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Phase III Trial to Evaluate the Efficacy of Maintaining Hemoglobin Levels above 120 G/DL with Erythropoietin vs above 100 G/DL without Erythropoietin in Anemic Patients Receiving Concurrent Radiation and Cisplatin for Cervical Cancer: A Gynecologic Oncology Group Study

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Abstract

Purpose—To determine whether maintaining Hgb levels ≥ 12.0 g/dL with recombinant human erythropoietin (R-HUEPO) compared to “standard” treatment (transfusion for Hgb ≤ 10.0 g/dL) improves progression-free survival (PFS), overall survival (OS) and local control (LC) in women receiving concurrent weekly cisplatin and radiation (CT/RT) for carcinoma of the cervix. In addition, to determine whether platinum-DNA adducts were associated with clinical characteristics or outcome.

Methods—Patients with stage IIB-IVA cervical cancer and Hgb < 14.0 g/dL were randomly assigned to CT/RT $\pm$ R-HUEPO (40,000 units SC weekly). R-HUEPO was stopped if Hgb > 14.0 g/dL. Endpoints were PFS, OS and LC. Platinum-DNA adducts were quantified using immunocytochemistry assay in buccal cells.

Results—Between 08/01 and 09/03, 109 of 114 patients accrued were eligible. Fifty-two received CT/RT and 57 CT/RT plus R-HUEPO.

The study closed prematurely, with less than 25% of the planned accrual, due to potential concerns for thromboembolic event (TE) with R-HUEPO. Median follow-up was 37 months (range 9.8-50.4 months). PFS and OS at three years were 66% and 74% for CT/RT and 58% and 60% for CT/RT + R-HUEPO, respectively. TE occurred in 4/52 receiving CT/RT and 11/57 with CT/RT + R-HUEPO, not all considered treatment related. No deaths occurred from TE. High platinum-adducts were associated with inferior PFS and LC.

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Conclusion—TE are common in cervical cancer patients receiving CT/RT. Difference in TE rate between the two treatments was not statistically significant. The impact of maintaining Hgb level > 12.0 g/dL on PFS, OS & LC remains undetermined.

Keywords

erythropoietin; carcinoma of cervix; thromboembolism; Platinum-DNA adducts

Introduction

Concurrent cisplatin-containing chemotherapy with pelvic irradiation has become a standard of care for the management of patients with advanced cervical cancer.¹ Despite the gains made in local control and survival with the addition of concurrent chemotherapy to irradiation, results are still unsatisfactory, particularly for those with advanced or bulky disease in the pelvis.

More recently, research efforts in multiple solid tumors have focused on attempts to identify strategies that target specific molecular or clinical features expressed by the tumor. In patients with carcinoma of the cervix, anemia and tumor hypoxia are two such specific relevant targets. A significant proportion of patients with carcinoma of the cervix treated with irradiation have a hemoglobin level at presentation ≤ 12.0 g/dL.² In the US, 44% of patients present with hemoglobin levels of 10.0-12.0 g/dL, 15% with 8.0-10.0 g/dL and 10% with 6.0-8.0 g/dL. One randomized study examining the role of concurrent cisplatin and radiation in advanced cervical cancer has demonstrated that the addition of concurrent cisplatin significantly reduces hemoglobin levels during treatment compared to those observed when irradiation alone is used.³

The prognostic significance of anemia, both at presentation of disease and during radical radiation treatment, has been examined across many solid tumors, but particularly in head and neck and cervical cancer. Almost all studies that have examined anemia as a prognostic factor have shown that anemia predicts for poorer local control and overall survival in patients treated with radical irradiation with or without chemotherapy. It is unknown whether low hemoglobin levels are causally related to poor outcomes or whether they are simply an epiphenomenon accompanying more aggressive tumors with an inherently bad prognosis or those in whom response to treatment is poor. If low hemoglobin levels are causally related to poor prognosis, the mechanism by which this occurs is unclear but several postulates have been put forward. Tumor hypoxia results in relative irradiation and chemotherapy resistance.⁴⁻⁷ In addition, hypoxia is an important stimulus for angiogenesis; it drives the process of malignant progression resulting in the up regulation of enzymes and proteins which lead to tumor invasion, the down regulation of cell surface antigens resulting in cell detachment, genomic instability with mutagenic properties and the increased clonal selection of cells with apoptosis enhanced survival.⁸ It has been assumed that low hemoglobin levels may therefore result in impaired tumor oxygenation, although tumors exposed to low levels of oxygen as seen in the chronic anemia of malignancy are able to adapt to the microenvironment and restore tumor oxygenation.⁴⁻⁷ Acute anemia, for example from bleeding, is also likely to cause tumor hypoxia. Nevertheless, multiple studies have demonstrated a relationship between decreasing hemoglobin levels and increasing levels of tumor hypoxia.⁹⁻¹¹ Similarly, studies have demonstrated a relationship between hemoglobin levels and various markers of angiogenesis.^{12,13}

