritoneal cavity. We thus injected  $2.5 \times 10^6$  cells into one of the mice that did not form a tumor and imaged the mouse immediately after injection; as shown in **Figure 2**, this confirmed that the cells were indeed spread throughout the peritoneal cavity.

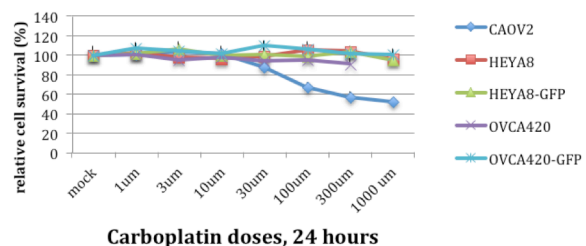
The variability in tumor formation was discussed with other investigators at Duke and colleagues at other institutions who work with cancer xenografts in mice and was reported by all others to be quite common. The only potential solution offered was to inject more mice than required for the experiment, aiming to get the number needed with tumors to test.

Due to the difficulties with imaging multiple mice simultaneously (solved), the lack of effective response to the six rounds of carboplatin treatment in all but one mouse and the variability in tumor formation, we ordered nine additional mice to conduct more preliminary studies. These mice were four weeks old on arrival. They were acclimated for two days, then injected IP with  $2.5 \times 10^5$  HEYA8-GFP/LUC cells. They were imaged six days later, and 8 showed detectable tumor. Carboplatin treatment was initiated at 60 mg/kg every other day for three days. The mice showed no decrease in tumor signal after these treatments, so we increased the carboplatin to 80 mg/kg for another three cycles. The tumors continued to increase in size over the second round of carboplatin treatment. At this point, we opted to return to *in vitro* experiments to test the response of the HEYA8, HEYA8-GFP/LUC, OVCA420, OVCA420-GFP/LUC and CAOV2 to carboplatin. We used the engineered (containing the pLenti-Fire construct) and the native cells to determine if the construct was influencing chemosensitivity. We also tested the response to paclitaxel and combined

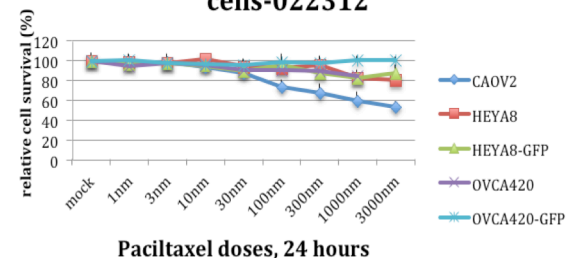
One of the mice showed no detectable tumor signal after the six rounds of carboplatin, so we tested for tolerance of Oltipraz administered by oral gavage (200  $\mu$ l at 25 mg/ml). The mouse tolerated the dosing well and there were no obvious ill effects.

The remaining mice all showed high variability in tumor signal, and some did not form visible tumor at all. We thought this might be due to the injected cells being inadvertently delivered to locations other

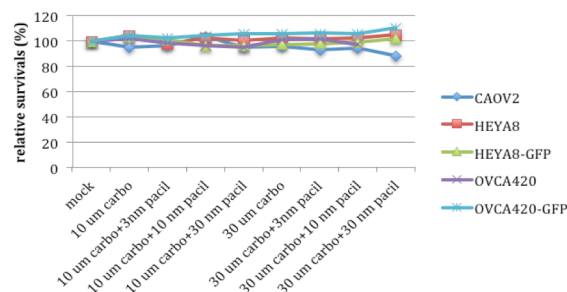
### Carboplatin treatment in OV cells-022312



### Paclitaxel treatment in OC cells-022312



### Carboplatin and Paclitaxel-022312



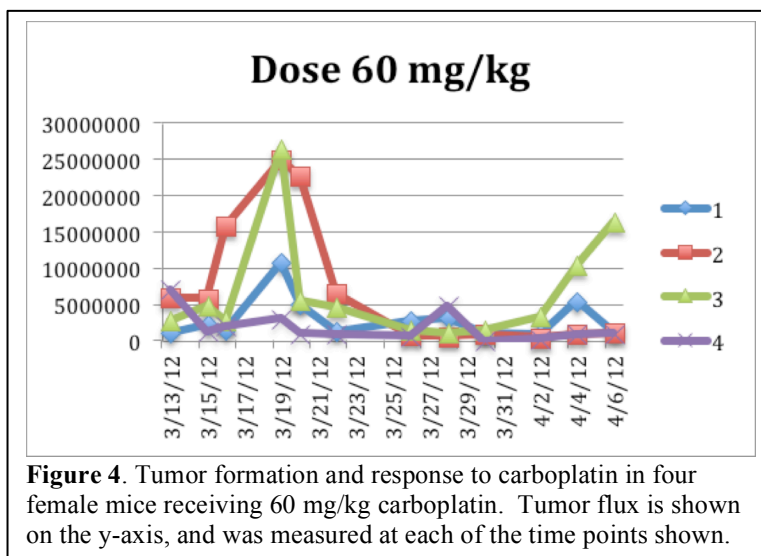
**Figure 3.** *In vitro* experiments to test response to carboplatin, paclitaxel and combined treatment for HEYA8 and OVCA420 pLenti-Fire-containing or native cells, and CAOV2 cells.

treatment to try and determine to which drug or combination these cells would best respond.

The results are shown in **Figure 3**. The HEYA8 and OVCA420 cells (with and without the pLENTI-Fire construct) remained resistant across the spectrum of doses used for cisplatin, and showed relative resistance to paclitaxel. They were similarly resistant to combined therapy with both drugs, explaining the lack of efficacy seen in the mice for carboplatin treatment against HEYA8-GFP/LUC derived tumors. CAOV2 cells showed a reasonable response to carboplatin and paclitaxel, but combined treatment was less effective. We therefore opted to engineer the CAOV2 cells with the pLENTI-Fire construct to enable their use for the xenograft tumor formation and carboplatin response. We first confirmed that the CAOV2 cells were free of murine pathogens before engineering the cells with this construct.

We ordered an additional 16 four-week old female mice to test with the CAOV2-GFP/LUC cells, which arrived February 22 2012. Twelve of the mice were injected IP on March 9, 2012 with  $3.5 \times 10^5$  CAOV2-GFP/LUC cells each. Visible tumors were formed (detected using live imaging) by March 15, 2012 and carboplatin treatment was initiated on the 17th, using three doses (4 mice per group): 20 mg/kg, 40 mg/kg and 60 mg/kg. Six cycles of carboplatin were delivered IP from March 17 through March 26. Tumors were monitored over this time period with tumor growth peaking on March 19<sup>th</sup>, ten days post-injection of the cells. Three of the four mice receiving 60 mg/kg carboplatin showed response to treatment, with tumor flux after carboplatin treatment below that when the tumors were first detected. Tumor recurrence was evident on day 20-post cell injection (~1.5 weeks remission time) (**Figure 4**).

On April 16 and 17, we injected the same group of 12 mice with 7.5 mg/kg, 10 mg/kg and 20 mg/kg UCN-01 to test for toxicity. Note we were not testing for response since most of these mice had recurrent tumors. The mice tolerated this treatment at all three doses very well. The mice were euthanized on April 20<sup>th</sup> and tumors were samples and stored in OCT compound at -80 degrees Celsius.



**Figure 4.** Tumor formation and response to carboplatin in four female mice receiving 60 mg/kg carboplatin. Tumor flux is shown on the y-axis, and was measured at each of the time points shown.

The four remaining mice from this group were injected with  $3.5 \times 10^5$  CAOV2-GFP/LUC cells on March 31. Carboplatin treatment was initiated on April 7 but two of the mice succumbed due to problems with the injections. This experiment was abandoned due to having only two mice remaining, although these mice were also used to test for toxicity to UCN-01, which was again well-tolerated.

**Task 5.** Aim 1: test efficacy of UCN-01 and Oltipraz in duplicate against xenografts derived from spheres of established cell lines, months 7-18.

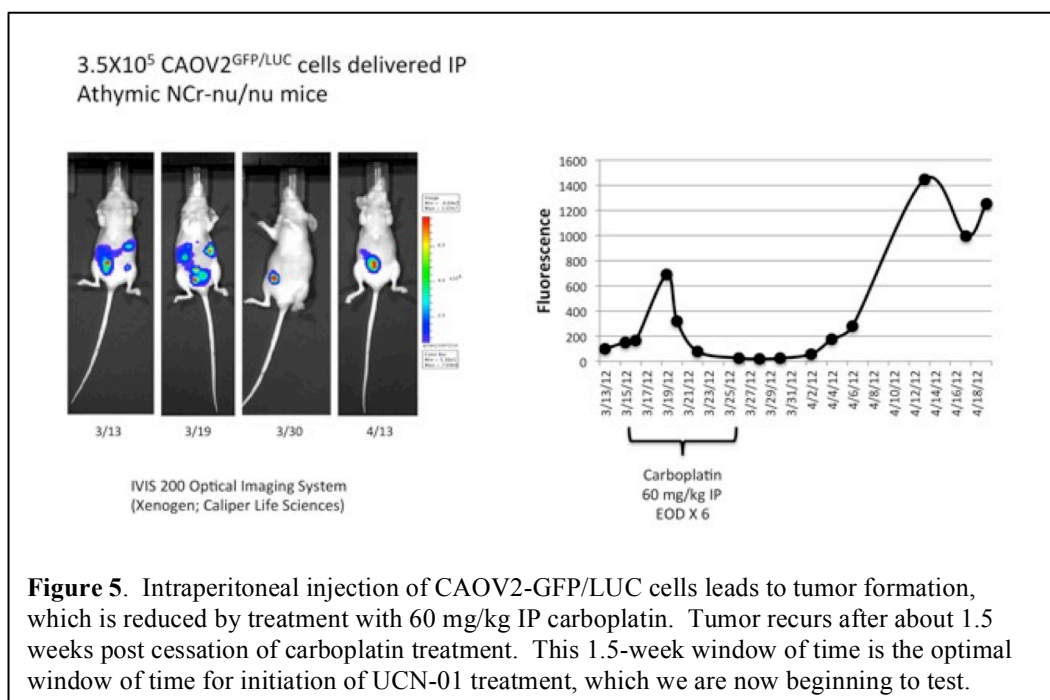
**5a.** Propagate spheres from established cell lines, HEYA8 and OVCA420, months 7-8.

One of the unresolved questions about the use of spheres as a mechanism to enrich for cancer stem cells *in vitro* is how the spheres arise. There are a substantial number of published reports that describe utilization of spheres for this purpose with selection by growth in stem cell selective media on low attachment plates. We were also using this technique, and observed sphere formation for many of our cell lines. However, we noted when checking the incubator two hours after plating cells in this manner that spheres were already

formed. This raised the question of whether “sphere formation” was due from clonal expansion of single cells able to survive under the stem cell-selective conditions as we had assumed, or whether this represents a phenomenon of aggregation. We therefore cultured the cells under sphere-forming conditions and photographed the cells at five-minute intervals for 24 hours, using a Zeiss Axio Observer microscope. All cell lines tested showed aggregation within hours of plating in the stem cell selective conditions, some within minutes (even before we could transfer them from the lab, following trypsinization to single cell suspensions, to the core facility to use the Axio Observer microscope, which is located in the same building as the lab). These results were presented at the 2012 AACR meeting and the abstract is appended to this report (**Appendix Item 1**). We concluded that although aggregation is probably an important attribute in the context of tumor cell metastasis and survival, it is likely not enriching for cancer stem cells, supported by our finding that stem cell marker CD133 positive and negative cells were equally able to form these sphere aggregates. We therefore opted to not use cells grown as spheroids as our starting material for tumor initiation in the xenograft model of ovarian cancer. Instead, we will use the *same* cells but grown in standard culture conditions, explained below.

The cells we are using can be grown on standard tissue culture plates in regular culture media with serum - which allows them to rapidly proliferate, or they can be grown on "low-attachment" plates on which they do not adhere in reduced serum conditions, in so-called "stem cell-selective" media, in which we now know from the work described above that the cells do not proliferate and instead aggregate. The treatment regimen will be the same as was originally planned - first we will treat tumors with carboplatin, then following objective reduction of tumor volume we will either not treat with secondary therapy, treat with additional carboplatin as secondary therapy, or treat with UCN-01 or Oltipraz as secondary therapy. Another arm, in which the mice do not receive primary or secondary treatment, will also be used as a control for tumor formation. These arms are exactly as originally planned, and thus the scope of the proposed work has not been altered, only the manner in which the ovarian cancer cells will be grown has changed.

