Biologics in Cervical Cancer Therapy

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Cervical cancer, VEGF, bevacizumab, EGFR, erlotinib, cetuximab

Abstract
Though cervical cancer incidence and prevalence have decreased in the United States, the disease remains a very important cause of morbidity and mortality worldwide. Current therapy for early-stage disease is surgical with adjuvant therapy being administered according to histopathologic findings. Pelvic radiation with concomitant platinum-based chemotherapy is used to treat locally advanced disease, whereas metastatic and recurrent lesions continue to be difficult to effectively treat and cure. Clinical trials in this latter scenario have suggested that clinical benefit may be associated with biologic therapies. This article focuses on the use of targeted therapies in cervical cancer, specifically evaluating antiangiogenesis and endothelial growth factor receptor–related treatments. (JNCCN 2010;8:1417–1423)

Epidemiology
In the United States, 11,270 new cases of cervical cancer and 4070 associated deaths occurred in 2009. These numbers represent a significant decline in incidence and prevalence that is attributed to excellent screening protocols and treatment of preinvasive conditions. These numbers are in sharp contrast to worldwide statistics, which show that cervical cancer is the second leading cause of cancer-related mortality in women. When cervical cancer is detected early, the treatment protocol is surgical resection, typically with radical hysterectomy and pelvic lymph node dissection. Cure rates are good in this instance. However, more advanced disease is a greater challenge to treat effectively. The American Cancer Society estimated that, in 2006, cervical cancer at diagnosis was locally advanced or associated with regional nodal metastases in 32% of cases, and associated with distant spread in 8%. These higher-stage lesions were associated with a significant decline in outcome, with 5-year survival rates of 55% in the former and 17% in the latter.

Interestingly, although overall rates have declined in the United States, certain ethnic groups remain at increased risk for poor outcomes. African American women have an increased incidence of disease compared with white women (11.5 vs. 7.3 cases/100,000 population), and have twice the mortality. Hispanic women experience nearly double the incidence (14.2/100,000) of non-Hispanic whites. Other populations are also at increased risk, such as the increased disease burden seen among immigrant populations from Asia, Eastern Europe, and South America. Women with limited access to health care and therefore limited access to preventative care, and those of lower socioeconomic status are also at increased risk for disease and its associated morbidities.

Angiogenesis
Angiogenesis is the development of new blood vessels in areas of novel tissue growth. This process is a normal physiologic phenomenon and is associated with routine processes, such as embryogenesis and wound healing.
This essential process occurs universally in solid tumors secondary to expansion of the cancer mass and subsequent growth away from the existing blood supply. Because of this outward growth, the oxygen tension falls beneath levels required for oxidative metabolism, creating areas of hypoxia.\(^5\)

In this hypoxic state, an interplay of proangiogenic signaling occurs. One protein involved in this cascade is hypoxia-inducible factor (HIF)-1\(\alpha\), which is stabilized in these conditions. This protein subsequently enters the nucleus where it can form a complex with HIF-1\(\beta\); this complex then acts as a transcription factor allowing upregulation of several growth factors, one of which is vascular endothelial growth factor (VEGF).\(^6,7\) The VEGF family includes 6 closely related proteins, but the most important member in angiogenesis is VEGF-A.

The mechanism through which VEGF exerts its influence seems to be multifactorial. The initial hypothesis was that this action was mediated by a paracrine mechanism, whereby tumor cells produced VEGF that subsequently stimulated endothelial cells containing VEGF receptors.\(^8\) The current view is that its action is mediated by several mechanisms, including origination from host cells such as muscle cells and platelets.\(^8\) Stromal cells associated with tumors seem to generate VEGF\(^9,10\). Another theory includes an autocrine action, whereby tumor cells produce VEGF that subsequently triggers receptors located on their own cell surface.\(^11–13\)

Sprouting-type angiogenesis is an important mechanism in tumor neovascularization. In this process, endothelial remodelling occurs after endothelial cell proliferation and migration toward tumor cells, leading to tubule and loop vessel formation. Pericytes are recruited, allowing maturation of the new vasculature through stabilization of the new vessels.\(^14\) After this maturation, growth factors are no longer integral in the survival of the endothelium, but in neoplastic growth the maturation is usually incomplete secondary to the constant vascular remodelling into tumor stroma. Because of this immaturity, endothelial cells within these vessels require growth signals for survival to avoid apoptosis. Examples of these mechanisms include vasculogenesis, glomeruloid angiogenesis, vascular remodeling, and vascular mimcry.\(^15\)

Although the process of angiogenesis is common among most solid tumors, cervical cancers are unique in that they are associated with the secondary down-regulation of the tumor suppressor gene, p53. This is essentially the loss of p53 function without the necessity of a p53 mutation. p53 is an important component of cervical carcinogenesis and is deregulated in almost all epithelial cervical cancers. This deregulation occurs through interaction with the oncogenic protein human papillomavirus (HPV) E6 through 2 mechanisms (Figure 1).\(^16\) The first involves blockage of p53 induction that typically occurs after DNA damage, which would normally allow the cell to enter arrest and undergo DNA repair or, if fatally injured, apoptosis. The second mechanism occurs when E6 induces ubiquitination of p53 and subsequent protein degradation.

The effects of angiogenesis and hypoxia as independent risk factors in cervical cancer have been evaluated in multiple prior studies. Cooper et al.\(^17\) evaluated formalin-fixed paraffin-embedded tumor biopsies for intratumoral microvessel density (IMD) and distance to the closest microvessel in specimens from 111 patients who were treated with pelvic irradiation. They reported that high vascularity was associated with 50% 5-year survival, whereas low vascularity had a 65% survival. IMD was a significant prognostic marker on multivariate analysis, because high tumor vascularity was associated with lower locoregional control and overall survival.