With the assumption that low hemoglobin levels were causally related to poor outcomes, clinicians have often corrected the anemia of patients with carcinoma of the cervix undergoing chemo-radiation (CT/RT) therapy by administering blood transfusions. Usually, however, the common practice was to offer blood transfusions only if the hemoglobin dropped below 10.0

g/dL. One study did examine local control rates for patients with the hemoglobin maintained by transfusion not greater than 12.5 g/dL.^{6,14} While this study is widely quoted as showing benefits, the specific effect of transfusion was not evaluable given the relatively small number of patients and the lack of a multivariate analysis controlling for other prognostic factors.

Thus, the question remains as to whether raising and maintaining hemoglobin during treatment can positively influence local control and survival. Two studies of the effect of hemoglobin on patients treated with radiation alone or radiation plus cisplatin-containing concurrent chemotherapy have demonstrated a stepwise increment in survival associated with each 1.0 g/dL of hemoglobin above 10.0 g/dL with optimal survival levels above 12.0g/dL and not significantly increasing beyond 12.0 g/dL.^{2,15}

It appeared from the retrospective analyses that the level of hemoglobin maintained during radiation treatment (rather than the presenting hemoglobin level) was specifically and independently related to outcome. The outcome was independent of whether the patient's hemoglobin was spontaneously above 12.0 g/dL or whether it was raised by transfusion.²

Given this background, the current study was designed to investigate the efficacy of raising and maintaining patient hemoglobin levels above 12.0 g/dL using erythropoietin compared to maintenance above 10.0 g/dL without erythropoietin.

To obtain additional information on potential prognostic and predictive markers in this patient population, platinum-DNA adducts in buccal cells were examined affecting response, the measurement of several biomarkers was also planned in this study, including cisplatin DNA-adduct. It is hypothesized that high levels of platinum-DNA adducts indicates less efficient DNA repair and would be a good prognostic factor if the high level of DNA adducts triggered cell cycle arrest and apoptosis (sensitive tumor) but would be a poor prognostic factor if the high level of platinum-DNA adducts failed to trigger cell cycle arrest and apoptosis (resistant tumor).

Information relating the level of platinum-DNA adducts in peripheral blood leukocytes (PBL) to disease response in a number of cancers are conflicting. While some report a positive correlation in testicular, breast, colon, lung, esophageal, and ovarian cancer, others report no association or a negative association.¹⁶⁻²³ A positive association has also been observed between platinum-DNA adducts in buccal cells and either disease response or better overall survival in a variety of cancers.^{24,25} DNA-adducts had not yet been studied in cervical cancer. The purpose was to test the predictive value of platinum-DNA adducts with regard to outcome in cervical cancer.

Materials and Methods

Patients with primary previously untreated, histologically confirmed invasive squamous cell carcinoma, adenocarcinoma or adenosquamous carcinoma of the uterine cervix stage IIB, IIIB and IVA were eligible for this study (GOG #191). The patients were to have a hemoglobin level at presentation of less than 14.0 g/dL. Patients were to have negative para-aortic lymph nodes as determined by lymphangiogram, computerized tomography (CT), magnetic resonance imaging (MRI) or surgical staging. All patients were to have adequate hematologic, renal and hepatic function as well as performance status for study entry.

This study received local institutional review board approval and all patients signed an approved informed consent. The primary clinical objective was to determine the efficacy of raising and maintaining patient hemoglobin levels above 12.0 g/dL using erythropoietin compared to maintenance above 10.0 g/dL without erythropoietin on PFS, OS and LC.

