# Circulating Tumor Cells In Advanced Cervical Cancer: NRG Oncology—Gynecologic Oncology Group Study 240 (NCT 00803062)

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## ABSTRACT

To isolate circulating tumor cells (CTC) from women with advanced cervical cancer and estimate the impact of CTCs and treatment on overall survival and progression-free survival (PFS). A total of 7.5 mL of whole blood was drawn pre-cycle 1 and 36 days post-cycle 1 from patients enrolled on Gynecologic Oncology Group 0240, the phase III randomized trial that led directly to regulatory approval of the antiangiogenesis drug, bevacizumab, in women with recurrent/metastatic cervical cancer. CTCs (defined as anti-cytokeratin$^{+}$/anti-CD45$^{-}$ cells) were isolated from the buffy coat layer using an anti-EpCAM antibody-conjugated ferrofluid and rare earth magnet, and counted using a semiautomated fluorescence microscope. The median pre-cycle 1 CTC count was 7 CTCs/7.5 mL whole blood (range, 0–18) and, at 36 days posttreatment, was 4 (range, 0–17). The greater the declination in CTCs between time points studied, the lower the risk of death [HR, 0.87; 95% confidence interval (CI), 0.79–0.95)]. Among patients with high (≥ median) pretreatment CTCs, bevacizumab treatment was associated with a reduction in the hazard of death (HR, 0.57; 95% CI, 0.32–1.03) and PFS (HR, 0.59; 95% CI, 0.36–0.96). This effect was not observed with low (< median) CTCs. CTCs can be isolated from women with advanced cervical cancer and may have prognostic significance. A survival benefit conferred by bevacizumab among patients with high pretreatment CTCs may reflect increased tumor neovascularization and concomitant vulnerability to VEGF inhibition. These data support studying CTC capture as a potential predictive biomarker.

## Introduction

Infection by high-risk subtypes of human papillomavirus (HPV) and their malignant sequelae continue to represent a global epidemic. Approximately 500,000 women are diagnosed each year with invasive cervical cancer, with over one-half dying annually. The disease burden is predominantly felt in impoverished nations of Central and South America, sub-Saharan Africa, and Southeast Asia including India (1). In developed countries, cytologic screening with or without oncogenic HPV DNA testing has led to dramatic reductions in both incidence and mortality rates (1). In the United States, there are expected to be 13,800 new cases and 4,290 deaths in 2020 (2). Should HPV vaccination campaigns lead to widespread adoption, the incidence will decline further. Patients with early-stage disease (up to 2018 FIGO stage $IB_2$) are often cured with either fertility-preserving surgery or radical hysterectomy with lymphadenectomy and tailored adjuvant therapy, while those diagnosed with locally advanced cancers (2018 FIGO $IB_3$-IVA) may be salvaged with chemoradiation and high-dose-rate intracavitary brachytherapy (3).

For years, patients with recurrent/persistent cervical cancer not amenable to pelvic exenterative procedures and those who presented with metastatic disease (i.e., 2018 FIGO IVB) have constituted a high, unmet clinical need (1, 4–6). Some progress was made when, in February 2013, the NCI Data Safety and Monitoring Board stopped the Gynecologic Oncology Group (GOG) 240 phase III randomized clinical trial when it was determined that the arms administering antiangiogenesis therapy were associated with a significant improvement in overall survival (OS); (median 17.0 vs. 13.3 months; HR, 0.71; 97% CI, 0.54–0.94; $P = 0.0035$), progression-free survival (PFS), and objective response rate (ORR) by RECIST (7, 8). GOG-240 led directly to FDA approval of bevacizumab for advanced cervical cancer on August 14, 2014. Regulatory approval by the European Medicines Agency for the European Union followed on April 8, 2015. Both triplet regimens studied (cisplatin–paclitaxel–bevacizumab and topotecan–paclitaxel–bevacizumab) are designated Category 1 in the

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**Note:** Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

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National Cancer Center Network Cervical Cancer Treatment Guidelines (9).

The introduction of novel therapies into practice drives the need to identify predictive biomarkers of response. Serial imaging modalities are cost-prohibitive and no validated serum tumor markers for cervical cancer exist. In addition, malignant tissues are often not readily accessible for biomarker interrogation to guide second-line therapy upon progression following anti-VEG therapy. Finally, prognostic theranostic markers have not been defined.

Circulating tumor cells (CTC) are minimally invasive liquid biopsies and their presence has been correlated with survival in several malignancies. The protocol-specified translational objective of GOG-240 was to determine whether CTCs could be isolated from women with advanced cervical cancer. If detectable, we sought to determine their association with survival, whether intervening therapy leads to declination of CTCs, and if their enumeration could serve as a predictive biomarker for anti-VEGF therapy selection.