Instead, we have developed a recurrent mouse model of ovarian cancer that more closely mimics the course of disease in women by first forming rapidly proliferating tumors that largely respond to carboplatin. However, some of the injected cancer cells survive the carboplatin treatment and go on to cause disease recurrence, just as is frequently observed in women with epithelial ovarian cancer. To do this, ovarian cancer cells are injected intraperitoneally into female nude mice with tumor formation monitored using the IVIS *in vivo* imaging system. Once tumors are detected, we initiate treatment with carboplatin and monitor tumor regression. In this model, tumors recurred approximately 1.5 weeks after carboplatin treatment had stopped. See **Figure 5**.





**5b. Inject ~~sphere-derived~~ monolayer cells into NCr-nu/nu mice, months 7-8.**

Note that the change to use of monolayer cells in work task 5b reflects a change in methodology; there is no change in the scope of proposed work. As described above, we have determined that sphere formation does not enrich for an ovarian cancer stem cell-like population of cells and therefore decided that instead of growing the cells on the low attachment plates with reduced serum to form spheres, we would grow the same cells under standard culture conditions, on regular tissue culture plates in RPMI1640 media with 10% serum. The decision to use the same cells grown under monolayer culture conditions versus under non-adherent sphere-forming conditions does not alter the scope of the proposed work; it is simply a change in the methodology used to grow the same cells. The following experiments were conducted using cells grown as monolayers under standard culture conditions.

We ordered 60 5-week old female athymic Ncr-nu/nu mice, which arrived on May 2, 2012. These mice were treated as two independent groups since we required 30 mice per experiment. CAOV2-GFP/LUC cells were grown and sorted by flow cytometry as described above. Mice were injected with  $4 \times 10^5$  cells IP. Imaging showed tumor formation in 27/30 mice in group 1 and 26/30 mice in group 2 by May 14, 2012. Carboplatin treatment (60 mg/kg) was started on May 15 but was extended to seven rounds in both groups as tumor volume was not showing response as it had in pilot studies. Group 2 mice received an additional dose on June 1. Group 1 mice showed the best response to carboplatin, so consolidation therapy testing was initiated on June 2, 2012. The mice were divided into five arms according to the proposal, with Arm 1 receiving no additional treatment during the consolidation phase, Arm 2 receiving UCN-01 (10mg/kg/day), Arm 3 receiving Oltipraz PO at 250 mg/kg/day, Arm 4 receiving additional carboplatin (60mg/kg/EOD) and Arm 5 serving as a positive control with no treatment in either the primary or consolidation setting. The results of these experiments are currently being analyzed.

Group 2 mice exhibited robust tumor growth despite the carboplatin treatment, and were thus re-grouped to select the mice that showed an objective response ( $>50\%$  tumor flux reduction from initial tumor burden at the time of tumor detection, prior to carboplatin treatment). Of these eight mice, three received UCN-01, three received Oltipraz and two received no additional treatment, all per the same regimen as delivered to the Group 1 mice. This data is also currently being analyzed.

**5c. Complete treatment regimens and collect data, months 8-11.**

**5d. Analyze tumors by immunohistochemistry, month 12.**

All tumors thus far have been collected and frozen in OCT compound for future immunohistochemistry analyses.

**5e. Repeat steps 5a to 5d, months 8-13.**

**Task 6. Prepare manuscript(s) detailing established cell line data, months 12-15.**

**Task 7. Aim 2: test efficacy of UCN-01 and Oltipraz in duplicate against xenografts derived from ~~spheres of~~ primary ascites, months 6-24.**

**7a. Propagate spheres from ascites as available for up to 10 patients, months 6-18.**

For the reasons described above, we have opted to use live *in vivo* imaging to monitor tumor formation. We therefore have been working to deliver SV40 T Antigen to primary human epithelial ovarian cancer cells to try and immortalize these cells so that we can generate large quantities for delivery to the mice. It appears we now have two lines derived from primary ovarian cancers that are showing growth of colonies under selection. The next step, after we confirm that these are cancer cells that have been selected, is to transduce the cells with the pLENTI-Fire construct to engineer these to express GFP and LUC as we have done for the primary cultures. Although we could have opted to inject primary cells taken directly from

surgery, this would not have allowed for monitoring of tumor formation and regression as we have shown is highly effective above, and the ability of any given patient's tumor cells to form xenografts is actually quite low, so the number of patient samples and the number of cells required to inject 30 mice, and have them all form tumors to enable testing, was felt to be consistent with a very small likelihood of success. We realize that SV40 T Antigen-mediated immortalization of the cells may lead to other changes that are not going to reflect precisely the phenotype of the primary cells themselves, but feel that the advantage gained by doing this, if successful, outweighs the potential deviation in phenotype that might result. If few are unsuccessful at generating immortalized lines from the primary cancers that can be used, we will revert to the original plan, but without the use of sphere-derived cells as we discuss above.

**7b.** Inject sphere-derived cells into NCr-nu/nu mice from two patients, months 6-18.

**7c.** Complete treatment regimens and collect data, months 7-24.

**7d.** Analyze tumors by immunohistochemistry, months 22-24.

**Task 8.** Prepare manuscript(s) detailing primary ascites data, months 22-24.

## KEY RESEARCH ACCOMPLISHMENTS

- We have determined that sphere formation does not select for a cancer stem-cell-enriched population, but rather is a phenomenon of cell aggregation.
- We have engineered ovarian cancer cells to stably express green fluorescent protein and luciferase to enable live imaging, per the reviewer's recommendations.
- We have successfully used live *in vivo* imaging to monitor tumor formation, response to treatment, and recurrence, and improved the technique through modifications in imaging procedures, including the monitoring of only a single mouse at a time.
- We have shown that HEYA8 and OVCA420 cells originally proposed for this work (due to their sphere forming capacity and response to UCN-01) are resistant to carboplatin, making it difficult to use these cells in a model of recurrent disease.
- We have shown that CAOV2 ovarian cancer cells are responsive to carboplatin and show evidence that they are capable of inducing recurrent disease within a two-week time span following apparent remission after carboplatin treatment.

## REPORTABLE OUTCOMES

(1) Huang, Z., Lowery, W.J., Berchuck, A. and S.K. Murphy. 2012. Aggregation rather than monoclonal expansion explains ovarian cancer spheroid formation. American Association for Cancer Research Annual Meeting, Philadelphia, PA. Abstract 5363. (**Appendix item 1**)

(2) Development of cell lines:

- (a) CAOV2-GFP/LUC
- (b) OVCA420-GFP/LUC
- (c) HEYA8-GFP/LUC

(3) A Department of Defense OCRP Synergistic Translational Leverage Award application was invited for submission from Dr. Ashley Chi and Dr. Susan Murphy as Co-PIs; the preliminary data showing our ability to use live imaging to monitor tumor formation and response to treatment in the CAOV2 lines is a large component of this application. This application is due July 18, 2012.

(4) Korch, C., Spillman, M.A., Jackson, T.A., Jacobsen, B.M., Murphy, S.K., Lessey, B.A., Jordan, V.C. and A.P. Bradford. 2012. DNA profiling analysis of endometrial and ovarian cell lines reveals misidentification, redundancy and contamination. *Gynecologic Oncology* (in press). (**Appendix item 2**)

(5) Invited presentation: “Toward Preventing Ovarian Cancer Recurrence.” 2011. Gail Parkins Memorial Ovarian Cancer Awareness Educational Forum, Raleigh, NC

## CONCLUSION

We have taken the first steps in developing a mouse model of recurrent epithelial ovarian cancer for which we can monitor tumor formation, response and recurrence *in vivo* using live imaging. We are now trying to test the efficacy of two drugs that we had identified as more effective at targeting slow-proliferating cells *in vitro* (1) in the consolidation setting using this model. Despite demonstration of responsiveness of CAOV2-GFP/LUC cells to carboplatin, there remains variability in response between experiments, which we now believe has to do with the age of the mice and/or their size when first injected with tumor cells (smaller mice do not respond as well and look less healthy, so drug response may be compromised), as well as the number of cells injected (fewer cells may form smaller or less dense tumors that are more easily targeted by the IP chemotherapy). Next experiments will use slightly older and heavier mice and fewer injected cells. We are also making progress toward developing the primary cells for use in xenograft formation that can be monitored through live imaging. To our knowledge, there are no animal models of recurrent ovarian cancer reported in the literature, yet recurrent disease is usually the lethal event for humans. Finding drugs that can target residual cells during remission is perhaps the best hope for delaying or preventing recurrence (2). If we fail to show such efficacy for UCN-01 and/or Oltipraz, the development of this mouse model will open new opportunities for testing a wide variety of other drugs in this setting.

## REFERENCES

1. E. Kondoh *et al.*, Targeting slow-proliferating ovarian cancer cells. *Int J Cancer* **126**, 2448 (May 15, 2010).
2. S. K. Murphy, Targeting ovarian cancer-initiating cells. *Anticancer Agents Med Chem* **10**, 157 (Feb, 2010).

## APPENDICES

List:

**Appendix Item 1:** Huang, Z., Lowery, W.J., Berchuck, A. and S.K. Murphy. 2012. Aggregation rather than monoclonal expansion explains ovarian cancer spheroid formation. American Association for Cancer Research Annual Meeting, Philadelphia, PA. Abstract 5363.

**Appendix Item 2:** Korch, C., Spillman, M.A., Jackson, T.A., Jacobsen, B.M., Murphy, S.K., Lessey, B.A., Jordan, V.C. and A.P. Bradford. 2012. DNA profiling analysis of endometrial and ovarian cell lines reveals misidentification, redundancy and contamination. *Gynecologic Oncology* (in press).

## Presentation Abstract

Abstract Number: 5363

Presentation Title: Aggregation rather than monoclonal expansion explains ovarian cancer spheroid formation

Presentation Time: Wednesday, Apr 04, 2012, 8:00 AM -12:00 PM

Location: McCormick Place West (Hall F), Poster Section 18

Poster Section: 18

Poster Board Number: 30

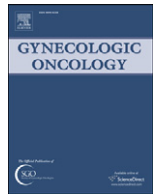
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**Abstract Body:** Purpose: It is thought that a subpopulation of ovarian cancer cells possess stem cell like properties and are responsible for both the emergence of cancer and for subsequent chemoresistance and recurrence. A common approach used to enrich for cancer stem cells in vitro involves culture in selective media on low attachment plates resulting in non-attached multicellular spheres. Spheroids are present in ascites fluid of women with ovarian cancer, and may contain stem cells responsible for metastasis and recurrence. Our objective was to address whether spheroids are truly monoclonal in origin and arise from expansion of a single common progenitor cell.

Procedures: We cultivated 5 ovarian cancer cell lines (DOV13, HEYC2, OVCAR2, OVCAR3, and PEO4) to confluence in monolayer cultures. The cell lines were chosen for their ability to form spheroids as well as variation in expression of the stem cell marker CD133 (OVCAR2, OVCAR3 CD133+, others negative). The cells were trypsinized to form single cell suspensions and plated at densities ranging from 100 to 5000 cells per plate in stem cell-selective media in ultra-low attachment culture dishes. A Zeiss Axio Observer microscope was used for time-lapse photography of the plated cells at 5-minute intervals over a 24-hour period. In addition, single cell suspensions were subjected to flow activated cell sorting to plate one cell in each well of 96-well Costar ultra-low cluster plates to monitor clonal formation of spheroids.

Results: Cells were dissociated and plated under stem cell-selective conditions and 288 images of each cell line were recorded over 24 hours. The cells were found to begin to aggregate within two to four hours, regardless of cell numbers plated. By 24 hours post-plating, there was pronounced aggregation in 5/5 (100%) cell lines with formation of tightly compacted spheroid structures in 4/5 (80%) cell lines. Single cells in the 96-well plates showed no evidence of spheroid formation over a 14-day observation period.

Conclusion: We observed spheroid formation in both CD133 positive and negative ovarian cancer cell lines. Formation occurred due to cellular aggregation rather than clonal expansion from a single progenitor with stem cell properties. Although cellular aggregation may be relevant to ovarian cancer biology, these data suggest that spheroid formation should be viewed with caution if used as a proxy for monoclonal expansion of ovarian cancer stem cells.



# DNA profiling analysis of endometrial and ovarian cell lines reveals misidentification, redundancy and contamination

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## ABSTRACT

**Objectives.** Cell lines derived from human ovarian and endometrial cancers, and their immortalized non-malignant counterparts, are critical tools to investigate and characterize molecular mechanisms underlying gynecologic tumorigenesis, and facilitate development of novel therapeutics. To determine the extent of misidentification, contamination and redundancy, with evident consequences for the validity of research based upon these models, we undertook a systematic analysis and cataloging of endometrial and ovarian cell lines.

**Methods.** Profiling of cell lines by analysis of DNA microsatellite short tandem repeats (STR), p53 nucleotide polymorphisms and microsatellite instability was performed.

