Title

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Journal

Molecular Cancer Therapeutics, 19(11)

ISSN

1535-7163

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Publication Date

2020-11-01

DOI

10.1158/1535-7163.mct-20-0276

Published in final edited form as:

Mol Cancer Ther. 2020 November ; 19(11): 2363–2370. doi:10.1158/1535-7163.MCT-20-0276.

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

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Conflicts of Interest/Disclosure:
Dr(s) Tewari, Monk, Penson, and Birrer disclose that they have attended Advisory Boards held by Genentech/Roche.

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UNSTRUCTURED ABSTRACT

To isolate circulating tumor cells (CTCs) from women with advanced cervical cancer and estimate the impact of CTCs and treatment on overall survival (OS) and progression-free survival (PFS). 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 anti-angiogenesis drug, bevacizumab, in women with recurrent/metastatic cervical cancer. CTCs (defined as anti-cytokeratin positive/anti-CD45 negative cells) were isolated from the buffy coat layer using an anti-EpCAM antibody-conjugated ferrofluid and rare earth magnet, and counted using a semi-automated fluorescence microscope. The median pre-cycle 1 CTC count was 7 CTCs/7.5 mL whole blood (range, 0–18) and, at 36 days post-treatment, 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% CI, 0.79–0.95). Among patients with high (≥ median) pre-treatment CTCs, bevacizumab treatment was associated with a reduction in the hazard of death (HR 0.57; 95% CI, 0.32–1.03) and progression (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 pre-treatment CTCs may reflect increased tumor neovascularization and concomitant vulnerability to VEGF inhibition. These data support studying CTC capture as a potential predictive biomarker.

Keywords

Cervical cancer; circulating tumor cells; angiogenesis; bevacizumab

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_1) 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_2-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 National Cancer Institute's 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 anti-angiogenesis 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 RESIST.[7,8] GOG-240 led directly to U.S. Food and Drug Administration approval of bevacizumab for advanced cervical cancer on August 14, 2014. Regulatory approval by the European Medicines Agency for the European Union followed in April 8, 2015. Both triplet regimens studied (cisplatin-paclitaxel-bevacizumab and topotecan-paclitaxel-bevacizumab) are designated Category 1 in the 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. Additionally, 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 (CTCs) 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 of 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 anti-angiogenesis 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, sampled 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 anitbodies and transferred to a viewing chamber in a CellTracks magnetic cartridge. Following a short incubation, CTCs (defined as anti-cytokeratin positive/anti-CD45 negative cells) were counted using a semi-automated fluorescence microscope.

Statistical Analysis

The identification of CTCs at each time point were studied with the exact Pearson Chi-Square 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's 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 post-therapy samples were collected.[17] OS was defined from the time of randomization (for analyses on samples collected strictly pre-treatment) or 36 days after initiating the first treatment (for analyses that used post-therapy 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 pre-treatment and post-cycle 1 CTCs were studied using deviance residual plots.[18] The impact of pre-treatment 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 2 or 3 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, Figure 1). CTCs were identified in nearly every case prior to therapy and in 81% of samples procured after cycle 1 (Figure 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 vs non-Hispanic ethnicity, there was a non-suggestive trend (99% vs 83%) for higher CTC detection rates among non-Hispanic patients (Table 2). Post-cycle 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 suggested 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 pre-treatment CTCs on OS is depicted in the Kaplan-Meier curves of Figure 2. Patients with pre-treatment CTCs above and below the median of 7 CTCs/7.5 mL were stratified by treatment with and without bevacizumab. Among patients with low pre-treatment 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) (Figure 3A). Those patients with high levels of pre-treatment CTCs who did not receive bevacizumab experienced a median survival of 16.2 months, similar to those patients with low CTC counts.

PFS according to low vs high pre-treatment CTCs and stratified according to bevacizumab use is plotted in Figure 3B. While low levels of pre-treatment 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 hazard ratio of PFS in patients

with low levels of pre-treatment 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 pre-treatment CTCs who received bevacizumab (10.8 vs 6.9 months; HR 0.59; 95% CI 0.36–0.96). For women with high pre-treatment CTCs treated with the cisplatin-paclitaxel chemotherapy backbone, median PFS with and without bevacizumab was 14.6 vs 6.4 months, respectively (HR 0.26; 95% CI, 0.12–0.55) (Figure 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) (Figure 4A) for treatment with bevacizumab to no bevacizumab among the high levels of post therapy 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 vs 7.4 months, respectively (HR 0.79; 95% CI, 0.49–1.27) (Figure 4B). Similarly, the HR was 1.07 (95% CI 0.62~1.85) among patients with the lower levels of post-cycle 1 CTC.