## Materials and Methods

### Study design

GOG-240 was a phase III, randomized trial that enrolled 452 women with recurrent/persistent and metastatic cervical cancer. Patients were randomized to one of two different chemotherapy backbones (i.e., cisplatin 50 mg/m$^2$ plus paclitaxel 135 mg/m$^2$ or 175 mg/m$^2$ and topotecan 0.75 mg/m$^2$ days 1–3 plus paclitaxel 175 mg/m$^2$) with and without bevacizumab 15 mg/kg. Cycles were repeated every 21 days until progression, intolerability, complete response, or voluntary patient withdrawal. Primary endpoints were OS and toxicology, and the secondary endpoints were PFS and ORR. The study was stopped at the second interim analysis when a survival advantage conferred by antiangiogenesis therapy was recognized. Clinical endpoints (including the final protocol-specified analysis of OS), along with the secondary objectives of patient reported outcomes and prospective validation of pooled prognostic factors, have been reported previously (8, 10–13). The identification of CTCs constituted the sole translational objective of the trial. The study protocols were approved by the NCI's central institutional review board (cIRB) and local IRBs when indicated. All patients provided written, informed consent according to study procedures.

Peripheral whole blood samples measuring 7.5 mL each were collected for CTC analysis at baseline (i.e., within 28 days of commencing therapy) and 36 days post-cycle 1. The specimens were drawn into a special Cell Save Vacutainer tube and shipped directly for next morning delivery to Brigham and Women's Hospital in Boston for immediate processing, isolation, enumeration and characterization of CTCs according to standard operating procedures, good clinical laboratory practice, and previously published protocols using microfluidic technologies (14). Prospectively, acquired data included age, race/ethnicity, performance status, histology, tumor grade, prior exposure to radiosensitizing cisplatin, presence/absence of pelvic disease, and survival parameters.

### CTC Analysis

Peripheral blood samples (7.5 mL per tube) were collected into CellSave (Veridex LLC) tubes and processed within 48 hours using the CellSearch instrument with the CTC enumeration kit, according to the manufacturer's instructions. Briefly, samples were centrifuged at low speed to separate blood components. CTCs were isolated from the buffy coat layer using an anti-EpCAM antibody-conjugated ferrofluid and rare earth magnet. Isolated cells were washed and incubated with DAPI and fluorescently tagged anti-cytokeratin and anti-CD45 antibodies and transferred to a viewing chamber in a CellTracks magnetic cartridge. Following a short incubation, CTCs (defined as anti-cytokeratin$^+$ positive/anti-CD45$^-$ cells) were counted using a semiautomated fluorescence microscope.

### Statistical analysis

The identification of CTCs at each time point was studied with the exact Pearson $\chi^2$ test (15) for associations with previously established clinical prognostic factors, including age, race, ethnicity, and performance status among other covariates. Changes in the number of CTCs over time were assessed using the paired Students $t$ test (16), and also examined for differences in the change across the various regimens

Landmark exploratory analyses were conducted with patients surviving (or having PFS) for at least 36 days after treatment when posttherapy samples were collected (17). OS was defined from the time of randomization (for analyses on samples collected strictly pretreatment) or 36 days after initiating the first treatment (for analyses that used posttherapy samples this time point was referred to as "36 days post-cycle 1") to death and PFS was determined similarly for disease progression using RECIST v1.0. The prognostic impact of pretreatment and post-cycle 1 CTCs were studied using deviance residual plots (18). The impact of pretreatment CTC and changes in CTC were examined on OS and PFS using predominantly a univariate Cox proportional hazards model (19). Other covariates were included to estimate the effect of CTC after stratification for bevacizumab and/or topotecan backbone treatment. Patients with CTC counts equal to or above the median CTC count were considered to have high CTC counts, with those below the median identified as having low CTC counts. Some analyses used continuous CTC counts whereas others used CTC cut points to divide the population into two or three groups. Kaplan–Meier estimates were used for plots of the survival functions (20).

The sample size was calculated for the trial's primary endpoint of OS assuming four treatments in patients enrolled onto a study using a 2 × 2 factorial design with the assumption of no interaction (8). The impact of CTCs on OS and PFS were assessed by a log-rank test (21) with a one-sided alpha of 0.05. There were 91 deaths included, giving the study 80% power to detect a HR of 0.56 for the analysis of chemotherapy vs. chemotherapy plus bevacizumab. For the PFS endpoint, there were 137 events, giving the study 80% power to detect a HR of 0.62.

## Results

Nearly 39% ($n = 176$) of the entire study population ($n = 452$) participated in CTC analysis pre-cycle 1 and approximately 37% ($n = 167$) provided whole blood for CTC analysis after having received the first cycle of therapy (see CONSORT diagram, **Fig. 1**). CTCs were identified in nearly every case prior to therapy and in 81% of samples procured after cycle 1 (**Fig. 2**). The median CTC count pre-cycle 1 was 7 CTCs per 7.5 mL whole blood and the median CTC count post-cycle 1 was 4 CTCs per sample, with the per patient difference in means being suggestive (95% CI on change is −3.9 to −2.2 cells; $P < 0.0001$; **Table 1**). The magnitude of the change in CTCs was not dependent on the treatment administered.