**Results.** Fifty-one ovarian cancer lines were profiled with ten found to be redundant and five (A2008, OV2008, C13, SK-OV-4 and SK-OV-6) identified as cervical cancer cells. Ten endometrial cell lines were analyzed, with RL-92, HEC-1A, HEC-1B, HEC-50, KLE, and AN3CA all exhibiting unique, uncontaminated STR profiles. Multiple variants of Ishikawa and ECC-1 endometrial cancer cell lines were genotyped and analyzed by sequencing of mutations in the p53 gene. The profile of ECC-1 cells did not match the EnCa-101 tumor, from which it was reportedly derived, and all ECC-1 isolates were genotyped as Ishikawa cells, MCF-7 breast cancer cells, or a combination thereof. Two normal, immortalized endometrial epithelial cell lines, HES cells and the hTERT-EEC line, were identified as HeLa cervical carcinoma and MCF-7 breast cancer cells, respectively.

**Conclusions.** Results demonstrate significant misidentification, duplication, and loss of integrity of endometrial and ovarian cancer cell lines. Authentication by STR DNA profiling is a simple and economical method to verify and validate studies undertaken with these models.

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## Introduction

Cell lines, immortalized from normal human tissues or derived from tumors, are widely used models to address molecular mechanisms underlying the physiology and pathology of the female reproductive tract, and to evaluate novel therapeutics or preventive strategies [1–3]. Verification of the provenance and integrity of such cell lines is clearly of paramount importance, but historically, has rarely been undertaken by investigators. The problem of cross-contamination, identified and characterized by examination of isozyme patterns, karyotyping, and cytogenetics, dates back to the establishment of the prototypical HeLa cell

line in culture in 1951 and remains a significant concern [4–7]. Over one-third (18–50%) of cell lines may be mixtures, misidentified or intra-species contaminants [2,8–15]. Furthermore, there are many examples of redundancy among reportedly unique cell lines, and instances of contamination during original derivations, such that the intended novel cell line was never established [5,10,16–19]. Thus, it is evident that authentication of cell line origins and integrity is crucial to validate results and conclusions obtained using these model systems.

Short tandem repeat (STR) profiling or ‘DNA fingerprinting’ identifies variants in tetranucleotide microsatellite loci on multiple human chromosomes and is the accepted international standard for genetic analysis of cell lines for authentication by comparison to established STR databases [20–24].

A comprehensive analysis of cell lines commonly used in the study of ovarian and endometrial cancer had not been undertaken, particularly with respect to those cell lines not obtained from established cell repositories. We used STR profiling, sequencing of p53 mutations, and

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human papilloma virus screening to examine cell lines of purported ovarian and endometrial origins. We observed examples of cross-contamination, misidentification of lines and/or tissue of origin, and redundancy among established cancer cells, and found evidence that immortalized normal endometrial epithelial cell lines are genetically identical to previously established cervical and breast cancer cells. We provide reference DNA profiles for women's cancer cell lines that are not currently in public cell banks and extend the number of loci for profiles currently available through central repositories.

## Materials and methods

### DNA isolation and STR profiling

Cell lines were grown in appropriate specific standard media. Genomic DNA was isolated from  $0.5$  to  $5 \times 10^6$  cells using a Zymo Research ZR genomic DNA II kit and quantified by gel electrophoresis and ethidium bromide staining by comparison to a DNA mass ladder. Multiplex PCR amplified products were generated using 1–2 ng of genomic DNA with an Applied Biosystems Identifier kit and ABI 3730 capillary sequencer as described [2,18]. STR loci were analyzed with Gene Mapper 4.0. Profiles were compared to published reports [22,25], consolidated (ATCC, DSMZ, JCRB and RIKEN) databases, and an in-house database, using a custom search algorithm designed to facilitate comparison of cell lines with related profiles and identify individual cell lines in a mixture (C. Korch and J. West, Vanderbilt University, unpublished). STR profiles of the ovarian and endometrial cancer cells analyzed in this study are available online at <http://DNAsequencingcore.UCDenver.edu>.

### TP53 sequence analysis and microsatellite instability assays

PCR amplification was used to generate overlapping products spanning the Variable Number Tandem Repeat (VNTR; a pentanucleotide repeat of A<sub>4</sub>T) in intron 1, through the protein encoding exons 2–11, including intervening introns 2–8 and 10 [26]. Sequencing primers and p53 gene structure are shown in Fig. S1. DNAs were screened for microsatellite instability [27] using Promega MSI analysis system version 1.2 according to the manufacturers' protocol.

### HPV testing

Aliquots of cells were placed into ThinPrep (Hologic) solution. DNA was isolated and tested in the University of Colorado Hospital Clinical Laboratory using the hybrid capture PCR, Digene HC2 High Risk HPV test (Qiagen).

### Ovarian and endometrial cell lines

We obtained cell lines from multiple institutions in the United States, Europe and Japan, including, where possible, the originating laboratories. Multiple independent samples of the earliest available passages from each institution were analyzed and, if available, profiles of each individual cell line were compared from several sources. Ovarian cancer cell lines are listed in Table S1. Ishikawa cells were obtained from Dr. K.K. Leslie (University of Iowa), Dr. B.A. Lessey (Greenville Hospital System, SC), Dr. M. Brown (Dana Farber Cancer Institute, Harvard University) and Drs H. Philpott and P. Thraves (European Collection of Cell Cultures, ECACC). ECC-1 cells were from Drs. B.A. Lessey, M. Brown and V.C. Jordan (Lombardi Comprehensive Cancer Center, Georgetown University). EnCa-101 tumors were provided by Drs. V.C. Jordan and G. Balburski (Fox Chase Cancer Center). HES cells were from Dr. D. Kniss (Ohio State University) and hTERT-EECs from Dr. T. Klonisch (University of Manitoba, Canada). KLE and HEC-50 cells were from Dr. K.K. Leslie. RL-95-2, HEC-1A, HEC-1B and AN3CA cells were from the American Type Culture Collection (ATCC, Manassas, VA).

## Results

### Analysis of endometrial cancer cell lines

Endometrial carcinomas are derived from glandular epithelium and are typically divided into two subtypes based on clinical, histological and molecular characteristics [28–30]. Cell lines derived from type I (Ishikawa, ECC-1 and RL-95-2) and type II (HEC-1, HEC-50, KLE and AN3CA) tumors have been widely used as models to investigate molecular genetics and mechanisms underlying their development, progression and response to therapeutics [31–35].

HEC-1A and HEC-1B cell lines, the first to be derived from a human endometrial carcinoma [32,36,37], both exhibited a unique profile consistent with their derivation from the same patient (Table S3). HEC-1A cells are predominantly diploid, while the HEC-1B line is tetraploid [38,39]. HEC-50 cells [38,40], also have a unique profile consistent with that on file with the Japanese Collection of Research Bioresources (JCRB: 1145).

Similarly, KLE (CRL-1622) and AN3CA (HTB-111) cells, originating from peritoneal and lymph node metastases, respectively [34,41,42], and RL-95-2 cells (CRL-1671) derived from a moderately differentiated (Grade 2) endometrial adenosquamous carcinoma [35], all have STR profiles consistent with those reported by the ATCC (Table S3).

Ishikawa cells were established from the epithelial component of a moderately differentiated, stage 2, endometrial adenocarcinoma [43,44]. At least three variants of Ishikawa cells, the original line, 3-H-4 and 3-H-12, differing in their reported degree of differentiation, relative expression of estrogen (ER) and progesterone (PR) receptors, growth and colony formation rates, were distributed to investigators [45].

We profiled multiple isolates of the original Ishikawa cells and 3-H-12 variants obtained from a number of laboratories as detailed in the Materials and methods section. Samples with unique profiles, which may represent the 3-H-4 variant based upon their date of origin are designated '3-H-4'. The results are summarized in Table 1.

Overall the Ishikawa cell lines exhibit very similar profiles, indicative of their origin from the same patient. Identical alleles were present at several loci (CSF1PO, D5S818, D16S539, D21S11, TH01 and TPOX). Others reflect loss or gain of alleles (D8S1179, D13S317 and FGA) or alterations in the number of repeats (D2S1338, D3S1358, D19S433 and vWA). At the D7S820 locus, the original Ishikawa isolate exhibits 8.3- and 11-repeat alleles, while subsequent sublines display 9- or 10-repeats. The D18S51 locus was found to be highly polymorphic in most Ishikawa lines.

Minor differences in the number of repeats at certain loci are consistent with the known microsatellite instability (MSI) of these lines, due to mutations in mismatch repair systems [46–48], and suggest that these variants arose by genetic drift between different clonal isolates over hundreds of cell passages. Accordingly, all Ishikawa cell lines exhibited high variability/instability at microsatellite loci (Table S2). Defective mismatch repair also underlies allelic variation in AN3CA cells (Table S3) [49]. In contrast, EnCa-101 tumors and MCF-7 cells were MSI stable.

We also profiled a variant of Ishikawa cells lacking ER [50]. Previous reports implied that these cells, also known as Ishikawa B, were derived from a different patient [51,52]. The STR profile of ER-negative Ishikawa cells exhibits minor variations from other Ishikawa sublines (Table 1), but overlap at the majority of loci indicates a common origin.

A second type 1, ER and PR positive cell line, ECC-1, was established from a grade 2, well-differentiated, endometrial carcinoma adenocarcinoma [42,53,54]. The line was derived by passage of the tumor, designated EnCa-101, in nude mice and subsequent isolation of PR positive cells from an epithelial monolayer culture [42,55]. ECC-1 cells were described as a well-differentiated, steroid responsive line with a phenotype characteristic of luminal surface epithelium, distinct from Ishikawa cells, which expressed markers of glandular endometrial epithelium [33].



**Table 1**

Summary of STR profiles of Ishikawa and ECC-1 endometrial cancer cells and EnCa-101 tumor.

Cell line	Amelogenin	CSF1PO	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	TH01	TPOX	vWA
Ishikawa original	X	11, 12	18, 20	17, 18	10, 11	8, 3, 11	12, 16	9, 12	9	14, (19) 20, 21 polymorphic	12, 2, 14	28	21	9, 10	8	14, 18
Ishikawa '3-H-4'	X	11, 12	19, 20	16, 17	10, 11	9, 10	12, 16	9, 12	9	13, 21, 22	12, 2, 14	28	21, 22	9	8	14, 17
Ishikawa 3-H-12	X	11, 12, (13)	19, 20	16, 17	10, 11	9, 10	12, 13, 16	9, 12, 13	9	12, 19, 20	13, 2, 14	28	20, 21	9, 10	8	14, 17
Ishikawa 3-H-12	X	11, 12	20	16, 17, (18)	10, 11, (12)	9, 10	12, (13), 16	9, 12	9, (10)	13, 20	12, 2, 14, (15)	28	21	9, 10 or 11	8	14, 17 or 18
Ishikawa ER -ve	X	11, 13	20	16, 17	10, 11	9, 10	12, 13, 16	9, 12	8, 9	13, 19	12, 2, 14	28	20	9, 10	8	14, 17
Ishikawa, ECACC, this report	X	11, 12, (13)	20	15, 17	10, 11, 12	9, 10	12, 16	9, 12, 13	9	13, 19, (14, 20)	12, 2, 14	28	21	9, 10	8	14, 17
Ishikawa, ECACC	X	11, 12	NT	NT	10, 11	9, 10	NT	9, 12	9	NT	NT	NT	NT	9, 10	8	14, 17
ECC-1	X	11, 12	20	16, 17	10, 11	9, 10	13, 16	9, 12,	9	12, 19	12, 2, 14 or 15	28	21	9, 10	8	14, 17
ECC-1 ATCC CRL-2923	X	11, 12	NT	NT	10, 11	9, 10	NT	9, 12	9	NT	NT	NT	NT	9, 10	8	14, 17
EnCa-101	X	13, 14	23, 27	15, 21	14, 15	11, 3, 12	18, 21	10, 13	12, 13	16	13, 2, 14 or 15	27, 30	21	9, 9, 3	8	18, 23

Number of STRs at each of 16 surveyed loci. Numbers after decimal point indicate number of bases in an incomplete STR. Commas separate allele calls for multiple peaks. Alleles in parenthesis indicate low amplitude peaks suggesting only a minor fraction of the cells in the population carry that allele. ECACC: DNA profile from European Collection of Cell Cultures; ATCC: DNA profile from American Type Culture Collection. NT: locus not tested.