Among patients treated with anti-VEGF therapy, higher pre-treatment CTCs were associated with a lower hazard of death (HR 0.90; 95% CI, 0.81–0.99) (Supplementary Figure 1A). Conversely, higher post-cycle 1 CTCs were associated with an increased hazard of death (HR 1.16; 95% CI, 1.043 ~ 1.286) (Supplementary Figure 1B). Supplementary Figure 1C 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).[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 vascular endothelial growth factor (VEGF)-induced angiogenesis. Following infection, linearization of native, episomal, high-risk human papillomavirus (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 free, bidirectional passage of small molecules, gases, and plasma proteins.[27] Angiogenesis leads to a hyper-permeable 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 hyper-permeable 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 pro-angiogenic 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 non-polarized 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).[31–35] A meta-analysis by Bidard et al collected individual patient data from 21 studies involving early breast cancer patients 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 et al 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 et al 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 *et al* 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 have 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 cervical cancer patients treated outside of a clinical trial setting, have reported successful CTC capture and correlations survival.[40–42] Our analysis constitutes the primary translational endpoint of a phase 3 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 vs 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 pre-treatment 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 (eg., grade) that were collected, it is possible that molecular biomarkers that directly participate in the VEGF axis (eg., 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 (eg., 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. Circulating tumor cell-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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

The following Gynecologic Oncology Group member institutions participated in the primary treatment studies: Roswell Park Cancer Institute, University of Alabama at Birmingham, Duke University Medical Center, Abington Memorial Hospital, Walter Reed Army Medical Center, Wayne State University, University of Minnesota Medical School, Northwestern Memorial Hospital, University of Mississippi Medical Center, Colorado Gynecologic Oncology Group P.C., University of Washington, University of Pennsylvania Cancer Center, Milton S. Hershey Medical Center, University of Cincinnati, University of North Carolina School of Medicine, University of Iowa Hospitals and Clinics, University of Texas Southwestern Medical Center at Dallas, Indiana University School of Medicine, Wake Forest University School of Medicine, University of California Medical Center at Irvine, Rush-Presbyterian-St. Luke's Medical Center, Magee Women's Hospital, SUNY Downstate Medical Center, University of Kentucky, University of New Mexico, The Cleveland Clinic Foundation, State University of New York at Stony Brook, Washington University School of Medicine, Memorial Sloan-Kettering Cancer Center, Cooper Hospital/University Medical Center, Columbus Cancer Council, MD Anderson Cancer Center, University of Massachusetts Medical School, Fox Chase Cancer Center, Women's Cancer Center, University of Oklahoma, University of Virginia Health Sciences Center, University of Chicago, Mayo Clinic, Case Western Reserve University, Tampa Bay Cancer Consortium, Yale University, University of Wisconsin Hospital, Cancer Trials Support Unit, University of Texas - Galveston, Women and Infants Hospital, The Hospital of Central Connecticut, Georgia Core, Aurora Women's Pavilion of West Allis Memorial Hospital, Grupo Espanol de Investigacion en Cancer de Ovario, University of California, San Francisco-Mt. Zion, St. Joseph's Hospital and Medical Center (Arizona), and Community Clinical Oncology Program.

FINANCIAL INFORMATION

This study was supported by National Cancer Institute grants to the GOG Tissue Bank (U10 CA27469 and U24 CA114793), NRG Oncology (1U10 CA180822) and NRG Operations (U10CA180868).