Eligible patients were randomly assigned to receive either R-HUEPO if their hemoglobin level was <12.0 g/dL in order to raise and maintain the hemoglobin level to ≥ 13.0 g/dL during treatment versus standard supportive management in the control arm. In the latter arm recommendation was made (that a transfusion be given to raise the level to greater than 10.0 g/dL) if it fell to less than 10.0 g/dL. The chosen eligibility hemoglobin level of less than 14.0 g/dL would capture the majority of patients whose hemoglobin would fall to less than 12.0 g/dL during the course of radiation treatment.² The maintenance level of ≥ 13.0 g/dL was chosen to insure that a hemoglobin level of greater than 12.0 g/dL would likely be maintained even in those continuing to undergo bleeding from the tumor. For patients in the intervention arm where there was insufficient time to raise the hemoglobin level to greater than 12.0 g/dL before the start of radiation treatment, blood transfusion was given and thereafter R-HUEPO. If the patients in the intervention arm have vaginal bleeding resulting in fall in hemoglobin below 12.0g/dL blood, transfusion was to be used to elevate the level to greater than 12.0 g/dL in addition to continued administration of erythropoietin.

STUDY MODALITY – Radiation Therapy—The external beam radiation dose prescription to the whole pelvis was 45 Gy in 25 fractions at the isocentre. Intracavitary brachytherapy dose could be performed with either low dose rate (LDR), 40 Gy in one to two fractions or high dose rate (HDR), 30 Gy in five fractions. Tandem and ovoid or tandem and ring applicators were recommended for brachytherapy. HDR brachytherapy was to be started where possible in the fourth week of external beam therapy with at least one fraction per week. After completion of external beam irradiation, two fractions of HDR were given per week. Parametrial boosts of 5.4-9.0 Gy in 3 to 5 fractions of 1.8Gy were to be given for involved parametria. The overall radiation treatment time was not to exceed eight weeks.

STUDY MODALITY – Chemotherapy—All patients were to receive cisplatin 40 mg/m², not to exceed 70 mg, on days 1, 8, 15, 22 and 29 of external beam irradiation and once during the parametrial boost for a total of six weekly cycles. Cisplatin was reduced by 25% for grade IV nausea and vomiting or grade II neurotoxicity and was discontinued for creatinine greater than 2.0 mg% or grade III-IV neurotoxicity. Cisplatin was withheld for an absolute neutrophil count less than 1500 or platelet count less than 100, 000.

In the intervention arm, the patients were to receive R-HUEPO at presentation and during radiation to raise and maintain their hemoglobin ≥ 13.0 g/dL but ≤ 14.0 g/dL. The starting dose was 40,000 units subcutaneously weekly. The first dose was administered as soon as possible after randomization and dosing discontinued if the hemoglobin was above 14.0 g/dL on two consecutive weeks. R-HUEPO would be recommenced if the hemoglobin level dropped below 13.0 g/dL. If the hemoglobin level of greater than 12.0 g/dL could not be reached before commencing chemoradiation or fell abruptly to ≤ 12.0 g/dL, transfusion was to be given to raise the hemoglobin to greater than 12.0 g/dL. Cisplatin-DNA adducts in buccal cell samples: Buccal cells were collected using cotton swabs before and two hours after the completion of the first and second course of cisplatin. The harvested cells were suspended in ethanol (70 %) and stored until shipment and analysis.

The preparation of cytopins slides and the immunocytochemical staining procedure for cisplatin-adducts was performed as reported earlier, with some modifications.²⁰ Immunofluorescence using Cy5-Streptavidine was applied as final staining procedure instead of peroxidase and image analysis was performed with CCD camera instead of a scanning spot method. In addition, pooled buccal cells from nine healthy volunteers were incubated in vitro with different doses of cisplatin (0, 0.25, 0.5, 1, 2, and 4 μ g/ml for 2 hours). In each experiment, complete sets of buccal cell slides per patient (pre- & post-cycle 1 and pre- and post-cycle 2), together with a aliquot of the reference pool dose response buccal cells were stained and

analyzed simultaneously. Inclusion of common reference pool cells served as an internal control for each staining run.

Images were recorded using a digital CCD camera (10× magnification), with separate acquisition of Cy5 (adduct staining) and DAPI signals (nuclear staining). All images were stored on a central server and analyzed with Image Pro-Plus software (Media Cybernetics Inc., USA). To eliminate nonspecific extra-nuclear staining, nuclei were located by means of DAPI staining using automatic thresholding, and used as a mask for the subsequent Cy5 staining intensity measurements. The mean intensity of all stained buccal cell nuclei was recorded per sample and expressed as “cisplatin-dose equivalent.” This was calculated by fitting a second degree polynomial curve through the dose response curve for the reference buccal cells, stained concurrently, and using this fit to convert staining intensity of the test sample to cisplatin dose ($\mu\text{g} \cdot \text{ml}^{-1}$; 2h). This procedure corrects for batch differences in staining.