Upon STR and MSI analyses, ECC-1 samples exhibited DNA profiles essentially identical to Ishikawa 3-H-12 cells (Tables 1 and S2). In addition, the ATCC profile for ECC-1 also closely matched that of earlier Ishikawa cells on file with the European Collection of Cell Cultures (ECACC). Other 'ECC-1' cell lines were found to be identical to MCF-7 breast cancer cells or consist of a mixture of Ishikawa and MCF-7 cells (not shown). Unfortunately, following the death of Dr. Satyaswaroop, records and cell lines from his laboratory were lost or destroyed (Zaino, R. and Lessey, B., personal communication). Thus, we could not obtain reference samples of the original ECC-1 line or EnCa-101 tumor from which it was purportedly derived. However, the EnCa-101 tumor has been continuously maintained in mice [56] and we obtained and analyzed 3 independent samples. Profiling of these tumors showed minor variations, but results indicated that they were derived from the same human patient. In contrast, the unique EnCa-101 profiles did not match ECC-1, Ishikawa or MCF-7 cell lines (Table 1). These data are inconsistent with the reported origins of ECC-1 cells and suggest that the original line has been lost. Our results show that currently available ECC-1 cells are Ishikawa cells, MCF-7 breast cancer cells, or a mixture of both.

#### Sequencing of p53 mutations in endometrial cancer cells

To confirm the apparent equivalence of Ishikawa and ECC-1 cells, we screened for p53 mutations by PCR amplification and sequencing of the Variable Number Tandem Repeat (VNTR) region in intron 1, and the protein encoding exons and introns (Fig. S1). Table 2 lists the observed p53 mutations and SNPs compared to the reference/normal sequence.

In agreement with previous reports [31,57], Ishikawa original and 3-H-12 cells harbor a Met 246 Val mutation in exon 7. These two lines are also homozygous in the VNTR region with 8 repeats of A<sub>4</sub>T, heterozygous in exon 4 for the Asp 49 Val mutation (nucleotide G12069S), and heterozygous in intron 10 for deletion of the seventh T in a heptanucleotide repeat (17822delT). The original Ishikawa sample has two additional heterozygous mutations, 12724insA (intron 4) and 13764delA (intron 6), which are not present in the 3-H-12 line (Table 2).

Possible '3-H-4' sublines have a similar profile, but lack the intronic 12724insA and 13764delA mutations of poly A stretches, present in the original Ishikawa lines (Table 2). An additional heterozygous mutation in intron 4 (G12299K (G + T)) was detected in some Ishikawa 3-H-12 sublines. Interestingly, consistent with their closely matched STR profiles, the ER-negative Ishikawa cells, despite their purported distinct origin, exhibit TP53 mutations identical to Ishikawa 3-H-12 and '3-H-4' (not shown). TP53 mutations unique to the original Ishikawa lines are insertions or deletions in homopolymer A or T stretches, which are consistent with microsatellite instability due to mutations in the mismatch repair system [46].

In agreement with their identical STR profiles, ECC-1 cells show the same TP53 mutations as Ishikawa 3-H-12 lines, further evidence that ECC-1 cells are misidentified Ishikawa cells. In contrast, EnCa-101 tumors have completely different TP53 mutations from the Ishikawa and ECC-1 lines (Table 2), again demonstrating that ECC-1 cells are not derived from the EnCa-101 tumor. 'ECC-1' cells shown to be contaminated with or identical to MCF-7 cells were not subjected to TP53 analysis.

Finally, our data suggest that only one copy of the p53 gene is expressed in Ishikawa cells. In the genomic DNA, both the A14063R (A + G) and G12069S (G + C) positions are heterozygous. However, only the 14063G mutation is present in the cDNA sequence [31,57], suggesting that the G12069C mutation is in the unexpressed copy of the gene.

#### Analysis of normal endometrial epithelial cells

Immortalized, non-transformed endometrial epithelial cells are a potentially valuable resource to investigate normal uterine physiology and tumorigenesis. We profiled two such lines, human endometrial (HES) cells [58] and hTERT-EEC [59], obtained from their developers, which have been extensively used as models of normal endometrium. Neither cell line was authenticated as they exhibited DNA profiles corresponding to HeLa and MCF-7 cancer cells, respectively.

HES cells were established, in 1989, from a primary culture of benign proliferative endometrium, which apparently underwent spontaneous transformation after serial passage [58,60]. Profiling of these cells

**Table 2**  
Summary of TP53 mutations and single nucleotide polymorphisms (SNPs).

TP53 reference sequence	Ishikawa original	Ishikawa '3-H-4'	Ishikawa 3-H-12	Ishikawa 3-H-12	ECC-1	EnCa-101 tumor
Intron 1: VNTR A <sub>4</sub> T repeats	Homozygous 8 repeats	Homozygous 8 repeats	Homozygous 8 repeats	Homozygous 8 repeats	Homozygous 8 repeats	Heterozygous 7 and 9 repeats
Exon 4: G12069 Asp 49	Heterozygous G12069S Asp49His	Heterozygous G12069S Asp49His	Heterozygous G12069S Asp49His	Heterozygous G12069S Asp49His	Heterozygous G12069S Asp49His	
Intron 4: G12299			Heterozygous G12299K			
Intron 4: Poly A <sub>7</sub> 12718–12724	Heterozygous 12724insA Poly A <sub>7</sub> /A <sub>8</sub>					
Intron 5: G12786						Homozygous G12786T SNP
Intron 5: C13253						Heterozygous C13253Y SNP
Intron 6: G13642						Heterozygous G13642K SNP
Intron 6: Poly A <sub>9</sub> 13756–13764	Heterozygous 13764delA Poly A <sub>9</sub> /A <sub>8</sub>					
Exon 7: A14063 Met246	Heterozygous A14063R Met246Val	Heterozygous A14063R Met246Val	Heterozygous A14063R Met246Val	Heterozygous A14063R Met246Val	Heterozygous A14063R Met246Val	
Intron 10: Poly T <sub>7</sub> 17816–17822	Heterozygous 17822delT Poly T <sub>7</sub> /T <sub>6</sub>	Heterozygous 17822delT Poly T <sub>7</sub> /T <sub>6</sub>	Heterozygous 17822delT Poly T <sub>7</sub> /T <sub>6</sub>	Heterozygous 17822delT Poly T <sub>7</sub> /T <sub>6</sub>	Heterozygous 17822delT Poly T <sub>7</sub> /T <sub>6</sub>	Homozygous/Hemizygous Poly T <sub>7</sub>

Tumor protein p53 (TP53) genomic DNA, from multiple independent samples of each cell line, was sequenced as described in the [Materials and methods](#) section. The normal reference normal is GenBank HSP53G, a.k.a. [X54156](#), which is used by the International Agency for Research on Cancer IARC (<http://www-p53.iarc.fr>). A blank cell in the table indicates the DNA sequence that matches the reference/normal sequence. VNTR: Variable Number Tandem Repeat. Symbols — K: G and T; R: A and G; S: G and C; Y: C and T; del: nucleotide deletion; ins: nucleotide insertion.

([Table 3](#)) indicated that they are identical at all loci to HeLa cervical carcinoma cells, specifically the HeLaS3 variant. HES cells are also identical to WISH cells, a cell line originally described as derived from human amnion [61] but subsequently also identified as HeLa [7,62,63]. These results were independently confirmed by the STR fragment analysis

facility at Johns Hopkins University (D. Kniss, Ohio State University; personal communication).

hTERT-EECs were isolated from normal proliferative phase endometrial epithelium and immortalized by stable transfection with the catalytic subunit of human telomerase (hTERT) [59]. Replicate STR

**Table 3**  
Summary of STR profiles of normal immortalized endometrial epithelial cells.

Cell line	Amelogenin	CSF1PO	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	TH01	TPOX	vWA
hTERT-EEC-B37	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
hTERT-EEC-15	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
hTERT-EEC-17	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
hTERT-EEC-38	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
hTERT-EEC-49	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
MCF-7 (HTB-22) this report	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
MCF-7 NCI-60	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
MCF-7 ATCC (HTB-22)	X	10	NT	NT	11, 12	8, 9	NT	11	11, 12	NT	NT	NT	NT	6	9, 12	14, 15
HES	X	9, 10	17	15, 18	11, 12	8, 12	12, 13	13.3	9, 10	16	13, 14	27, 28	21	7	8, 12	16, 18
HeLa this report	X	9, 10	17	15, 18	11, 12	8, 12	12, 13	12, 13.3	9, 10	16	13, 14	27, 28	18, 21	7	8, 12	16, 18
HeLa ATCC (CCL-2)	X	9, 10	NT	NT	11, 12	8, 12	NT	12, 13.3	9, 10	NT	NT	NT	NT	7	8, 12	16, 18
HeLaS3 ATCC (CCL-2.2)	X	9, 10	NT	NT	11, 12	8, 12	NT	13.3	9, 10	NT	NT	NT	NT	7	8, 12	16, 18
WISH ATCC (CCL-25)	X	9, 10	NT	NT	11, 12	8, 12	NT	13.3	9, 10	NT	NT	NT	NT	7	8, 12	16, 18

Number of STRs at each of 16 surveyed loci. Numbers after decimal point indicate number of bases in an incomplete STR. Commas separate allele calls for multiple peaks. NT: not tested. Numbers following hTERT-ECC indicate clones. Samples were analyzed in duplicate independent reactions. MCF-7 breast cancer cells reference STR profiles from ATCC (HTB-22) and NCI-60 panel [25]. HeLa and WISH reference profiles from ATCC database.



profiling of the earliest available passages of multiple clonal lines indicated all isolates of hTERT-EEC cells to be genetically identical to MCF-7 breast cancer cells (Table 3). As for HES cells, this was not attributable to contamination as no other profiles were detected in the samples.

#### Analysis of ovarian cancer cell lines

We obtained and genotyped fifty-one ovarian cancer cell lines (Table S1), many of which are not available from public repositories. Two of the lines (IGROV1 and OVCAR-10) gave mixed genotypes indicating cross-contamination and were excluded from further analysis. The mixed genotype for IGROV1 was confirmed in multiple isolates including those obtained directly from the National Cancer Institute.

Several purported 'ovarian cancer' lines were genotypically identical to other known, non-ovarian, cancer cells: BG-1[64] was identified as MCF-7 breast cancer cells, and CH1, CH1cisR, and 222 as the teratocarcinoma line PA1. C13, A2008 and OV2008 were identical to the

ME-180 (ATCC: HTB-33) cervical cancer cell line, and confirmed to be HPV positive (Table 4). The genotypically distinct 2008 cell line [65], obtained directly from the originating laboratory of Dr. Peter Disaia [66], was HPV negative. Finally, SK-OV-4 and SK-OV-6 lines matched HPV-negative C-33A (HTB-31) cervical cancer cells (Table 4).

Two 'normal ovarian' cell lines, NOSE06 and NOSE07, were genotyped as the ovarian cancer line DOV-13. Similarly, Caov-2 was identical to the earlier NIH:OVCAR-2 line (Table S4) and some samples of COLO-720E were found to be COLO-704 (not shown). Ovary1847 cells were genotyped as NIH:OVCAR-8.

The remaining ovarian cancer cell lines exhibited unique, uncontaminated genotypes and are listed with their STR profiles in Table S4.

We noted disparate genotypes for several cell lines with similar names; 2008 cells are distinct from A2008 and OV2008, and 167 differs from OV167 cells. In contrast, the TOV-112D cell line is identical to TOV-21D, which appears to have arisen via transposition of numbers and letters in the name. Some isolates of TOV-112D were misidentified and matched TOV-21G cells.