The detection of CTCs prior to therapy was not influenced by age, race, or performance status, with high levels of CTCs detected in all fields. When stratified by Hispanic versus non-Hispanic ethnicity, there was a nonsuggestive trend (99% vs. 83%) for higher CTC detection rates among non-Hispanic patients (**Table 2**). Post-cycle

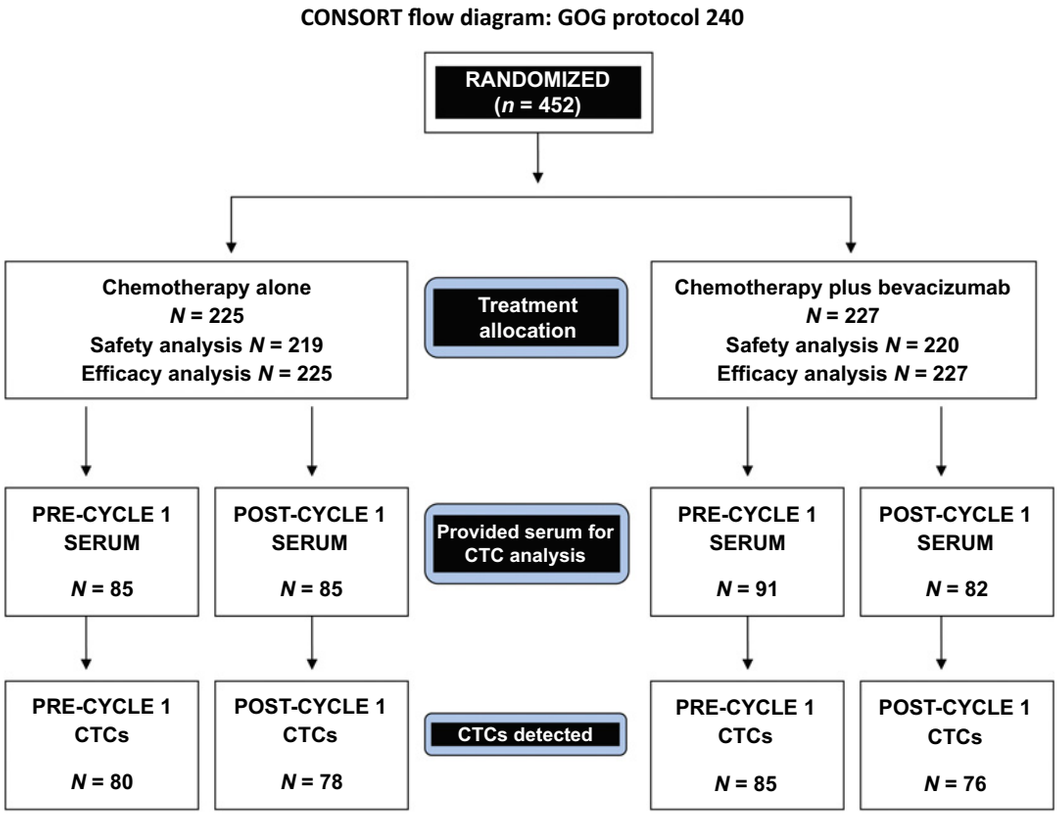


**Figure 1.**
CONSORT diagram indicating sample collection according to the schema of GOG protocol 240.

1, fewer CTCs were detected in nearly all fields and, with the exception of age, CTC counts were not associated with clinical factors under evaluation. Women 48 years or older were observed to have higher numbers of CTCs post-cycle 1 than women under 48 years (**Table 2**).

The enumeration of baseline CTCs was not associated with tumor-related factors including histology, grade, cisplatin exposure, or pelvic disease (**Table 3**). Following treatment, CTCs were more likely to be detected in adenocarcinoma compared with tumors of squamous cell or adenosquamous histology (**Table 3**), but the amount of CTC decrease was not dependent on cell type.

The association or impact of pretreatment CTCs on OS is depicted in the Kaplan–Meier curves of **Fig. 3**. Patients with pretreatment CTCs above and below the median of 7 CTCs/7.5 mL were stratified by treatment with and without bevacizumab. Among patients with low pretreatment CTC counts, the curves with and without bevacizumab were similar, with median survivals of 15.8 and 17.1 months, respectively (HR, 1.06; 95% CI, 0.59–1.92; **Fig. 3A**). Those patients with high levels of pretreatment CTCs who did not receive bevacizumab experienced a median survival of 16.2 months, similar to those patients with low CTC counts.

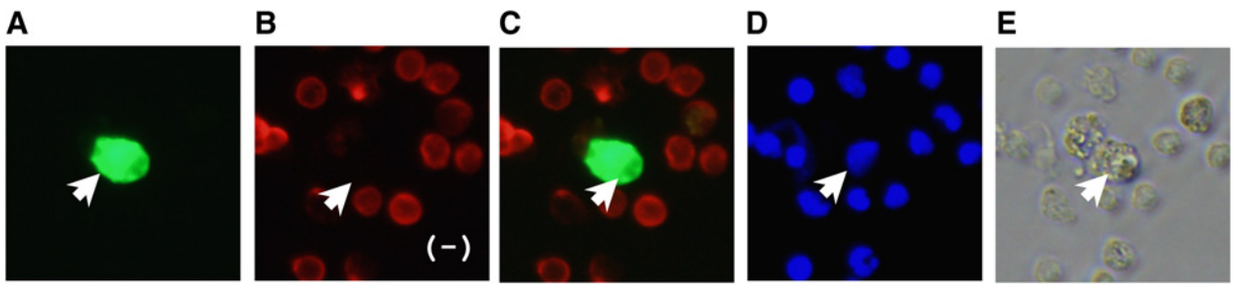


**Figure 2.**
CTC is indicated by the arrow. Pan-cytokeratin positive (**A**), CD45 (leukocyte common antigen) negative (**B**) were counted as CTC. **C** merges panels A and B. Cells were stained with DAPI (**D**) to assess fluorescence excitation/emission. The bright-field image is also depicted (**E**). Images kindly provided by M. Takakura from Kanazawa Medical University, Uchinada, Japan.