Due to early closure of GOG #191, the study did not provide the information necessary to address other translational research, biomarker, and quality of life objectives.

Statistical Methods and End Points

The primary clinical endpoints were PFS, OS and LC. Progression was defined as a 50% or greater increase in the cross-product of the existing primary tumor relative to the smallest cross-product from all previous exams. The defined PFS was calculated as the time in months from enrollment onto GOG #0191 to disease progression or death for non-censored events or to the date of last contact for censored events, that is, for patients alive with no evidence of disease progression. OS was calculated as the time from enrollment to death for non-censored events; or to the date of last contact for censored events, that is, for patients alive, regardless of disease status. Recurrence site was considered local if within the pelvic field and distant if outside the pelvic field. LC is coded as successful if no disease developed in the pelvic field and that disease remained controlled within the pelvic field. LC was coded as a failure if there was loss of local control either by progression or persistent disease within the pelvic field.

The study design, based on the ability to detect a 33% decrease in the progression hazard rate (59% versus 70% at three years) suggested 165 total recurrences be observed in order to test the primary objective of the study at 83% statistical power (probability of a true-positive study) and at the 0.05 significance level (probability of type I error). Calculations using the Gompertz model suggested enrolling 230 patients per arm with an additional follow-up of approximately two years would provide the required 165 total recurrences.²⁶

An Interim analysis was scheduled to occur that reports for the first time 55 or more patients having had a recurrence, however, the study closed prematurely and no interim analysis was conducted. Early reporting of results was permitted if a significant difference was observed between the two treatment arms (stopping for efficacy) or if the R-HUEPO arm demonstrated any degree of excess risk of recurrence (stopping for futility).

The GOG Statistical and Data Center randomly assigned eligible patients to treatment regimens. Randomization was carried out by a block arrangement; balanced within 12 strata defined by three factors; FIGO stage (IIB, III, and IVA), the type of brachytherapy (LDR and HDR) and the optional surgical procedure (para-aortic lymph node sampled: Yes or No). Canadian patients entered through the NCI Canada, were randomized through the NCIC Clinical Trials Group Central Office. Patients were to be followed quarterly for two years, semiannually for the next three years and then annually until death.

Information collected on this study was monitored for quality assurance. Data analysis (based on the intent-to-treat principle) and reporting employed the utility of SAS version 9.1, R version

2.2.0 and SPSS version 10.0. With regard to translational research results reported here, only cases with a paired buccal specimen (pre-cycle 1 and post-cycle 1 and/or pre-cycle 2 and post-cycle 2) available for testing were evaluable. Product-Limit estimates were calculated according to the method of Kaplan and Meier and differences in PFS and OS were assessed utilizing the log-rank test.²⁷⁻²⁹ The Cox model was used to adjust for prognostic factors and to estimate hazard ratios (and 95% confidence interval) of PFS and OS.³⁰ Fisher's exact test was implemented to evaluate differences in the incidence of adverse events (including TE) by treatment.³¹ Baseline patient characteristics including hemoglobin (HGB) level, tumor size, tumor cell type, stage and performance status were summarized and tabulated by treatment. Descriptive statistics and graphs summarize changes in HGB levels over duration of treatment. Assessment of treatment attribution for each observed TE resulted from attentive monitoring (of patients) and subsequent reviews (of case report forms) by the study's principal investigator. Lists and tables document in detail all observed TEs with corresponding baseline and clinical characteristics. Statistical significance of association between DNA adducts level and baseline characteristics were assessed by chi-square analysis. Exact logistic regression was used to adjust for baseline performance status and to estimate relations between relative odds for local control and incidence of severe leukopenia with respect to and platinum-DNA adducts level.^{31,32}

Results

The study closed prematurely at the request of the sponsor, after less than 25% of the planned accrual due to potential concerns for TE with R-HUEPO and the subsequent withdrawal of study drug and study support by the sponsor. However, because of concerns about a possible negative impact on survival and an increased risk of TE from previous analyses of the use of erythropoietin in other tumor sites, we offer an analysis of the TE as observed in this study.