**Table 4**  
STR profiles of cervical and other cancer cell lines misclassified as ovarian.

Cell line	Amelogenin	CSF1PO	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	TH01	TPOX	vWA	HPV
A2008	X	11	18	16	12	9, 10	14	11, 12	12, 13	12	13, 15.2	30, 31	23	8, 9.3	8, 10	15, 17	+
C13	X	11	18	16	12	9	14	11, 12	12, 13	12	15.2	30, 31	23	8, 9.3	8, 10	15, 17	NT
ME-180	X	11	18	16	12	9, 10	14	11, 12	12, 13	12	13, 15.2	30, 31	23	8, 9.3	8, 10	15, 17	NT
OV2008	X	11	18	16	12	9, 10	14	11, 12	12, 13	12	13, 15.2	30, 31	23	8, 9.3	8, 10	15, 17	+
<b>ME-180</b> <b>ATCC</b> <b>(HTB-33)</b>	<b>X</b>	<b>11</b>	<b>NT</b>	<b>NT</b>	<b>12</b>	<b>9, 10</b>	<b>NT</b>	<b>11, 13</b>	<b>12, 13</b>	<b>NT</b>	<b>NT</b>	<b>NT</b>	<b>NT</b>	<b>8, 9.3</b>	<b>8, 10</b>	<b>15, 17</b>	<b>+</b>
SKOV4	X	12	23, 25	16	11, 12	10	10, 14	13	13, 14	15, (17), 18	11, 13	29, 31, 32	21, 26	7, 8	9	18, 20 (19)	NT
SKOV6	X	12	23, 25	16	11, 12	10	10, 14	13	13, 14	15, (17), 18	11, 13	29, 30, 31, 32	21, 26	7, 8	9	18, 20	NT
C-33 A	X	12	23, 25	16	11, 12	10	10, 14	13	13, 14	15, (17), 18	11, 13, 14	29, 30, 31	21, 26	7, 8	9	18, 20	NT
<b>C-33</b> <b>ATCC</b> <b>(HTB-31)</b>	<b>A X</b>	<b>12</b>	<b>NT</b>	<b>NT</b>	<b>11, 12</b>	<b>10</b>	<b>NT</b>	<b>13</b>	<b>13, 14</b>	<b>NT</b>	<b>NT</b>	<b>NT</b>	<b>NT</b>	<b>7, 8</b>	<b>9</b>	<b>18, 20</b>	<b>–</b>
BG-1	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 24, 25	6	9, 12	14, 15	NT
<b>MCF-7</b> <b>NCI-60</b>	<b>X</b>	<b>10</b>	<b>21, 23</b>	<b>16</b>	<b>11, 12</b>	<b>8, 9</b>	<b>10, 14</b>	<b>11</b>	<b>11, 12</b>	<b>14</b>	<b>13, 14</b>	<b>30</b>	<b>23, 25</b>	<b>6</b>	<b>9, 12</b>	<b>14, 15</b>	<b>NT</b>
<b>MCF-7</b> <b>ATCC</b> <b>(HTB-22)</b>	<b>X</b>	<b>10</b>	<b>NT</b>	<b>NT</b>	<b>11, 12</b>	<b>8, 9</b>	<b>NT</b>	<b>11</b>	<b>11, 12</b>	<b>NT</b>	<b>NT</b>	<b>NT</b>	<b>NT</b>	<b>6</b>	<b>9, 12</b>	<b>14, 15</b>	<b>NT</b>
CH1	X	9, 12, 13	24	15	11	9	14, 15	9, 10	9, 12	15, 18	13	29, 31.2	24	7, 9	11	15, 17	NT
CH1-cisR	X	9, 13	24	15	11	9	14, 15	9, 10	9, 12	15, 18	13	29, 31.2	24	7, 9	11	15, 17	NT
222	X	9, 13	24	15	11	9	14, 15	9, 10	9, 12	15, 18	13	29, 31.2	24	7, 9	11	15, 17	NT
<b>PA-1</b> <b>JCRB</b> <b>(9061)</b>	<b>X</b>	<b>9, 12</b>	<b>NT</b>	<b>NT</b>	<b>11</b>	<b>9</b>	<b>NT</b>	<b>9, 10</b>	<b>9, 12</b>	<b>NT</b>	<b>NT</b>	<b>NT</b>	<b>NT</b>	<b>7, 9</b>	<b>11</b>	<b>15, 17</b>	<b>NT</b>
NOSE06	X	8, 10	20, 24	14, 16	11	10	14	11	10, 13	12, 16	13, 14	32.2, 33.2	21, 24	6, 9.3	6, 8	19	NT
NOSE07	X	8, 10	20, 24	14, 16	11	10	14	11	10, 13	12, 16	13, 14	32.2, 33.2	21, 24	6, 9.3	6, 8	19	NT
DOV-13	X	8, 10	20, 24	14, 16	11	10	14	11	10, 13	12, 16	13, 14	32.2, 33.2	21, 24	6, 9.3	6, 8	19	NT

Number of STRs at each of 16 surveyed loci. Numbers after decimal point indicate number of bases in an incomplete STR. Commas separate allele calls for multiple peaks. Alleles in parentheses indicate low amplitude peaks suggesting only a minor fraction of the cells in the population carry that allele. NT: allele not tested. ATCC is a reference DNA profile from the American Type Culture Collection. HPV: human papilloma virus status (+: positive; -: negative). MCF-7 breast cancer cells reference STR profiles from ATCC (HTB-22) and NCI-60 panel.

The heterogeneity of ovarian tumor cells in ascitic fluid has previously leads to the establishment of several cell lines with different phenotypic characteristics [67]. We profiled very early passages of OV429 and OV433 [68,69] and found identical genotypes, indicative of either a common patient origin or early cross-contamination (Table S4). Of historical note, OV433 was the cell line used originally to select for reactivity to the OC125 monoclonal antibody to the ovarian tumor marker CA125.

The cluster of PEO1/PEO4/PEO6 cells is known to originate from the same patient [70], and genotype accordingly. Similarly, HEY/HEYA8/HEY2 cells [71] are derived from the same original line, and share identical genotypes (Table S4).

#### Chemotherapy resistant derivatives mirror parental cell line genotypes

We tested five original and cisplatin-resistant paired cell lines and all five parent and derivative combinations were confirmed by genotyping. However, as shown earlier (Table 4), the OV2008/C13 cells are cervical, not ovarian cancer cells and the CH1/CH1cisR lines [72] are PA1 teratocarcinoma cells. Table S5 shows STR profiles of the matched cisplatin-sensitive/-resistant ovarian cancer cell lines. The 41M/41McisR, TYKnu/TYKnuR and A2780/A2780cisR pairs each have unique profiles. The paired lines demonstrate some genetic instability, consistent with cisplatin-induced MSI [73]. Cisplatin-resistant A2780 cells have lost alleles at the D3S1358, FGA, D8S1179, D5S818, D7S820, CSF1PO, and D2S1338 loci, and gained an allele at the D18S51 locus. The 41M/41McisR pair is more stable, with the cisplatin-resistant line differing only at the vWA locus. The original derivation of the 41M cisplatin-resistant lines lists three isolates (41McisR2, 41McisR4 and 41McisR6), which differed in their IC<sub>50</sub> [74]. The subline profiled herein is unknown, as the identifying number has been lost.

#### Discussion

Gynecologic cancer research is critically dependent on the use of cell culture models, to investigate molecular mechanisms underlying the development and progression of tumors, to design and test novel therapeutic strategies, and to identify potential diagnostic or prognostic markers. In this report, we profiled the most widely used endometrial and ovarian cell lines and discovered several examples of misidentification, redundancy and cross-contamination.

Genotyping and HPV testing of ovarian cancer cell lines identified eight (BG-1 [64], CH1/CH1cisR [72], 222 [75], C13 [76], A2008 [77,78], OV2008, SKOV-4 and SKOV-6 [79]) as previously existing, breast cancer, teratocarcinoma or cervical cancer cell lines. In addition, two 'normal ovarian' cell lines, NOSE06 and NOSE07 [80], were genotyped as the ovarian cancer line DOV-13 [81]. We also highlight the possibility for confusion of several ovarian cancer cell lines with similar names, but distinct genotypes; e.g. 167 and OV167, 2008 and A2008/OV2008.

We profiled a number of variants of Ishikawa endometrial cancer cells. Results are consistent with a common origin for these sublines, with variations and polymorphisms in some STR loci attributable to genetic instability, mismatch repair defects, and high passage number [75–77]. Analyses of mutations in the p53 gene (TP53) are consistent with previous reports [31,57] and provide additional genetic markers to perhaps distinguish the original, 3-H-4 and 3-H-12 Ishikawa lines. Furthermore, STR profiling, TP53 sequencing, and MSI analysis confirm that currently available isolates of ECC-1 cells are not authentic but are identical to Ishikawa cells, specifically the 3-H-12 line. This conclusion is reinforced by evidence that the EnCa-101 tumor, from which the original ECC-1 line was purportedly derived [42,55], is genetically distinct from both Ishikawa and ECC-1 cells. We also observed several ECC-1 isolates to be misidentified MCF-7 cells or a cross-contaminated mixture of Ishikawa and MCF-7 lines.

ECC-1 cells were initially characterized as distinct from Ishikawa lines based on differential expression of cytokeratin 13 and osteopontin

[33]. However, both markers were present in the two lines, which otherwise showed identical patterns of expression of steroid hormone receptors and their coactivators [33]. The karyotypes of Ishikawa and ECC-1 cells also exhibit some apparent differences [31,33], but chromosomal number and structural rearrangements in both lines were complex with high intercellular variability [31,33]. Comparative cytogenetic analysis found that, given the evident heterogeneity and differential capabilities of the techniques used (FISH or SKY) to detect abnormalities in small chromosomal segments, the karyotypic similarity was likely underestimated, and is consistent with the two lines sharing a common origin.

Thus, we conclude that the original ECC-1 cell line has been lost, although the persistence of the EnCa-101 tumor [56] provides an opportunity for its re-derivation. ECC-1 cells have been extensively used as models of ER positive, type 1, endometrial cancers. Since Ishikawa cells are also representative of such endometrioid tumors, our evidence that the two lines are identical may not significantly impact conclusions drawn from these studies, beyond the use of two redundant cell lines. However, the possible misidentification of MCF-7 breast cancer cells as ECC-1, or cross contamination with the former, should be considered in interpreting results using ECC-1 cells.

We identified the normal endometrial epithelial cell line (HES) as HeLa cervical carcinoma cells. HES cells have been used as a model of benign endometrial epithelium to study mucosal immunity [82], implantation [83,84], decidualization [85] and endometriosis [86], and have served as 'normal' controls for novel chemotherapeutics [87,88] and analysis of signaling pathways in the endometrium [89–93]. Similarly, the telomerase immortalized endometrial epithelial cell line, hTERT-EEC [59], was an exact genotypic match to MCF-7 breast cancer cells. hTERT-EEC has been proposed as model to study steroids in normal endometrial physiology, including, endometriosis and implantation [59,94,95]. Clearly, conclusions derived from studies utilizing HES cells (HeLa) or hTERT-EEC (MCF-7) should be interpreted with caution, in the light of evidence that they are neither normal nor endometrial in origin.

Cell line authentication is essential for their meaningful use in research. We recommend that cell lines be quarantined and authenticated by DNA profiling prior to use, and periodically evaluated by STR genotype, to check for cross-contamination and validate construction of stably transfected, genetically modified or clonally selected variants. Derivation of novel cell lines should be accompanied, where possible, by STR profiles of the patient germ line, tumor or tissue, and cell line DNA. We also suggest the use of histological or phenotypic markers to verify the tissue of origin, since STR profiling cannot provide this information resulting in debate as to the tissue type of some cancer cell lines [2,96].

The origins and mechanisms of cell line contamination, including poor tissue culture technique, inadequate quality control, clerical and labeling errors, and aerosol transfer of cells, have been reviewed previously [63] and, despite best laboratory practices, are probably unavoidable. Accordingly, even among cell lines that exhibited unique profiles, we found examples, from all sources, of individual aliquots that were misidentified or contaminated, indicating a widespread and pervasive problem. STR profiling is a simple, widely available and relatively inexpensive method to document and authenticate cell lines, and has been recommended as an internationally accepted standard for human cells [22,63,97,98]. Despite repeated calls for journals to require DNA profiling of cells for publication, this practice has not been widely adopted [63,99]. Complacency and denial of the existence and extent of the problem with validation and authenticity of cell lines, while prevalent [7,24,63,99], are antithetical to the conduct of responsible research in gynecologic oncology.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ygyno.2012.06.017>.

#### Conflict of interest statement

No conflict of interest.

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Presentation Abstract

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Presentation Title: Aggregation rather than monoclonal expansion explains ovarian cancer spheroid formation

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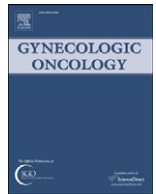
Author Block: Zhiqing Huang, William J. Lowery, Andrew Berchuck, Susan K. Murphy. Duke Univ. Medical Ctr., Durham, NC

Abstract Body: Purpose: It is thought that a subpopulation of ovarian cancer cells possess stem cell like properties and are responsible for both the emergence of cancer and for subsequent chemoresistance and recurrence. A common approach used to enrich for cancer stem cells in vitro involves culture in selective media on low attachment plates resulting in non-attached multicellular spheres. Spheroids are present in ascites fluid of women with ovarian cancer, and may contain stem cells responsible for metastasis and recurrence. Our objective was to address whether spheroids are truly monoclonal in origin and arise from expansion of a single common progenitor cell.

Procedures: We cultivated 5 ovarian cancer cell lines (DOV13, HEYC2, OVCAR2, OVCAR3, and PEO4) to confluence in monolayer cultures. The cell lines were chosen for their ability to form spheroids as well as variation in expression of the stem cell marker CD133 (OVCAR2, OVCAR3 CD133+, others negative). The cells were trypsinized to form single cell suspensions and plated at densities ranging from 100 to 5000 cells per plate in stem cell-selective media in ultra-low attachment culture dishes. A Zeiss Axio Observer microscope was used for time-lapse photography of the plated cells at 5-minute intervals over a 24-hour period. In addition, single cell suspensions were subjected to flow activated cell sorting to plate one cell in each well of 96-well Costar ultra-low cluster plates to monitor clonal formation of spheroids.