**Table 1.** Submission of whole blood for CTC enumeration and percentage of specimens with CTCs identified.

| | Pre-cycle 1 | 36 days Post-cycle 1 |
|---|---|---|
| % (*N*)[a] whole blood submitted (7.5 mL) | 38.5 (174) | 36.95 (167) |
| % (*N*) with CTCs identified | 96.6 (168) | 81.4 (136) |
| Median CTC count per 7.5 mL (range) | 7 (0–18) | 4 (0–17) |

Abbreviation: CTCs, circulating tumor cell.
[a]Denominator = entire GOG 240 population ($n$ = 452 patients).

PFS according to low versus high pretreatment CTCs and stratified according to bevacizumab use is plotted in **Fig. 3B**. While low levels of pretreatment CTCs, irrespective of bevacizumab treatment, and high levels of CTCs without bevacizumab treatment were associated with similar median PFS (6.2–7.3 months; the HR of PFS in patients with low levels of pretreatment CTCs for bevacizumab to no bevacizumab therapy was 0.95; (95% CI, 0.58–1.55), there was a significant improvement in PFS among women with high pretreatment CTCs who received bevacizumab (10.8 vs. 6.9 months; HR, 0.59; 95% CI, 0.36–0.96). For women with high pretreatment CTCs treated with the cisplatin–paclitaxel chemotherapy backbone, median PFS with and without bevacizumab was 14.6 versus 6.4 months, respectively (HR, 0.26; 95% CI, 0.12–0.55; **Fig. 3C**). This effect was not observed among women with high CTCs treated on the topotecan–paclitaxel backbone.

Bevacizumab treatment was not found to impact OS or PFS when analyzing subsets of patients by low and high levels of 36-day post-cycle 1 CTCs. The median OS estimates ranged from 16.4 to 17.2 months and associated with a hazard of death of 1.12 (95% CI, 0.64–1.98; Supplementary Fig. S1A) for treatment with bevacizumab to no bevacizumab among the high levels of posttherapy CTC patients. Similarly, the HR was 0.90 (95% CI, 0.46–1.75) among patients with the lower levels of post-cycle 1 CTCs. Women with high post-cycle 1 CTCs treated with and without bevacizumab experienced a median PFS of 8.2 versus 7.4 months, respectively (HR, 0.79; 95% CI, 0.49–1.27; Supplementary Fig. S1B). Similarly, the HR was 1.07 (95% CI, 0.62–1.85) among patients with the lower levels of post-cycle 1 CTC.

**Table 2.** Identification of CTCs according to known clinical prognostic factors.

| | % (*N*) Pre-cycle 1 | *P* | % (*N*) 36 days Post-cycle 1 | *P* |
|---|---|---|---|---|
| Age < 48 years | 99 (78) | NS | 74 (55) | 0.045 |
| Age ≥ 48 years[a] | 95 (90) | | 87 (81) | |
| White | 96 (129) | NS | 80 (104) | NS |
| Black | 96 (25) | | 88 (21) | |
| Asian | 100 (6) | | 100 (5) | |
| Pacific Islander | 100 (1) | | 0 (0) | |
| Native American | 100 (2) | | 100 (2) | |
| Unknown | 100 (5) | | 66 (4) | |
| Non-Hispanic | 99 (141) | 0.006 | 80 (111) | NS |
| Hispanic | 83 (20) | | 82 (18) | |
| Unknown | 100 (7) | | 100 (7) | |
| PS 0 | 98 (94) | NS | 81 (58) | NS |
| PS 1 | 95 (74) | | 82 (78) | |

Abbreviation: CTCs, circulating tumor cell.
[a]Median age of patients in GOG 240 = 48 years.