Between August 2001 and September 2003, GOG #191 accrued 114 patients. Five of these patients were subsequently found to be ineligible for the following reasons: one patient received chemotherapy prior to registration; one had wrong stage and three for inadequate pathology. Among the remaining 109 eligible and evaluable patients, there were 52 on the control arm and 57 on the intervention arm (i.e. the R-HUEPO arm).

Table I illustrates the distribution of baseline patient characteristics by treatment regimen. The median age at time of diagnosis for patients was 50 versus 46 years, with 27% versus 49% being less than or equal 45 years, in the control arm versus the R-HUEPO arm respectively. Some imbalance was seen in performance status in the control versus the R-HUEPO arm; 77 versus 65% had GOG performance status 0, 19 versus 33% had GOG status 1 and 4 versus 2% had GOG performance status 2, respectively. FIGO stage was somewhat imbalanced; 75% of the control versus 65% of the R-HUEPO patients had stage IIB disease, 25 of the control versus 35% of the erythropoietin arm had stage IIIB and IV disease. Imbalance was also seen between the treatment arms with respect to tumor size; 52% in the control arm versus 33% in the R-HUEPO arm had tumors up to 5 cm in diameter; 29% in the control arm versus 44% in the R-HUEPO arm had tumors 6-7 cm; 19% in the control arm versus 23% had tumors in excess of 8 cm. The majority of tumors in both arms were squamous cell carcinoma with 10 and 7% in the control and R-HUEPO arms respectively, having adenocarcinoma or adenosquamous carcinoma.

Vital Status and Disease Outcome

As of April 2006, 68 women were alive with no evidence of disease; five women were alive with disease progression and 36 women died. Among those who died, 25 of the deaths resulted from disease progression, two of the deaths were due to treatment (one from each arm) and nine were due to causes other than disease or treatment. The median follow-up period is 37

months (range 9.8 - 50.4 months). Maximum time to progression observed was 44.4 months in each arm. Figures 1 and 2 depict Kaplan Meier estimates of PFS and OS by treatment regimens respectively. HGB levels (from baseline to first follow-up) by treatment and mean HGB level by TE occurrence are shown in figures 3A and 3B respectively. Table II shows patients' recurrence status by treatment regimen. In reference to patients on the control arm versus the R-HUEPO arm, 75% versus 68 % did not recur (DNR), 17% versus 21% had local recurrences (at least one recurrence within the pelvic field) and 8% versus 12% had only distant recurrences (recurrences outside of the pelvic field). The observed differences with respect to recurrence status between treatment regimens are not statistically significant ($p = 0.6471$).

Thromboembolic Events (TEs)

Table III provides a complete listing of all observed TEs by treatment regiment together with associated grade, time of incidence (with respect to treatment completion date), treatment attribution, patient's baseline traits and hemoglobin levels at time of TE incident.

Among the 109 evaluable patients, 33 (30.3%) were obese (BMI>30). TEs were more prevalent among obese patients, occurring in 18.2% (6/33) of the obese but in only 11.8% (9/76) of non-obese. The differences are not statistically significant ($p > 0.379$). This study did not record the smoking history.

TEs were recorded in four patients on the control arm of whom two were obese, and 11 in the erythropoietin arm, of which four were obese. One event in the control arm was a cerebrovascular incident occurring 183 days after the end of treatment and was not considered by the study chair to be related to treatment with chemoradiation. Of the eleven TEs recorded with the use of R-HUEPO, three occurred 222, 38, and 896 days after treatment completion and were not considered to be treatment related. In one other TE occurring 36 days prior to treatment completion the TE was not considered likely to be related to treatment. There were two TEs in 52 patients in the control arm and seven in 57 patients in the R-HUEPO arm that were considered to be possibly or probably related to the respective therapy. Although the incidence of TEs was larger in the R-HUEPO arm (19.3 versus 7.7%), this difference was not statistically significant (Fisher's exact test; $P = 0.0987$).

The maximum hemoglobin levels (over duration of treatment) for patients who experienced a TE and deemed possibly related to erythropoietin use was 13.2, 8.9, 12.0, 11.4, 13.5, 13.3, & 14.0 g/dL. Observed TEs were not related to excessive hemoglobin levels nor to a rapid rise in hemoglobin level (data not shown). No TE assessed as possibly related to treatment led to patient death. Among patients with stage IIB disease, 10.5% experienced a TE vs. 21.2% in those with stage IIIB-IVA ($p = 0.137$).