Results: Cells were dissociated and plated under stem cell-selective conditions and 288 images of each cell line were recorded over 24 hours. The cells were found to begin to aggregate within two to four hours, regardless of cell numbers plated. By 24 hours post-plating, there was pronounced aggregation in 5/5 (100%) cell lines with formation of tightly compacted spheroid structures in 4/5 (80%) cell lines. Single cells in the 96-well plates showed no evidence of spheroid formation over a 14-day observation period.

Conclusion: We observed spheroid formation in both CD133 positive and negative ovarian cancer cell lines. Formation occurred due to cellular aggregation rather than clonal expansion from a single progenitor with stem cell properties. Although cellular aggregation may be relevant to ovarian cancer biology, these data suggest that spheroid formation should be viewed with caution if used as a proxy for monoclonal expansion of ovarian cancer stem cells.



# DNA profiling analysis of endometrial and ovarian cell lines reveals misidentification, redundancy and contamination

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## ABSTRACT

**Objectives.** Cell lines derived from human ovarian and endometrial cancers, and their immortalized non-malignant counterparts, are critical tools to investigate and characterize molecular mechanisms underlying gynecologic tumorigenesis, and facilitate development of novel therapeutics. To determine the extent of misidentification, contamination and redundancy, with evident consequences for the validity of research based upon these models, we undertook a systematic analysis and cataloging of endometrial and ovarian cell lines.

**Methods.** Profiling of cell lines by analysis of DNA microsatellite short tandem repeats (STR), p53 nucleotide polymorphisms and microsatellite instability was performed.

**Results.** Fifty-one ovarian cancer lines were profiled with ten found to be redundant and five (A2008, OV2008, C13, SK-OV-4 and SK-OV-6) identified as cervical cancer cells. Ten endometrial cell lines were analyzed, with RL-92, HEC-1A, HEC-1B, HEC-50, KLE, and AN3CA all exhibiting unique, uncontaminated STR profiles. Multiple variants of Ishikawa and ECC-1 endometrial cancer cell lines were genotyped and analyzed by sequencing of mutations in the p53 gene. The profile of ECC-1 cells did not match the EnCa-101 tumor, from which it was reportedly derived, and all ECC-1 isolates were genotyped as Ishikawa cells, MCF-7 breast cancer cells, or a combination thereof. Two normal, immortalized endometrial epithelial cell lines, HES cells and the hTERT-EEC line, were identified as HeLa cervical carcinoma and MCF-7 breast cancer cells, respectively.

**Conclusions.** Results demonstrate significant misidentification, duplication, and loss of integrity of endometrial and ovarian cancer cell lines. Authentication by STR DNA profiling is a simple and economical method to verify and validate studies undertaken with these models.

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## Introduction

Cell lines, immortalized from normal human tissues or derived from tumors, are widely used models to address molecular mechanisms underlying the physiology and pathology of the female reproductive tract, and to evaluate novel therapeutics or preventive strategies [1–3]. Verification of the provenance and integrity of such cell lines is clearly of paramount importance, but historically, has rarely been undertaken by investigators. The problem of cross-contamination, identified and characterized by examination of isozyme patterns, karyotyping, and cytogenetics, dates back to the establishment of the prototypical HeLa cell

line in culture in 1951 and remains a significant concern [4–7]. Over one-third (18–50%) of cell lines may be mixtures, misidentified or intra-species contaminants [2,8–15]. Furthermore, there are many examples of redundancy among reportedly unique cell lines, and instances of contamination during original derivations, such that the intended novel cell line was never established [5,10,16–19]. Thus, it is evident that authentication of cell line origins and integrity is crucial to validate results and conclusions obtained using these model systems.

Short tandem repeat (STR) profiling or ‘DNA fingerprinting’ identifies variants in tetranucleotide microsatellite loci on multiple human chromosomes and is the accepted international standard for genetic analysis of cell lines for authentication by comparison to established STR databases [20–24].

A comprehensive analysis of cell lines commonly used in the study of ovarian and endometrial cancer had not been undertaken, particularly with respect to those cell lines not obtained from established cell repositories. We used STR profiling, sequencing of p53 mutations, and

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human papilloma virus screening to examine cell lines of purported ovarian and endometrial origins. We observed examples of cross-contamination, misidentification of lines and/or tissue of origin, and redundancy among established cancer cells, and found evidence that immortalized normal endometrial epithelial cell lines are genetically identical to previously established cervical and breast cancer cells. We provide reference DNA profiles for women's cancer cell lines that are not currently in public cell banks and extend the number of loci for profiles currently available through central repositories.

## Materials and methods

### DNA isolation and STR profiling

Cell lines were grown in appropriate specific standard media. Genomic DNA was isolated from  $0.5$  to  $5 \times 10^6$  cells using a Zymo Research ZR genomic DNA II kit and quantified by gel electrophoresis and ethidium bromide staining by comparison to a DNA mass ladder. Multiplex PCR amplified products were generated using 1–2 ng of genomic DNA with an Applied Biosystems Identifier kit and ABI 3730 capillary sequencer as described [2,18]. STR loci were analyzed with Gene Mapper 4.0. Profiles were compared to published reports [22,25], consolidated (ATCC, DSMZ, JCRB and RIKEN) databases, and an in-house database, using a custom search algorithm designed to facilitate comparison of cell lines with related profiles and identify individual cell lines in a mixture (C. Korch and J. West, Vanderbilt University, unpublished). STR profiles of the ovarian and endometrial cancer cells analyzed in this study are available online at <http://DNAsequencingcore.UCDenver.edu>.

### TP53 sequence analysis and microsatellite instability assays

PCR amplification was used to generate overlapping products spanning the Variable Number Tandem Repeat (VNTR; a pentanucleotide repeat of A<sub>4</sub>T) in intron 1, through the protein encoding exons 2–11, including intervening introns 2–8 and 10 [26]. Sequencing primers and p53 gene structure are shown in Fig. S1. DNAs were screened for microsatellite instability [27] using Promega MSI analysis system version 1.2 according to the manufacturers' protocol.

### HPV testing

Aliquots of cells were placed into ThinPrep (Hologic) solution. DNA was isolated and tested in the University of Colorado Hospital Clinical Laboratory using the hybrid capture PCR, Digene HC2 High Risk HPV test (Qiagen).

### Ovarian and endometrial cell lines

We obtained cell lines from multiple institutions in the United States, Europe and Japan, including, where possible, the originating laboratories. Multiple independent samples of the earliest available passages from each institution were analyzed and, if available, profiles of each individual cell line were compared from several sources. Ovarian cancer cell lines are listed in Table S1. Ishikawa cells were obtained from Dr. K.K. Leslie (University of Iowa), Dr. B.A. Lessey (Greenville Hospital System, SC), Dr. M. Brown (Dana Farber Cancer Institute, Harvard University) and Drs H. Philpott and P. Thraves (European Collection of Cell Cultures, ECACC). ECC-1 cells were from Drs. B.A. Lessey, M. Brown and V.C. Jordan (Lombardi Comprehensive Cancer Center, Georgetown University). EnCa-101 tumors were provided by Drs. V.C. Jordan and G. Balburski (Fox Chase Cancer Center). HES cells were from Dr. D. Kniss (Ohio State University) and hTERT-EECs from Dr. T. Klonisch (University of Manitoba, Canada). KLE and HEC-50 cells were from Dr. K.K. Leslie. RL-95-2, HEC-1A, HEC-1B and AN3CA cells were from the American Type Culture Collection (ATCC, Manassas, VA).

## Results

### Analysis of endometrial cancer cell lines

Endometrial carcinomas are derived from glandular epithelium and are typically divided into two subtypes based on clinical, histological and molecular characteristics [28–30]. Cell lines derived from type I (Ishikawa, ECC-1 and RL-95-2) and type II (HEC-1, HEC-50, KLE and AN3CA) tumors have been widely used as models to investigate molecular genetics and mechanisms underlying their development, progression and response to therapeutics [31–35].

HEC-1A and HEC-1B cell lines, the first to be derived from a human endometrial carcinoma [32,36,37], both exhibited a unique profile consistent with their derivation from the same patient (Table S3). HEC-1A cells are predominantly diploid, while the HEC-1B line is tetraploid [38,39]. HEC-50 cells [38,40], also have a unique profile consistent with that on file with the Japanese Collection of Research Bioresources (JCRB: 1145).

Similarly, KLE (CRL-1622) and AN3CA (HTB-111) cells, originating from peritoneal and lymph node metastases, respectively [34,41,42], and RL-95-2 cells (CRL-1671) derived from a moderately differentiated (Grade 2) endometrial adenosquamous carcinoma [35], all have STR profiles consistent with those reported by the ATCC (Table S3).

Ishikawa cells were established from the epithelial component of a moderately differentiated, stage 2, endometrial adenocarcinoma [43,44]. At least three variants of Ishikawa cells, the original line, 3-H-4 and 3-H-12, differing in their reported degree of differentiation, relative expression of estrogen (ER) and progesterone (PR) receptors, growth and colony formation rates, were distributed to investigators [45].

We profiled multiple isolates of the original Ishikawa cells and 3-H-12 variants obtained from a number of laboratories as detailed in the Materials and methods section. Samples with unique profiles, which may represent the 3-H-4 variant based upon their date of origin are designated '3-H-4'. The results are summarized in Table 1.

Overall the Ishikawa cell lines exhibit very similar profiles, indicative of their origin from the same patient. Identical alleles were present at several loci (CSF1PO, D5S818, D16S539, D21S11, TH01 and TPOX). Others reflect loss or gain of alleles (D8S1179, D13S317 and FGA) or alterations in the number of repeats (D2S1338, D3S1358, D19S433 and vWA). At the D7S820 locus, the original Ishikawa isolate exhibits 8.3- and 11-repeat alleles, while subsequent sublines display 9- or 10-repeats. The D18S51 locus was found to be highly polymorphic in most Ishikawa lines.

Minor differences in the number of repeats at certain loci are consistent with the known microsatellite instability (MSI) of these lines, due to mutations in mismatch repair systems [46–48], and suggest that these variants arose by genetic drift between different clonal isolates over hundreds of cell passages. Accordingly, all Ishikawa cell lines exhibited high variability/instability at microsatellite loci (Table S2). Defective mismatch repair also underlies allelic variation in AN3CA cells (Table S3) [49]. In contrast, EnCa-101 tumors and MCF-7 cells were MSI stable.

We also profiled a variant of Ishikawa cells lacking ER [50]. Previous reports implied that these cells, also known as Ishikawa B, were derived from a different patient [51,52]. The STR profile of ER-negative Ishikawa cells exhibits minor variations from other Ishikawa sublines (Table 1), but overlap at the majority of loci indicates a common origin.

A second type 1, ER and PR positive cell line, ECC-1, was established from a grade 2, well-differentiated, endometrial carcinoma adenocarcinoma [42,53,54]. The line was derived by passage of the tumor, designated EnCa-101, in nude mice and subsequent isolation of PR positive cells from an epithelial monolayer culture [42,55]. ECC-1 cells were described as a well-differentiated, steroid responsive line with a phenotype characteristic of luminal surface epithelium, distinct from Ishikawa cells, which expressed markers of glandular endometrial epithelium [33].



**Table 1**

Summary of STR profiles of Ishikawa and ECC-1 endometrial cancer cells and EnCa-101 tumor.

Cell line	Amelogenin	CSF1PO	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	TH01	TPOX	vWA
Ishikawa original	X	11, 12	18, 20	17, 18	10, 11	8, 3, 11	12, 16	9, 12	9	14, (19) 20, 21 polymorphic	12, 2, 14	28	21	9, 10	8	14, 18
Ishikawa '3-H-4'	X	11, 12	19, 20	16, 17	10, 11	9, 10	12, 16	9, 12	9	13, 21, 22	12, 2, 14	28	21, 22	9	8	14, 17
Ishikawa 3-H-12	X	11, 12, (13)	19, 20	16, 17	10, 11	9, 10	12, 13, 16	9, 12, 13	9	12, 19, 20	13, 2, 14	28	20, 21	9, 10	8	14, 17
Ishikawa 3-H-12	X	11, 12	20	16, 17, (18)	10, 11, (12)	9, 10	12, (13), 16	9, 12	9, (10)	13, 20	12, 2, 14, (15)	28	21	9, 10 or 11	8	14, 17 or 18
Ishikawa ER -ve	X	11, 13	20	16, 17	10, 11	9, 10	12, 13, 16	9, 12	8, 9	13, 19	12, 2, 14	28	20	9, 10	8	14, 17
Ishikawa, ECACC, this report	X	11, 12, (13)	20	15, 17	10, 11, 12	9, 10	12, 16	9, 12, 13	9	13, 19, (14, 20)	12, 2, 14	28	21	9, 10	8	14, 17
Ishikawa, ECACC	X	11, 12	NT	NT	10, 11	9, 10	NT	9, 12	9	NT	NT	NT	NT	9, 10	8	14, 17
ECC-1	X	11, 12	20	16, 17	10, 11	9, 10	13, 16	9, 12,	9	12, 19	12, 2, 14 or 15	28	21	9, 10	8	14, 17
ECC-1 ATCC CRL-2923	X	11, 12	NT	NT	10, 11	9, 10	NT	9, 12	9	NT	NT	NT	NT	9, 10	8	14, 17
EnCa-101	X	13, 14	23, 27	15, 21	14, 15	11, 3, 12	18, 21	10, 13	12, 13	16	13, 2, 14 or 15	27, 30	21	9, 9, 3	8	18, 23

Number of STRs at each of 16 surveyed loci. Numbers after decimal point indicate number of bases in an incomplete STR. Commas separate allele calls for multiple peaks. Alleles in parenthesis indicate low amplitude peaks suggesting only a minor fraction of the cells in the population carry that allele. ECACC: DNA profile from European Collection of Cell Cultures; ATCC: DNA profile from American Type Culture Collection. NT: locus not tested.