**Table 3.** Identification of CTCs according to known tumor-related/pathologic prognostic factors.

| Pre-cycle | Mean CTC Cycle 1 | Mean CTC Cycle 2 | Mean CTC Cycle 3 | ΔCTC C2–C1 | Rate ΔCTC |
|---|---|---|---|---|---|
| SCCA | 7.28 | 4.47 | 3.39 | −3.10 | −0.09 |
| Adenocarcinoma | 7.24 | 4.94 | 3.48 | −2.93 | −0.13 |
| Adenosquamous | 6.10 | 3.68 | 3.38 | −2.78 | −0.07 |
| Other types | 7.33 | 1.83 | 2.25 | −5.50 | −0.14 |
| Grade 1 | 5.50 | 4.91 | 4.18 | −1.00 | −0.05 |
| Grade 2 | 7.55 | 4.22 | 3.56 | −3.66 | −0.12 |
| Grade 3 | 6.88 | 4.80 | 3.32 | −2.77 | −0.09 |
| No grade | 5.40 | 2.00 | 2.60 | −4.00 | −0.07 |
| Excluded | 7.43 | 4.67 | 1.91 | −1.60 | −0.11 |
| No tissue | 6.60 | 2.67 | 3.50 | −5.00 | −0.06 |
| No prior CDDP-RT | 6.94 | 4.24 | 3.77 | −2.76 | −0.09 |
| Prior CDDP-RT | 7.22 | 4.43 | 3.21 | −3.29 | −0.11 |
| No pelvic disease | 6.80 | 4.43 | 3.62 | −2.98 | −0.08 |
| Pelvic disease | 7.38 | 4.33 | 3.19 | −3.24 | −0.12 |

Abbreviations: CTCs, circulating tumor cell; CDDP-RT, cisplatin-based chemoradiation.

Among patients treated with anti-VEGF therapy, higher pretreatment CTCs were associated with a lower hazard of death (HR, 0.90; 95% CI, 0.81–0.99; Supplementary Fig. S2A). Conversely, higher post-cycle 1 CTCs were associated with an increased hazard of death (HR, 1.16; 95% CI, 1.043–1.286; Supplementary Fig. S2B). Supplementary Figure S2C depicts the change in CTCs from pre-cycle 1 to 36 days post-cycle 1. Those women with with greater reductions in CTCs had a lower risk of dying (HR, 1.16; 95% CI, 1.05–1.27).

## Discussion

The eminent French surgeon, Joseph-Claude-Anthelme Récamier (1774–1852) was appointed Professor at the "Collège de France" and physician to the last King of France, Louis-Philippe I (reigned 1830–1858; ref. 22). In addition to reinventing the vaginal speculum in 1812, providing the first clear description of a vaginal hysterectomy for carcinoma of the cervix on July 26, 1829, Récamier believed that cancer propagates through the veins and introduced the term "metastasis" to describe the spread of the disease via invasion of the bloodstream (23). The first publication of CTCs appeared in 1869 by Ashworth who described a case in which cells similar to those in a tumor were found in the blood after a patient's death (24). In 1955, using a cellblock technique, Engell reported the detection of CTCs in patients with advanced malignancies (25).

Cervical cancer is driven by VEGF-induced angiogenesis. Following infection, linearization of native, episomal, high-risk HPV subtype(s) through interruption of the HPV E2 regulatory gene and subsequent integration into host DNA is essential for malignant transformation. Disruption of E2 removes the block from viral oncogene transcription, with HPV E6 directly degrading host cellular tumor suppressor gene product, p53, and engagement of HPV E7 with host cellular tumor suppressor gene product, pRb, leading to its inactivation (26). These concerted effects manifest in increased thrombospondin-1 and increased hypoxia-inducible factor α, both ultimately resulting in increased VEGF expression and tumor angiogenesis via the VEGF-dependent axis.

To supply nutrients to the tumor and clear waste products, the ensuing neovascularization must be sufficiently permeable to allow