In the control arm, 11.5% of patients had average hemoglobin during treatment ≥ 12.0 g/dL versus 71.9% of patients in the R-HUEPO arm (Table IV). Twenty-nine (55.8%) patients in the control arm received transfusions versus 34 (59.6%) patients in the R-HUEPO arm. Among patients who experienced TE, one from the control arm and seven from the R-HUEPO arm had transfusions. Among patients who received transfusions, 1 (3.4%) from the control arm, 7 (20.6%) from the E-HUEPO arm, had TEs (Fisher's exact test; $P = 0.0598$)

Platinum-DNA adducts

Platinum-DNA adduct data was available for 53 patients pre- and post-cycle 1, and for 44 patients pre- and post-cycle 2 (Figure 4).

An ad hoc Martingale residual analysis was performed that supported the utility of this cut point. Thus, adduct levels were categorized as low when adducts were ≤ 0.6 dose equivalence

or high when adducts were > 0.6 dose equivalence. Exploratory analyses, suggested that the optimal cut point of dichotomization of adducts levels was 0.6 dose equivalence.

There was no evidence of a relationship between post-cycle 1 or post-cycle 2 platinum-DNA adducts level expressed as a continuous variable and clinical characteristics including baseline tumor size, histologic cell type or pre-treatment hemoglobin level (data not shown). An association was observed between categorized adduct level in post-cycle 1 buccal cells or post-cycle 2 buccal cells and race ($p=0.023$ and $p=0.028$, respectively), but not with patient age or GOG performance status. Categorized adducts were not associated with FIGO stage, histologic subtype or baseline tumor size. Post-cycle 2, but not post-cycle 1 platinum-DNA adducts was associated with tumor grade ($p=0.011$).

A positive association was observed between post-cycle 1 adduct level and PFS, but not with OS (Table V). A multiple regression analysis of PFS was performed that included the prognostic variables, age, FIGO stage, tumor size and PS. After adjustment for these four variables, the relative risk of disease progression for the high adducts level group were similar to estimates obtained after adjustment for PS alone. Adjusting for PS, High post-cycle 1 adducts (>0.6) was associated with an increased risk of disease progression (HR=3.373; 95% CI=1.094-10.398; $p=0.0343$). High post-cycle 1 adducts was associated with an increased odds of experiencing a local failure. (Table VI) The level of post-cycle 2 adducts was not associated with an altered risk of disease progression or death nor with a significant change in the odds of local failure. There was no evidence of an association between post-cycle 1 or post-cycle 2 adducts and any of the common severe adverse effects (data not shown), including leukopenia associated with concurrent chemo-radiation.

Discussion

There is no doubt that anemia and tumor hypoxia remain valid specific therapeutic targets in the treatment of cervical cancer. Both direct measurements and indirect studies have confirmed the presence of tumor hypoxia even in small cervical cancers. Overcoming tumor hypoxia could lead to enhanced radiosensitivity and chemo-sensitivity, as well as having the theoretical potential to turn off molecular downstream events which confer enhanced malignant potential on tumors. While the relationship between anemia and hypoxia is complex, both have been shown to be significant independent predictors for outcome with chemoradiation in solid tumors.³² The fact that a causal relationship between anemia and poor outcomes in cervical cancer has not been established formed the basis of the primary question of this clinical trial: Does raising hemoglobin >120 g/dL lead to improved outcomes when patients are treated with chemoradiation?

While it is recognized that the use of exogenous poietins may increase the risk of TE, the two main studies in which TE occurred at an excessive rate and led to a decision to close ongoing studies sought to raise hemoglobin levels to those in excess of normal.^{33,34} In cervical cancer, particularly in those with advanced pelvic disease and pelvic sidewall disease, it is well recognized that TE occur without erythrocyte stimulating agents (ESAs), particularly in the context of treatment with radiation and cisplatin chemotherapy. These events have been reported with an incidence of 0.1-16.7%.³⁵ The high TE rate in this disease is not surprising given that cervical cancer patients often have bulky pelvic disease which may compress pelvic veins and lead to lower limb deep vein thrombosis. Deep vein thrombosis in upper limbs has been associated with the use of PICC lines for administration of chemotherapy.³⁶ Multiple comorbidities have been previously correlated with risk of thromboembolic events. These may include age, body mass index and smoking history. In one non-randomized study in cervical cancer patients receiving chemoradiation, TE events were observed in 2/72 not receiving ~HuR-HUEPO vs 17/75 receiving ~HuR-HUEPO, 14 of the 17 were upper extremity

thromboses, all associated with the use of PICC lines.³⁷ A significant proportion of patients with cervical cancer are obese and smoke, both risk factors for TEs. In the present study, the rate of TEs was higher in the R-HUEPO arm compared to the control arm (although not statistically significant).