Upon STR and MSI analyses, ECC-1 samples exhibited DNA profiles essentially identical to Ishikawa 3-H-12 cells (Tables 1 and S2). In addition, the ATCC profile for ECC-1 also closely matched that of earlier Ishikawa cells on file with the European Collection of Cell Cultures (ECACC). Other 'ECC-1' cell lines were found to be identical to MCF-7 breast cancer cells or consist of a mixture of Ishikawa and MCF-7 cells (not shown). Unfortunately, following the death of Dr. Satyaswaroop, records and cell lines from his laboratory were lost or destroyed (Zaino, R. and Lessey, B., personal communication). Thus, we could not obtain reference samples of the original ECC-1 line or EnCa-101 tumor from which it was purportedly derived. However, the EnCa-101 tumor has been continuously maintained in mice [56] and we obtained and analyzed 3 independent samples. Profiling of these tumors showed minor variations, but results indicated that they were derived from the same human patient. In contrast, the unique EnCa-101 profiles did not match ECC-1, Ishikawa or MCF-7 cell lines (Table 1). These data are inconsistent with the reported origins of ECC-1 cells and suggest that the original line has been lost. Our results show that currently available ECC-1 cells are Ishikawa cells, MCF-7 breast cancer cells, or a mixture of both.

#### Sequencing of p53 mutations in endometrial cancer cells

To confirm the apparent equivalence of Ishikawa and ECC-1 cells, we screened for p53 mutations by PCR amplification and sequencing of the Variable Number Tandem Repeat (VNTR) region in intron 1, and the protein encoding exons and introns (Fig. S1). Table 2 lists the observed p53 mutations and SNPs compared to the reference/normal sequence.

In agreement with previous reports [31,57], Ishikawa original and 3-H-12 cells harbor a Met 246 Val mutation in exon 7. These two lines are also homozygous in the VNTR region with 8 repeats of A<sub>4</sub>T, heterozygous in exon 4 for the Asp 49 Val mutation (nucleotide G12069S), and heterozygous in intron 10 for deletion of the seventh T in a heptanucleotide repeat (17822delT). The original Ishikawa sample has two additional heterozygous mutations, 12724insA (intron 4) and 13764delA (intron 6), which are not present in the 3-H-12 line (Table 2).

Possible '3-H-4' sublines have a similar profile, but lack the intronic 12724insA and 13764delA mutations of poly A stretches, present in the original Ishikawa lines (Table 2). An additional heterozygous mutation in intron 4 (G12299K (G + T)) was detected in some Ishikawa 3-H-12 sublines. Interestingly, consistent with their closely matched STR profiles, the ER-negative Ishikawa cells, despite their purported distinct origin, exhibit TP53 mutations identical to Ishikawa 3-H-12 and '3-H-4' (not shown). TP53 mutations unique to the original Ishikawa lines are insertions or deletions in homopolymer A or T stretches, which are consistent with microsatellite instability due to mutations in the mismatch repair system [46].

In agreement with their identical STR profiles, ECC-1 cells show the same TP53 mutations as Ishikawa 3-H-12 lines, further evidence that ECC-1 cells are misidentified Ishikawa cells. In contrast, EnCa-101 tumors have completely different TP53 mutations from the Ishikawa and ECC-1 lines (Table 2), again demonstrating that ECC-1 cells are not derived from the EnCa-101 tumor. 'ECC-1' cells shown to be contaminated with or identical to MCF-7 cells were not subjected to TP53 analysis.

Finally, our data suggest that only one copy of the p53 gene is expressed in Ishikawa cells. In the genomic DNA, both the A14063R (A + G) and G12069S (G + C) positions are heterozygous. However, only the 14063G mutation is present in the cDNA sequence [31,57], suggesting that the G12069C mutation is in the unexpressed copy of the gene.

#### Analysis of normal endometrial epithelial cells

Immortalized, non-transformed endometrial epithelial cells are a potentially valuable resource to investigate normal uterine physiology and tumorigenesis. We profiled two such lines, human endometrial (HES) cells [58] and hTERT-EEC [59], obtained from their developers, which have been extensively used as models of normal endometrium. Neither cell line was authenticated as they exhibited DNA profiles corresponding to HeLa and MCF-7 cancer cells, respectively.

HES cells were established, in 1989, from a primary culture of benign proliferative endometrium, which apparently underwent spontaneous transformation after serial passage [58,60]. Profiling of these cells



**Table 2**  
Summary of TP53 mutations and single nucleotide polymorphisms (SNPs).

TP53 reference sequence	Ishikawa original	Ishikawa '3-H-4'	Ishikawa 3-H-12	Ishikawa 3-H-12	ECC-1	EnCa-101 tumor
Intron 1: VNTR A <sub>4</sub> T repeats	Homozygous 8 repeats	Homozygous 8 repeats	Homozygous 8 repeats	Homozygous 8 repeats	Homozygous 8 repeats	Heterozygous 7 and 9 repeats
Exon 4: G12069 Asp 49	Heterozygous G12069S Asp49His	Heterozygous G12069S Asp49His	Heterozygous G12069S Asp49His	Heterozygous G12069S Asp49His	Heterozygous G12069S Asp49His	
Intron 4: G12299			Heterozygous G12299K			
Intron 4: Poly A <sub>7</sub> 12718–12724	Heterozygous 12724insA Poly A <sub>7</sub> /A <sub>8</sub>					
Intron 5: G12786						Homozygous G12786T SNP
Intron 5: C13253						Heterozygous C13253Y SNP
Intron 6: G13642						Heterozygous G13642K SNP
Intron 6: Poly A <sub>9</sub> 13756–13764	Heterozygous 13764delA Poly A <sub>9</sub> /A <sub>8</sub>					
Exon 7: A14063 Met246	Heterozygous A14063R Met246Val	Heterozygous A14063R Met246Val	Heterozygous A14063R Met246Val	Heterozygous A14063R Met246Val	Heterozygous A14063R Met246Val	
Intron 10: Poly T <sub>7</sub> 17816–17822	Heterozygous 17822delT Poly T <sub>7</sub> /T <sub>6</sub>	Heterozygous 17822delT Poly T <sub>7</sub> /T <sub>6</sub>	Heterozygous 17822delT Poly T <sub>7</sub> /T <sub>6</sub>	Heterozygous 17822delT Poly T <sub>7</sub> /T <sub>6</sub>	Heterozygous 17822delT Poly T <sub>7</sub> /T <sub>6</sub>	Homozygous/Hemizygous Poly T <sub>7</sub>

Tumor protein p53 (TP53) genomic DNA, from multiple independent samples of each cell line, was sequenced as described in the [Materials and methods](#) section. The normal reference normal is GenBank HSP53G, a.k.a. [X54156](#), which is used by the International Agency for Research on Cancer IARC (<http://www-p53.iarc.fr>). A blank cell in the table indicates the DNA sequence that matches the reference/normal sequence. VNTR: Variable Number Tandem Repeat. Symbols — K: G and T; R: A and G; S: G and C; Y: C and T; del: nucleotide deletion; ins: nucleotide insertion.

([Table 3](#)) indicated that they are identical at all loci to HeLa cervical carcinoma cells, specifically the HeLaS3 variant. HES cells are also identical to WISH cells, a cell line originally described as derived from human amnion [61] but subsequently also identified as HeLa [7,62,63]. These results were independently confirmed by the STR fragment analysis

facility at Johns Hopkins University (D. Kniss, Ohio State University; personal communication).

hTERT-EECs were isolated from normal proliferative phase endometrial epithelium and immortalized by stable transfection with the catalytic subunit of human telomerase (hTERT) [59]. Replicate STR

**Table 3**  
Summary of STR profiles of normal immortalized endometrial epithelial cells.

Cell line	Amelogenin	CSF1PO	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	TH01	TPOX	vWA
hTERT-EEC-B37	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
hTERT-EEC-15	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
hTERT-EEC-17	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
hTERT-EEC-38	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
hTERT-EEC-49	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
MCF-7 (HTB-22) this report	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
MCF-7 NCI-60	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
MCF-7 ATCC (HTB-22)	X	10	NT	NT	11, 12	8, 9	NT	11	11, 12	NT	NT	NT	NT	6	9, 12	14, 15
HES	X	9, 10	17	15, 18	11, 12	8, 12	12, 13	13.3	9, 10	16	13, 14	27, 28	21	7	8, 12	16, 18
HeLa this report	X	9, 10	17	15, 18	11, 12	8, 12	12, 13	12, 13.3	9, 10	16	13, 14	27, 28	18, 21	7	8, 12	16, 18
HeLa ATCC (CCL-2)	X	9, 10	NT	NT	11, 12	8, 12	NT	12, 13.3	9, 10	NT	NT	NT	NT	7	8, 12	16, 18
HeLaS3 ATCC (CCL-2.2)	X	9, 10	NT	NT	11, 12	8, 12	NT	13.3	9, 10	NT	NT	NT	NT	7	8, 12	16, 18
WISH ATCC (CCL-25)	X	9, 10	NT	NT	11, 12	8, 12	NT	13.3	9, 10	NT	NT	NT	NT	7	8, 12	16, 18

Number of STRs at each of 16 surveyed loci. Numbers after decimal point indicate number of bases in an incomplete STR. Commas separate allele calls for multiple peaks. NT: not tested. Numbers following hTERT-ECC indicate clones. Samples were analyzed in duplicate independent reactions. MCF-7 breast cancer cells reference STR profiles from ATCC (HTB-22) and NCI-60 panel [25]. HeLa and WISH reference profiles from ATCC database.

profiling of the earliest available passages of multiple clonal lines indicated all isolates of hTERT-EEC cells to be genetically identical to MCF-7 breast cancer cells (Table 3). As for HES cells, this was not attributable to contamination as no other profiles were detected in the samples.

#### Analysis of ovarian cancer cell lines

We obtained and genotyped fifty-one ovarian cancer cell lines (Table S1), many of which are not available from public repositories. Two of the lines (IGROV1 and OVCAR-10) gave mixed genotypes indicating cross-contamination and were excluded from further analysis. The mixed genotype for IGROV1 was confirmed in multiple isolates including those obtained directly from the National Cancer Institute.

Several purported 'ovarian cancer' lines were genotypically identical to other known, non-ovarian, cancer cells: BG-1[64] was identified as MCF-7 breast cancer cells, and CH1, CH1cisR, and 222 as the teratocarcinoma line PA1. C13, A2008 and OV2008 were identical to the

ME-180 (ATCC: HTB-33) cervical cancer cell line, and confirmed to be HPV positive (Table 4). The genotypically distinct 2008 cell line [65], obtained directly from the originating laboratory of Dr. Peter Disaia [66], was HPV negative. Finally, SK-OV-4 and SK-OV-6 lines matched HPV-negative C-33A (HTB-31) cervical cancer cells (Table 4).

Two 'normal ovarian' cell lines, NOSE06 and NOSE07, were genotyped as the ovarian cancer line DOV-13. Similarly, Caov-2 was identical to the earlier NIH:OVCAR-2 line (Table S4) and some samples of COLO-720E were found to be COLO-704 (not shown). Ovary1847 cells were genotyped as NIH:OVCAR-8.

The remaining ovarian cancer cell lines exhibited unique, uncontaminated genotypes and are listed with their STR profiles in Table S4.

We noted disparate genotypes for several cell lines with similar names; 2008 cells are distinct from A2008 and OV2008, and 167 differs from OV167 cells. In contrast, the TOV-112D cell line is identical to TOV-21D, which appears to have arisen via transposition of numbers and letters in the name. Some isolates of TOV-112D were misidentified and matched TOV-21G cells.