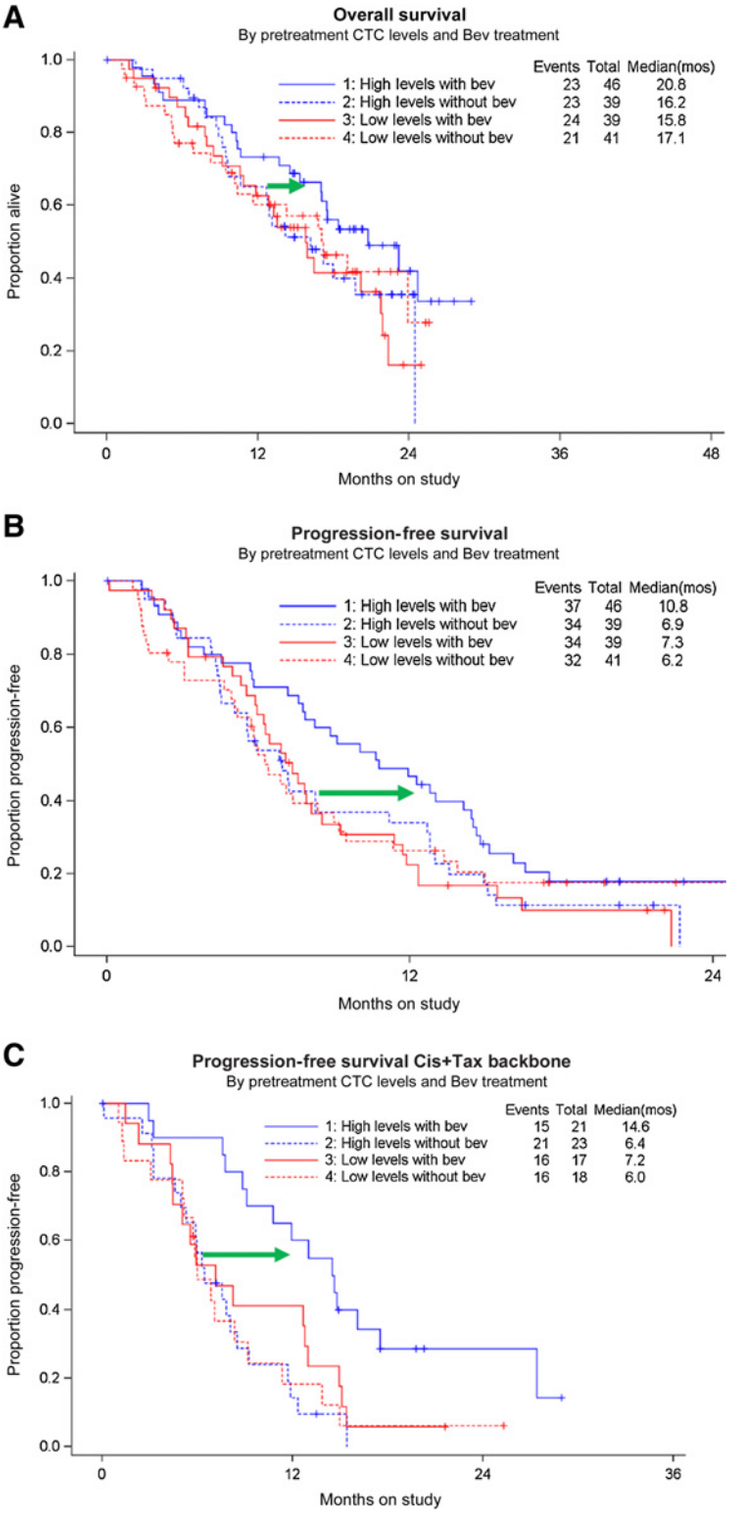


**Figure 3.**

Kaplan–Meier curves demonstrating OS (HR, 0.57; 95% CI, 0.32–1.03; **A**) and PFS (HR, 0.59; 95% CI, 0.36–0.96; **B**) among patients with high versus low levels of pre-cycle 1 circulating tumor cells stratified by treatment with and without bevacizumab. The effect of bevacizumab administration on PFS has its greatest impact among women treated with the cisplatin–paclitaxel chemotherapy backbone (HR, 0.26; 95% CI, 0.12–0.55; **C**). The green arrows that appear in each panel suggest that high levels of pretreatment CTCs may represent a *predictive biomarker* as treatment with bevacizumab shifts the survival curve to the right.

free, bidirectional passage of small molecules, gases, and plasma proteins (27). Angiogenesis leads to a hyperpermeable or "leaky" vasculature, the properties of which are mediated by chronic exposure to vascular permeabilizing agents, including VEGF. Prolonged VEGF-A stimulation transforms venular endothelium into mother vessels comprised of thin hyperpermeable cells with fewer vesiculo-vacuolar organelles (VVO), degraded basal lamina, and extensive loss of pericyte coverage (27). Protein-rich plasma exudates extravasate through VVO or through fenestrae and interact with tissue factor to trigger the clotting system and deposit fibrin, creating a proangiogenic

provisional stroma. Macromolecules may also extravasate through fenestrae with 3D reconstructions of serial electron microscopic sections revealing both intercellular and transcellular pores in tumor vasculature. Proangiogenic protein expression manifests concomitantly with epithelial–mesenchymal transition, characterized by loss of the epithelial marker E-cadherin, low regulation of specific cytokeritins, and transition of polarized, cubic, and immobile epithelial cells into nonpolarized and unstable spiculated cells with the capacity for invasion and migration (27). Highly angiogenic tumors may shed cells into the bloodstream via leaky vasculature and be preferentially susceptible to angiogenesis blockade.

Several barriers to drug discovery in advanced cervical cancer exist. As revealed by next-generation sequencing, spatial heterogeneity is characterized by extensive *inter*patient (and *intra*patient) heterogeneity with clonal diversity (28). Temporal heterogeneity induced by selective pressure over time from treatment results in acquired drug resistance. A lack of validated predictive biomarkers of response to guide personalized therapy and a paucity of readily accessible tissue for phenotypic interrogation remain problematic. Ideally, treatment of metastatic disease should be predicated on contemporary tumor samples. CTCs represent noninvasive, real-time, "liquid biopsies." Through identification of theranostic markers and provision of more sensitive monitoring of treatment efficacy, CTCs may guide drug selection.