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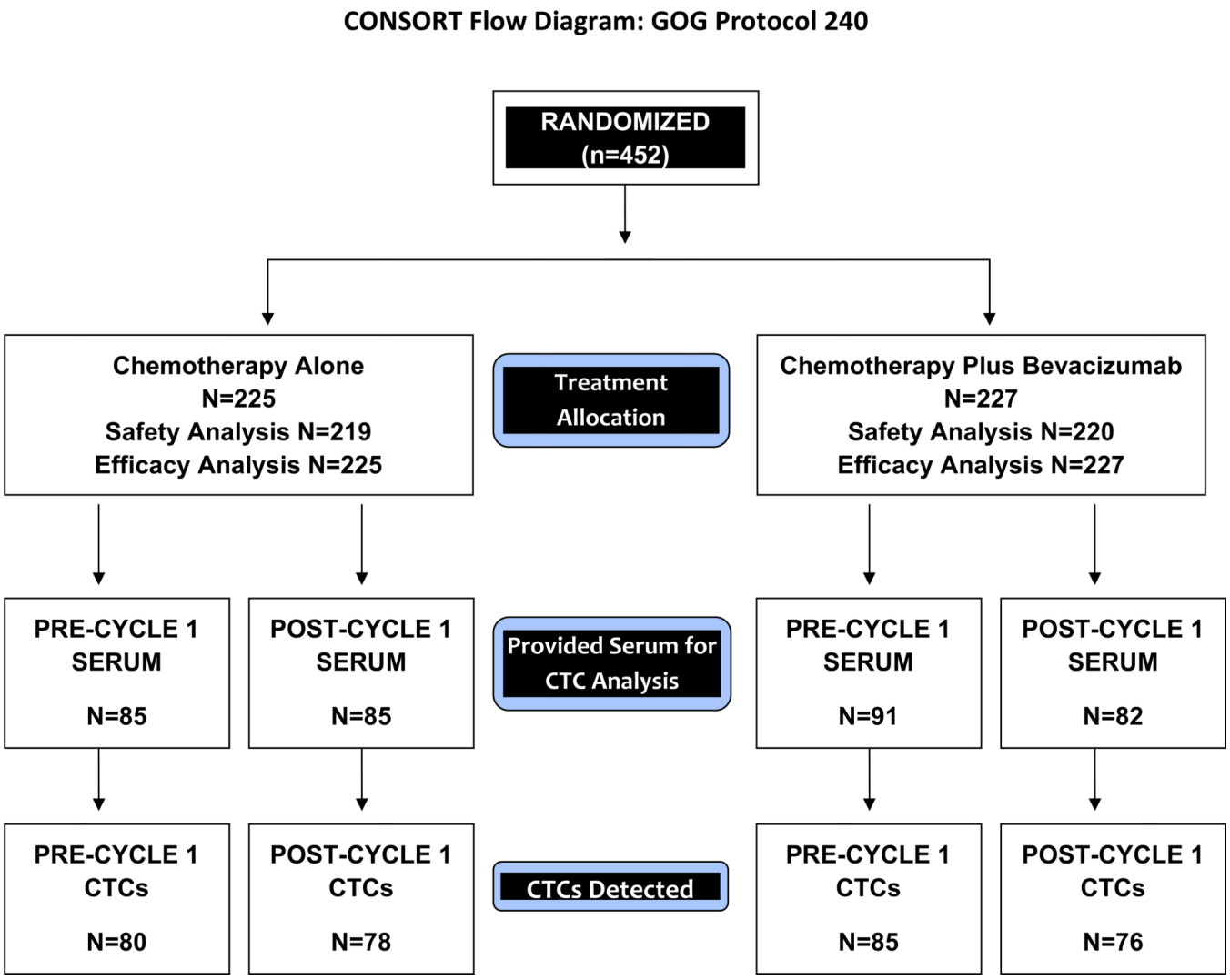

Figure 1:
CONSORT Diagram indicating sample collection according to the schema of Gynecologic Oncology Group protocol 240.

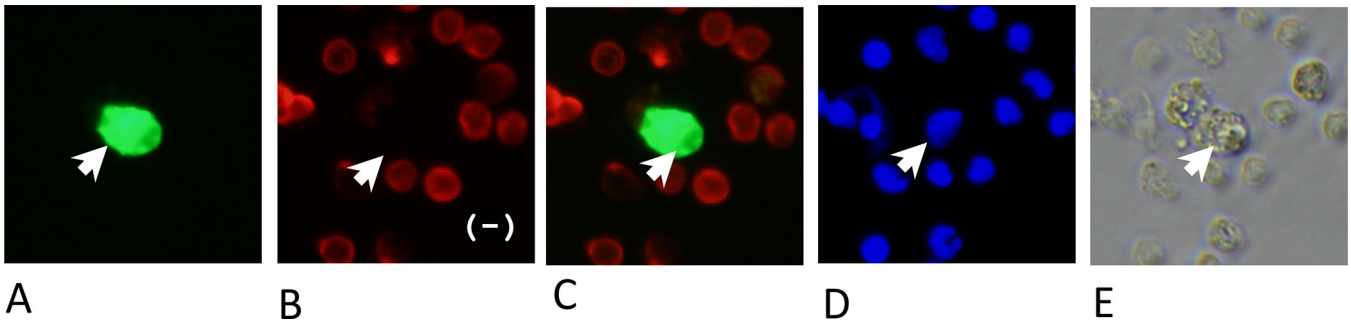

Figure 2: Circulating tumor cell (CTC) is indicated by the arrow.