It is unfortunate that this study was closed prior to its completion without clear indicators of possible positive or negative effects on outcomes and toxicities. Concern has been raised recently that ESAs may have a negative effect on local control and survival. Three recent randomized controlled trials including curative and palliative patients indicate that patients receiving ESA had worse outcomes than those in the control arm.³⁸⁻⁴⁰ DAHANCA 10 trial which evaluates ESA in head and neck cancer patients receiving curative radiotherapy, closed early because of worse local control and a trend to worse survival in patients in the ESA arm.^{41,42}

Many cancer therapies are associated with significant adverse events. Certain rates of events are deemed acceptable if the intervention results in significant improvements in treatment outcomes. In many studies of advanced ovarian cancer for example, high rates of complications are accepted for the use of intraperitoneal chemotherapy for non-curative treatments which prolong life significantly by a few months.⁴³ In cervical cancer improved outcomes are measured in terms of improved cure rates. Early closure of this study means that we are unlikely to understand the therapeutic ratio associated with the use of R-HUEPO to raise hemoglobin levels in the context of chemoradiation for cervical cancer. While the literature overall suggests an increased TE rate associated with the use of R-HUEPO, one might be prepared to accept an increased rate if the strategy had been shown to lead to improved survivals.

In the subset of patients in which we analyzed cisplatin-DNA adducts, the presence of high adducts in the post-cycle 1 buccal cell sample was associated with decreased PFS and locoregional control. This was not expected based on a similar but smaller study in NSCLC, where high buccal cell adducts were associated with better survival.¹³ However, other studies in the literature are conflicting concerning the relationship between adduct levels and outcome. Inconsistencies may be related to the range of different tumor and cell types studied, different chemotherapy schedules, and different measurement methods and assay times. The present study represents the only study on cervix cancer, and is one of largest. We conclude, that at present, buccal cell adducts do not provide a robust method of predicting outcome in this disease.

In future studies, in addition to adduct formation, collecting data on the expression of relevant DNA repair genes and other known cisplatin resistance genes, for both normal and tumor tissue, would be informative.

It is doubtful that this study will be repeated, but the current phase III study of the GOG continues to focus on hypoxia as a target for improving therapy. This study will examine the role of tirapazamine, a specific hypoxic cell cytotoxic, in combination with cisplatin and radiation in the management of patients with advanced cervical cancer.

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Figure 1. Progression-free survival by treatment regimen among all evaluable patients.

Figure 2.
Overall survival by treatment regimen among all evaluable patients.

Figure 3.

Figure 3A: Box plots of HGB (g/dL) levels over treatment span; Solid and broken lines demonstrate the trend in mean HGB levels for the control and R-HUEPO arm respectively. Time point 1 = baseline, time point 2=prior to cycle 1..., and time point 8 is first follow up after completion of therapy.

Figure 3B: Box plots showing distribution of mean HGB (g/dL) for subjects with TE and subjects without TE

Figure IV.
Box plots showing distribution of adduct level

Table I
Patients baseline characteristics by treatment regimen

		Control arm (N=52)	R-HUEPO arm (N=57)
Age (years)	<=45	14 (27)	28 (49)
	(45-65]	29 (56)	22 (39)
	> 65	9 (17)	7 (12)
	Median	50.0	46.0
	Range	32-78	25-77
Race	White	37 (71)	31 (54)
	Black	11 (21)	15 (26)
	Hispanic	1 (2)	8 (14)
	Other	3 (6)	3 (5)
Tumor size (cm)	<=3	2 (4)	4 (7)
	4-5	25 (48)	15 (26)
	6-7	15 (29)	25 (44)
	8-9	6 (12)	11 (19)
	>10	4 (8)	2 (4)
	Median	5.0	6.0
	Range	1-15	1-11
FIGO Stage	II-B	39 (75)	37 (65)
	III-B	11 (21)	17 (30)
	IV-A	2 (4)	3 (5)
Cell Type	Squamous cell carcinoma	47 (90)	53 (93)
	Adenocarcinoma, NS	3 (6)	2 (4)
	Adeno-squamous	2 (4)	2 (4)
GOG PS *	0	40 (77)	37 (65)
	1	10 (19)	19 (33)
	2	2 (4)	1 (2)