CTC capture exploits unique physical properties including larger size, differences in density, charge, deformability, and migratory properties, allowing them to be distinguished from normal circulating blood elements (29). Derived from malignant epithelium, most CTCs express epithelial cell markers including EpCAM (30). CTCs have previously been reported to have prognostic significance in metastatic breast cancer (MBC; refs. 31–35). A meta-analysis by Bidard and colleagues collected individual patient data from 21 studies involving patients with early breast cancer treated with neoadjvuant chemotherapy (31). CTC detection was shown to be an independent and quantitative prognostic marker for OS, distant disease-free survival, and locoregional relapse-free interval in this population. Several years earlier, Rack and colleagues had shown CTCs to have independent prognostic relevance before and after adjuvant chemotherapy for women with early breast cancer (32). In the Southwestern Oncology Group protocol S0500, Smerage and colleagues reported that for patients with persistently increased CTCs after 21 days of first-line therapy, early switching to a second-line regimen did not prolong OS (33). In another prospective study of 83 women, Cristofanilli and colleagues noted that patients with ≥5 CTCs at baseline and at first monthly follow-up had a worse prognosis than those with less than 5 CTCs (34). The value of baseline CTCs as a prognostic biomarker has also been reported in metastatic colorectal cancer (36), non–small cell lung cancer (37), stage III melanoma (38), and metastatic, castration-resistant prostate cancer (39).

Recently, investigators working with cervical cancer cell lines and cohorts of patients with cervical cancer treated outside of a clinical trial setting, have reported successful CTC capture and correlations with survival (40–42). Our analysis constitutes the primary translational endpoint of a phase III randomized trial and suggests that CTCs may serve as a prognostic biomarker in recurrent/metastatic cervical cancer. Interestingly, anti-VEGF treatment appears to shift the survival curves to the right among women with high levels of CTCs pre-cycle 1. CTCs may represent a predictive biomarker to guide antiangiogenesis therapy in this disease. Because we only measured CTC counts at two points, this work is not definitive. However, in nearly all other studies, baseline CTC count appears to be the strongest indicator of outcome.

The threshold defined for clinical validity may differ among tumor types. In women with MBC, the ≥5 CTC/7.5 mL threshold was initially optimized at baseline to distinguish two populations with improved versus worse survival outcome. This threshold provided a noteworthy HR and significant *P* value, leading to its selection for other studies of CTCs in MBC and extrapolation to other tumor types. However the ≥5 CTC/7.5 mL cut-off may not be applicable to every tumor type and may not represent even the best threshold for an early resistant screening test in MBC (43). Similar to previous studies by other investigators, we used the median baseline CTC count in our exploratory analyses. Prospective validating studies will be needed to determine whether indeed the median CTC count can serve as a marker to determine clinical significance.

Because precision cancer medicine relies on the ability to predict the future behavior of an individual tumor, there exists an urgent need for reliable prognostic and predictive biomarkers for advanced cervical cancer. While Darwinian selection is deterministic in nature, the acquisition of heritable alterations and genetic drift are both random processes with the end result being that cancer predictability is limited by stochasticity (44). Stated differently, unlike a deterministic process whose outcome is determined by the initial state, a stochastic process may have different outcomes even if the initial states are identical (44). CTC enumeration may fulfill the requirements of cancer evolutionary biology and inform on novel drug targets and reveal mechanisms of metastases through detection of minimal residual disease and putative culprit cells responsible for seeding and reseeding of metastatic foci.

Leaky vasculature resulting from tumor angiogenesis in cervical cancer may permit systemic distribution of CTCs through intratumoral vascular shunting. The improved survival associated with high pretreatment CTCs and treatment with bevacizumab is exploratory but may characterize a subpopulation of patients with increased tumor vascularization and concomitant vulnerability to anti-angiogenesis therapies. The observation that higher CTCs were detected among women 48 years and older is interesting when considered in light of the analysis of prognostic factors from the original GOG-0240 manuscript (8) in which treatment with bevacizumab tracked with improved survival among patients 48–56 years of age. This suggests that while the enumeration of CTCs was not associated with the tumor-related factors (e.g., grade) that were collected, it is possible that molecular biomarkers that directly participate in the VEGF axis (e.g., hypoxia-inducible factor α) may have been more informative. In addition, while high levels of baseline CTCs and bevacizumab intervention correlated with PFS, in GOG-0240, the median number of treatment cycles was seven. Measuring CTC levels later (e.g., post-Cycle 7 as opposed to post-cycle 1) may have been more informative with respect to clinical endpoints when anti-VEGF therapy was used.

CTC capture and enumeration, and possibly detection of circulating tumor DNA in women with advanced cervical cancer, may serve as a predictive biomarker to guide treatment selection. CTC-free DNA may represent waste byproducts of cancer and, unlike CTCs, may not necessarily be indicative of tumor burden. Future studies in cervical cancer should be designed to quantify CTCs not only during later cycles of therapy, but at several time points as continuous, dynamic CTC changes may be more meaningful. Nevertheless, early prediction of treatment efficacy may have important ramifications on quality of life in this high-risk population.