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

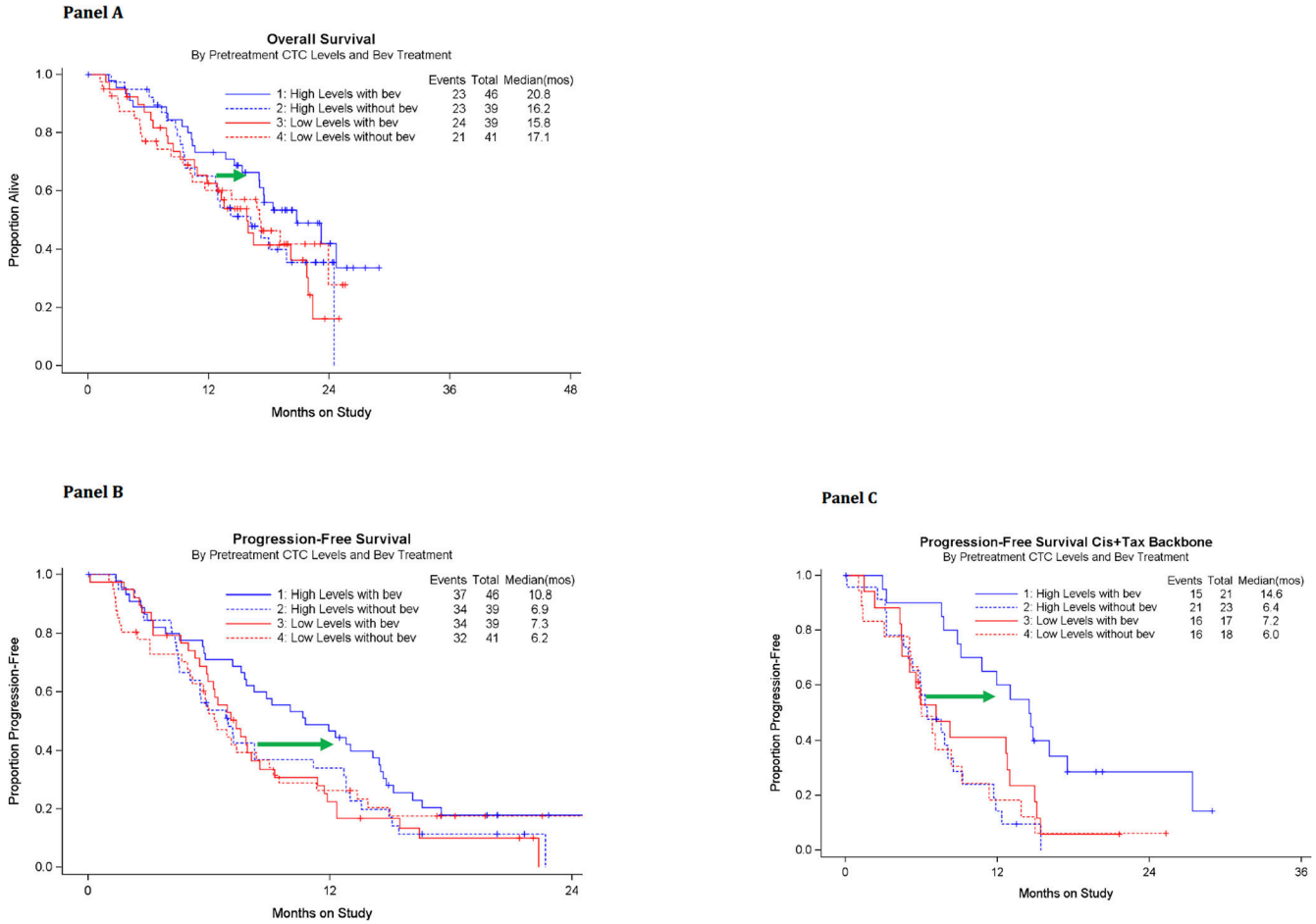

Figure 3:

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

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) * 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)

* Denominator = entire GOG 240 population (n=452 patients)

CTCs: circulating tumor cells

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 yrs	99 (78)		74 (55)	
Age ≥ 48 yrs *	95 (90)	NS	87 (81)	0.045
White	96 (129)		80 (104)	
Black	96 (25)		88 (21)	
Asian	100 (6)	NS	100 (5)	NS
Pacific Islander	100 (1)		0 (0)	
Native American	100 (2)		100 (2)	
Unknown	100 (5)		66 (4)	
Non-Hispanic	99 (141)		80 (111)	
Hispanic	83 (20)	0.006	82 (18)	NS
Unknown	100 (7)		100 (7)	
PS 0	98 (94)		81 (58)	
PS 1	95 (74)	NS	82 (78)	NS |

*Median age of patients in GOG 240 = 48 yrs

CTCs: circulating tumor cells

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

CTCs: circulating tumor cells; CDDP-RT: cisplatin-based chemoradiation