* GOG performance status

Table II
Recurrence status by treatment regimen

	Control arm (N=52)	R-HUEPO arm (N=57)
Recurrence status:		
Did Not Recur	39 (75.0)	38 (67.7)
Local*	9 (17.3)	12 (21.0)
Distant**	4 (7.7)	7 (12.3)

Fisher's exact test, P = 0.6471

* Patients with recurrence within the pelvis

** Patient with non-pelvic recurrence(s) only

Table III
Listing of observed thromboembolic events (TE) and corresponding patient traits

Regimen	TE	Grade	Time ^a (days)	Treatment Attribution	Max ^b HGB (g/dL)	HGB at TE (g/dL)	BMI	Tumor Size (cm)	Stage	P S ^c
Control arm	DVT	3	-6	Unlikely	13.2	10.5	61	7.0	IIB	0
	DVT	4	2	Probably	13.2	10.8	28	4.0	IIB	0
	Stroke	4	183	Unlikely	11.0	11.0	19	7.0	IIIB	0
	DVT	3	-15	Probably	13.2	10.4	34	4.0	IIIB	0
	DVT	4	-56	Possibly	13.2	13.2	49	9.0	IIIB	1
	Stroke	2	896	Unlikely	15.4	12.7	26	8.0	IIB	0
R-HUEPO Arm	DVT	4	-34	Possibly	13.2	8.9	18	7.0	IIB	1
	Stroke	4	-14	Possibly	13.2	12.0	22	8.0	IIIB	1
	DVT	4	-33	Unlikely	15.4	12.1	24	6.5	IIIB	1
	DVT	4	-13	Possibly	15.4	11.4	18	8.0	IIB	0
	DVT	4	222	Unlikely	13.2	13.4	32	4.5	IIB	0
	MI	5	38	Unlikely	13.2	11.7	23	4.0	IVA	1
	DVT	3	-49	Possibly	13.2	13.5	31	6.0	IIB	0
	DVT	3	-10	Possibly	13.2	13.3	34	8.0	IIB	0
	CNS	4	3	Possibly	15.4	14.0	29	8.0	IIIB	2

^aTime from treatment completion, (negative indicates that TE occurred before treatment completion)
^bMaximum HGB
^cPerformance Status
DVT= deep vein thrombosis; MI = Myocardial infarction; CNS H = CNS hemorrhage

Table IV
Mean HGB and Transfusion-TE category by treatment

	Control arm,N=52	R-HUEPO arm,N=57
Mean HGB level *		
< 12.0 g/dL	46 (88.5)	16 (28.1)
≥ 12.0 g/dL	6 (11.5)	41 (71.9)
Transfusions, TE **		
N, -TE	20	19
N,+TE	3	4
Y,-TE	28	27
Y,+TE	1	7

* Mean HGB level over treatment duration

** N=No or Y=Yes for transfusion; -TE and +TE for TE indicator

Numbers in parentheses are percentages of N

Table V
Relationship between Platinum-DNA Adducts and Overall Survival or Local Control

Endpoint	Platinum-DNA Adducts ^a	Statistic	Model	
			Unadjusted	Adjusted ^b
Overall Survival	Post-Cycle 1 High vs. Low	HR	2.273	2.733
		95% CI	(0.747 – 6.916)	(0.821 – 9.910)
		p-value	0.148	0.101
	Post-Cycle 2 High vs. Low	HR	3.862	3.761
		95% CI	(0.457 – 32.667)	(0.437 – 32.385)
		p-value	0.215	0.228
Local Control	Post-Cycle 1 High vs. Low	OR	7.530	28.993
		95% CI	(1.061 – 56.734)	(2.558 – 328.568)
		p-value	0.043	0.007
	Post-Cycle 2 High vs. Low	OR	2.368	2.429
		95% CI	(0.036 – 51.722)	(0.193 – 30.626)
		p-value	0.923	0.493

Hazard ratio (HR), Odds ratio (OR), 95% confidence interval (95% CI)

Platinum-DNA adducts were categorized as either low (≤ 0.60 platinum dose equivalence) or high (> 0.60 platinum dose equivalence)

^a Post-Cycle 1: Low (N=45) and High (N=8); Post-Cycle 2: Low (N=41) and High (N=3)

^b Adjusted for performance status (asymptomatic vs. symptomatic).