**Table 4**  
STR profiles of cervical and other cancer cell lines misclassified as ovarian.

Cell line	Amelogenin	CSF1PO	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	TH01	TPOX	vWA	HPV
A2008	X	11	18	16	12	9, 10	14	11, 12	12, 13	12	13, 15.2	30, 31	23	8, 9.3	8, 10	15, 17	+
C13	X	11	18	16	12	9	14	11, 12	12, 13	12	15.2	30, 31	23	8, 9.3	8, 10	15, 17	NT
ME-180	X	11	18	16	12	9, 10	14	11, 12	12, 13	12	13, 15.2	30, 31	23	8, 9.3	8, 10	15, 17	NT
OV2008	X	11	18	16	12	9, 10	14	11, 12	12, 13	12	13, 15.2	30, 31	23	8, 9.3	8, 10	15, 17	+
<b>ME-180</b> <b>ATCC</b> <b>(HTB-33)</b>	<b>X</b>	<b>11</b>	<b>NT</b>	<b>NT</b>	<b>12</b>	<b>9, 10</b>	<b>NT</b>	<b>11, 13</b>	<b>12, 13</b>	<b>NT</b>	<b>NT</b>	<b>NT</b>	<b>NT</b>	<b>8, 9.3</b>	<b>8, 10</b>	<b>15, 17</b>	<b>+</b>
SKOV4	X	12	23, 25	16	11, 12	10	10, 14	13	13, 14	15, (17), 18	11, 13	29, 31, 32	21, 26	7, 8	9	18, 20 (19)	NT
SKOV6	X	12	23, 25	16	11, 12	10	10, 14	13	13, 14	15, (17), 18	11, 13	29, 30, 31, 32	21, 26	7, 8	9	18, 20	NT
C-33 A	X	12	23, 25	16	11, 12	10	10, 14	13	13, 14	15, (17), 18	11, 13, 14	29, 30, 31	21, 26	7, 8	9	18, 20	NT
<b>C-33</b> <b>ATCC</b> <b>(HTB-31)</b>	<b>A X</b>	<b>12</b>	<b>NT</b>	<b>NT</b>	<b>11, 12</b>	<b>10</b>	<b>NT</b>	<b>13</b>	<b>13, 14</b>	<b>NT</b>	<b>NT</b>	<b>NT</b>	<b>NT</b>	<b>7, 8</b>	<b>9</b>	<b>18, 20</b>	<b>–</b>
BG-1	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 24, 25	6	9, 12	14, 15	NT
<b>MCF-7</b> <b>NCI-60</b>	<b>X</b>	<b>10</b>	<b>21, 23</b>	<b>16</b>	<b>11, 12</b>	<b>8, 9</b>	<b>10, 14</b>	<b>11</b>	<b>11, 12</b>	<b>14</b>	<b>13, 14</b>	<b>30</b>	<b>23, 25</b>	<b>6</b>	<b>9, 12</b>	<b>14, 15</b>	<b>NT</b>
<b>MCF-7</b> <b>ATCC</b> <b>(HTB-22)</b>	<b>X</b>	<b>10</b>	<b>NT</b>	<b>NT</b>	<b>11, 12</b>	<b>8, 9</b>	<b>NT</b>	<b>11</b>	<b>11, 12</b>	<b>NT</b>	<b>NT</b>	<b>NT</b>	<b>NT</b>	<b>6</b>	<b>9, 12</b>	<b>14, 15</b>	<b>NT</b>
CH1	X	9, 12, 13	24	15	11	9	14, 15	9, 10	9, 12	15, 18	13	29, 31.2	24	7, 9	11	15, 17	NT
CH1-cisR	X	9, 13	24	15	11	9	14, 15	9, 10	9, 12	15, 18	13	29, 31.2	24	7, 9	11	15, 17	NT
222	X	9, 13	24	15	11	9	14, 15	9, 10	9, 12	15, 18	13	29, 31.2	24	7, 9	11	15, 17	NT
<b>PA-1</b> <b>JCRB</b> <b>(9061)</b>	<b>X</b>	<b>9, 12</b>	<b>NT</b>	<b>NT</b>	<b>11</b>	<b>9</b>	<b>NT</b>	<b>9, 10</b>	<b>9, 12</b>	<b>NT</b>	<b>NT</b>	<b>NT</b>	<b>NT</b>	<b>7, 9</b>	<b>11</b>	<b>15, 17</b>	<b>NT</b>
NOSE06	X	8, 10	20, 24	14, 16	11	10	14	11	10, 13	12, 16	13, 14	32.2, 33.2	21, 24	6, 9.3	6, 8	19	NT
NOSE07	X	8, 10	20, 24	14, 16	11	10	14	11	10, 13	12, 16	13, 14	32.2, 33.2	21, 24	6, 9.3	6, 8	19	NT
DOV-13	X	8, 10	20, 24	14, 16	11	10	14	11	10, 13	12, 16	13, 14	32.2, 33.2	21, 24	6, 9.3	6, 8	19	NT

Number of STRs at each of 16 surveyed loci. Numbers after decimal point indicate number of bases in an incomplete STR. Commas separate allele calls for multiple peaks. Alleles in parentheses indicate low amplitude peaks suggesting only a minor fraction of the cells in the population carry that allele. NT: allele not tested. ATCC is a reference DNA profile from the American Type Culture Collection. HPV: human papilloma virus status (+: positive; -: negative). MCF-7 breast cancer cells reference STR profiles from ATCC (HTB-22) and NCI-60 panel.

The heterogeneity of ovarian tumor cells in ascitic fluid has previously leads to the establishment of several cell lines with different phenotypic characteristics [67]. We profiled very early passages of OV429 and OV433 [68,69] and found identical genotypes, indicative of either a common patient origin or early cross-contamination (Table S4). Of historical note, OV433 was the cell line used originally to select for reactivity to the OC125 monoclonal antibody to the ovarian tumor marker CA125.

The cluster of PEO1/PEO4/PEO6 cells is known to originate from the same patient [70], and genotype accordingly. Similarly, HEY/HEYA8/HEY2 cells [71] are derived from the same original line, and share identical genotypes (Table S4).

#### Chemotherapy resistant derivatives mirror parental cell line genotypes

We tested five original and cisplatin-resistant paired cell lines and all five parent and derivative combinations were confirmed by genotyping. However, as shown earlier (Table 4), the OV2008/C13 cells are cervical, not ovarian cancer cells and the CH1/CH1cisR lines [72] are PA1 teratocarcinoma cells. Table S5 shows STR profiles of the matched cisplatin-sensitive/-resistant ovarian cancer cell lines. The 41M/41McisR, TYKnu/TYKnuR and A2780/A2780cisR pairs each have unique profiles. The paired lines demonstrate some genetic instability, consistent with cisplatin-induced MSI [73]. Cisplatin-resistant A2780 cells have lost alleles at the D3S1358, FGA, D8S1179, D5S818, D7S820, CSF1PO, and D2S1338 loci, and gained an allele at the D18S51 locus. The 41M/41McisR pair is more stable, with the cisplatin-resistant line differing only at the vWA locus. The original derivation of the 41M cisplatin-resistant lines lists three isolates (41McisR2, 41McisR4 and 41McisR6), which differed in their IC<sub>50</sub> [74]. The subline profiled herein is unknown, as the identifying number has been lost.

#### Discussion

Gynecologic cancer research is critically dependent on the use of cell culture models, to investigate molecular mechanisms underlying the development and progression of tumors, to design and test novel therapeutic strategies, and to identify potential diagnostic or prognostic markers. In this report, we profiled the most widely used endometrial and ovarian cell lines and discovered several examples of misidentification, redundancy and cross-contamination.

Genotyping and HPV testing of ovarian cancer cell lines identified eight (BG-1 [64], CH1/CH1cisR [72], 222 [75], C13 [76], A2008 [77,78], OV2008, SKOV-4 and SKOV-6 [79]) as previously existing, breast cancer, teratocarcinoma or cervical cancer cell lines. In addition, two 'normal ovarian' cell lines, NOSE06 and NOSE07 [80], were genotyped as the ovarian cancer line DOV-13 [81]. We also highlight the possibility for confusion of several ovarian cancer cell lines with similar names, but distinct genotypes; e.g. 167 and OV167, 2008 and A2008/OV2008.

We profiled a number of variants of Ishikawa endometrial cancer cells. Results are consistent with a common origin for these sublines, with variations and polymorphisms in some STR loci attributable to genetic instability, mismatch repair defects, and high passage number [75–77]. Analyses of mutations in the p53 gene (TP53) are consistent with previous reports [31,57] and provide additional genetic markers to perhaps distinguish the original, 3-H-4 and 3-H-12 Ishikawa lines. Furthermore, STR profiling, TP53 sequencing, and MSI analysis confirm that currently available isolates of ECC-1 cells are not authentic but are identical to Ishikawa cells, specifically the 3-H-12 line. This conclusion is reinforced by evidence that the EnCa-101 tumor, from which the original ECC-1 line was purportedly derived [42,55], is genetically distinct from both Ishikawa and ECC-1 cells. We also observed several ECC-1 isolates to be misidentified MCF-7 cells or a cross-contaminated mixture of Ishikawa and MCF-7 lines.

ECC-1 cells were initially characterized as distinct from Ishikawa lines based on differential expression of cytokeratin 13 and osteopontin

[33]. However, both markers were present in the two lines, which otherwise showed identical patterns of expression of steroid hormone receptors and their coactivators [33]. The karyotypes of Ishikawa and ECC-1 cells also exhibit some apparent differences [31,33], but chromosomal number and structural rearrangements in both lines were complex with high intercellular variability [31,33]. Comparative cytogenetic analysis found that, given the evident heterogeneity and differential capabilities of the techniques used (FISH or SKY) to detect abnormalities in small chromosomal segments, the karyotypic similarity was likely underestimated, and is consistent with the two lines sharing a common origin.

Thus, we conclude that the original ECC-1 cell line has been lost, although the persistence of the EnCa-101 tumor [56] provides an opportunity for its re-derivation. ECC-1 cells have been extensively used as models of ER positive, type 1, endometrial cancers. Since Ishikawa cells are also representative of such endometrioid tumors, our evidence that the two lines are identical may not significantly impact conclusions drawn from these studies, beyond the use of two redundant cell lines. However, the possible misidentification of MCF-7 breast cancer cells as ECC-1, or cross contamination with the former, should be considered in interpreting results using ECC-1 cells.

We identified the normal endometrial epithelial cell line (HES) as HeLa cervical carcinoma cells. HES cells have been used as a model of benign endometrial epithelium to study mucosal immunity [82], implantation [83,84], decidualization [85] and endometriosis [86], and have served as 'normal' controls for novel chemotherapeutics [87,88] and analysis of signaling pathways in the endometrium [89–93]. Similarly, the telomerase immortalized endometrial epithelial cell line, hTERT-EEC [59], was an exact genotypic match to MCF-7 breast cancer cells. hTERT-EEC has been proposed as model to study steroids in normal endometrial physiology, including, endometriosis and implantation [59,94,95]. Clearly, conclusions derived from studies utilizing HES cells (HeLa) or hTERT-EEC (MCF-7) should be interpreted with caution, in the light of evidence that they are neither normal nor endometrial in origin.

Cell line authentication is essential for their meaningful use in research. We recommend that cell lines be quarantined and authenticated by DNA profiling prior to use, and periodically evaluated by STR genotype, to check for cross-contamination and validate construction of stably transfected, genetically modified or clonally selected variants. Derivation of novel cell lines should be accompanied, where possible, by STR profiles of the patient germ line, tumor or tissue, and cell line DNA. We also suggest the use of histological or phenotypic markers to verify the tissue of origin, since STR profiling cannot provide this information resulting in debate as to the tissue type of some cancer cell lines [2,96].

The origins and mechanisms of cell line contamination, including poor tissue culture technique, inadequate quality control, clerical and labeling errors, and aerosol transfer of cells, have been reviewed previously [63] and, despite best laboratory practices, are probably unavoidable. Accordingly, even among cell lines that exhibited unique profiles, we found examples, from all sources, of individual aliquots that were misidentified or contaminated, indicating a widespread and pervasive problem. STR profiling is a simple, widely available and relatively inexpensive method to document and authenticate cell lines, and has been recommended as an internationally accepted standard for human cells [22,63,97,98]. Despite repeated calls for journals to require DNA profiling of cells for publication, this practice has not been widely adopted [63,99]. Complacency and denial of the existence and extent of the problem with validation and authenticity of cell lines, while prevalent [7,24,63,99], are antithetical to the conduct of responsible research in gynecologic oncology.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ygyno.2012.06.017>.

#### Conflict of interest statement

No conflict of interest.

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