## Disclosure of Potential Conflicts of Interest

K.S. Tewari reports grants from University of California, Irvine during the conduct of the study; personal fees from Roche/Genentech (advisory board, consultant) outside the submitted work. B.J. Monk reports personal fees from Abbvie (honorarium/consultant), Advaxis (honorarium/consultant), Agenus (honorarium/consultant), Amgen (honorarium/consultant), Akeso Bio (honorarium/consultant), Aravive (honorarium/consultant), AstraZeneca (honorarium/consultant/speaker), Asymmetric Therapeutics (honorarium/consultant), Boston Biomedical (honorarium/consultant), ChemoCare (honorarium/consultant), ChemoID (honorarium/consultant), Circulogene (honorarium/consultant), Clovis (honorarium/consultant/speaker), Conjupro (honorarium/consultant), Dicepheria (honorarium/consultant), Easai (honorarium/consultant), Geistlich (honorarium/consultant), Genmab/Seatlle Genetics (honorarium/consultant), GOG Foundation (honorarium/consultant), ImmunoGen (honorarium/consultant), Immunomedics (honorarium/consultant), Incyte (honorarium/consultant), Iovance (honorarium/consultant), Janssen/Johnson & Johnson (honorarium/consultant/speaker), Laekna Health Care (honorarium/consultant), Mateon (formally Oxigene) (honorarium/consultant), Merck (honorarium/consultant/speaker), Mersana (honorarium/consultant), Myriad (honorarium/consultant), Nucana (honorarium/consultant), Oncomed (honorarium/consultant), Oncoquest (honorarium/consultant), Oncosec (honorarium/consultant), Perthera (honorarium/consultant), Pfizer (honorarium/consultant), Precision Oncology (honorarium/consultant), Puma (honorarium/consultant), Regeneron (honorarium/consultant), Roche/Genentech (honorarium/consultant/Sseaker), Samumed (honorarium/consultant), Takeda (honorarium/consultant), Tarveda (honorarium/consultant), TESARO/GSK (honorarium/consultant/speaker), Vavotar Life Sciences (honorarium/consultant), VBL (honorarium/consultant), and Vigeo (honorarium/consultant) outside the submitted work. R.T. Penson reports grants and personal fees from Genentech Roche during the conduct of the study; personal fees and other from AbbVie (DSMC); grants and personal fees from AstraZeneca, Clovis Oncology, Eisai Inc, Merck & Co, NewLink Genetics, Tesaro Inc, and Vascular Biogenics Ltd; nonfinancial support from Care4ward; personal fees from Curio Science, Janssen Oncology, Mersana Therapeutics, Nexus Global Group, Pieris Pharma Inc, and Sutro Biopharma; grants from Syndax Pharmaceuticals, outside the submitted work. L.M. Randall reports grants from University of California Irvine during the conduct of the study; grants and personal fees from GSK/Tesaro and Merck; grants from Pfizer, OnTarget Laboratories, Aivita Biopharma; personal fees from Clovis Oncology, Myriad, AstraZeneca, outside the submitted work. A. Oaknin reports personal fees from Roche, AstraZeneca, PharmaMar, Clovis Oncology, Tesaro, Inmunogen, and Genmab outside the submitted work. M.M. Leitao reports personal fees from Intuitive Surgical (ad hoc speaker) outside the submitted work. M.L. Pearl reports grants from GOG/NRG Oncology (NCI) during the conduct of the study; other from Vita-Tex, Inc (royalties via Stony Brook Foundation) outside the submitted work; and conducted NIH-funded research on circulating tumor cells in patients with ovarian cancer with researchers from Vita-Tex, a start-up company owned by my research collaborator. During that time, I received royalties ($500/year) from the Stony Brook Foundation. Vita-Tex was purchased last year and my collaboration is currently on hold. R. Salani reports other from Clovis (advisory board) and other from GlaxoSmithKline (advisory board) outside the submitted work. D.L. Richardson reports personal fees from AstraZeneca, Foundation Medicine, GSK/Tesaro, Mersana, Deciphera, Bayer, and Genentech outside the submitted work. No potential conflicts of interests were disclosed by the other authors.

## Authors' Contributions

**K.S. Tewari:** Conceptualization, resources, data curation, validation, investigation, visualization, methodology, writing-original draft, project administration, writing-review and editing. **M.W. Sill:** Conceptualization, data curation, software, formal analysis, supervision, visualization, methodology, writing-original draft, writing-review and editing. **B.J. Monk:** Conceptualization, resources, data curation, formal analysis, supervision, validation, investigation, methodology, writing-original draft, writing-review and editing. **R.T. Penson:** Methodology, writing-original draft, writing-review and editing. **D.H. Moore:** Data curation, writing-review and editing. **H.A. Lankes:** Resources, data curation, writing-original draft, writing-review and editing. **L.M. Ramondetta:** Resources, writing-review and editing. **L.M. Landrum:** Resources, writing-review and editing. **L.M. Randall:** Resources, writing-review and editing. **A. Oaknin:** Resources, data curation, formal analysis, methodology, writing-original draft, writing-review and editing. **M.M. Leitao:** Resources, writing-review and editing. **E.L. Eisenhauer:** Resources, writing-review and editing. **P. DiSilvestro:** Resources, writing-review and editing. **L. Van Le:** Resources, writing-review and editing. **M.L. Pearl:** Resources. **J.J. Burke:** Resources, writing-review and editing. **R. Salani:** Resources, writing-review and editing. **D.L. Richardson:** Resources, writing-review and editing. **H.E. Michael:** Validation, writing-review and editing. **D.W. Kindelberger:** Resources, writing-review and editing. **M.J. Birrer:** Conceptualization, resources, data curation, formal analysis, supervision, validation, investigation, methodology, writing-original draft, writing-review and editing.

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