Contrast-enhanced dynamic MRI (CD-MRI) is an imaging modality that can be used to measure vessel permeability, tumor cellular volume, and vascular volume. Rasila et al.\(^14\) evaluated CD-MRI–derived characteristics and histologic microvessel density counts in patients with primary or recurrent cervical cancer, and found that 2 of the parameters, amplitude and exchange rate constant, increased with increasing histologic microvessel density counts. Hawighorst et al.\(^18\) evaluated primary tumors treated with radical hysterectomy, and found that the exchange rate constant was a significant predictor of poor patient survival.

**VEGF Targeted Therapy**

Bevacizumab is a monoclonal antibody directed against VEGF-A. It was the first clinically available antiangiogenic agent in the United States\(^19\) and has been successfully studied in many solid tumors, including colon, lung, breast, renal, brain, and ovarian carcinomas. Its efficacy was clearly shown in a study
by Hurwitz et al., who evaluated the use of bevacizumab in first-line colorectal cancer therapy in combination with irinotecan, 5-fluorouracil, and leucovorin in patients with metastatic disease. This study found that the median survival in patients treated with bevacizumab was 20.3 months versus 15.6 for those in the control arm, which represented a hazard ratio of 0.65 favoring bevacizumab therapy. Statistically significant improvement in time to progression, which was delayed by 4 months, and overall response rate, which was increased by 10%, was witnessed in patients who underwent bevacizumab therapy. After the promising results in colorectal cancer, the use of bevacizumab therapy in other solid tumors has been evaluated.

The first trials of bevacizumab in patients with cervical cancer were performed in subjects for whom prior therapy failed. Wright et al. performed a retrospective study in patients who had been treated with a median of 3 prior regimens. Evaluation of 6 patients with multisite metastatic disease treated with either bevacizumab plus 5-fluorouracil (83%) or capecitabine (17%) found clinical benefit in 67% of patients, with 1 complete response, 1 partial response, and 2 patients with stable disease. Median time to progression in the 4 patients who displayed clinical benefit was 4.3 months. The therapy was well tolerated, with 1 grade 4 toxicity of neutropenic sepsis noted. The authors thus concluded that combination bevacizumab is well tolerated and showed encouraging antitumor activity in heavily pretreated recurrent cervical cancer.

After this promising result, the Gynecologic Oncology Group (GOG) performed a prospective phase II study of bevacizumab therapy in patients with cervical cancer who must have undergone 1 prior chemotherapeutic regimen (GOG protocol 227-C). The study protocol used bevacizumab at 15 mg/kg every 3 weeks until disease progression or adverse effects prohibited further therapy. Primary end points were progression-free survival (PFS) at 6 months and toxicity. The study enrolled 46 patients, of whom 38 had received prior radiation and either 1 (n = 34) or 2 (n = 12) prior cytotoxic regimens for recurrent disease. Toxicities classified as grade 3 or 4 adverse events possibly related to bevacizumab included hypertension, thromboembolism, gastrointestinal, anemia, cardiovascular, vaginal bleeding, neutropenia, and fistula. One grade 5 infection was observed. Of the patients studied, 11 survived progression-free for at least 6 months, and 5 had partial responses. In patients with response, the median duration was 6.21 months. The median PFS was 3.4 months and median overall survival was 7.29 months, which compared favorably with historical phase II GOG trials in this patient population (Figure 2). Thus, the authors recommended that, given the tolerability of the regimen and activity witnessed in patients undergoing second- and third-line therapy, the drug should be studied in a phase III setting.

Therefore, the GOG is currently testing bevacizumab in a phase III trial among patients with primary IVB cervical cancer or those with recurrent or
activated.

Activated EGFR can phosphorylate and subsequently activate several substrates, including PI3 kinase/AKT, which is important for cell survival, and mitogen-activated protein kinase (MAPK), which is important for cell growth.

Therefore, the cell functions mediated by EGFR may be critically important in tumor progression. EGFR has been found to be produced in approximately 75% of squamous cell cancers of the cervix, but its impact on prognosis is controversial. Inhibition of tumor-associated EGFR with monoclonal antibodies leads to growth arrest.

A prior study in head and neck tumors found that inhibition of EGFR by C225, a monoclonal antibody specific against EGFR (discussed in detail later), was active when coadministered with cisplatin in patients whose tumors had a cisplatin-resistant phenotype. Therefore, the cell functions mediated by EGFR may be critically important in tumor progression. EGFR has been found to be produced in approximately 75% of squamous cell cancers of the cervix, but its impact on prognosis is controversial. Inhibition of tumor-associated EGFR with monoclonal antibodies leads to growth arrest.

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**Endothelial Growth Factor Receptor–Targeted Therapy**

Endothelial growth factor receptor (EGFR) is a 170 kDa transmembrane glycoprotein that promotes cell growth in various normal and transformed tissues when activated. Activated EGFR can phosphorylate and subsequently activate several substrates, including PI3 kinase/AKT, which is important for cell survival, and mitogen-activated protein kinase (MAPK), which is important for cell growth.

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**Table 1 GOG 240 Schema**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Paclitaxel, 135 mg/m² (or 175 mg/m²), plus cisplatin, 50 mg/m²</td>
</tr>
<tr>
<td>II</td>
<td>Paclitaxel, 135 mg/m² (or 175 mg/m²), plus cisplatin, 50 mg/m², plus bevacizumab, 15 mg/kg</td>
</tr>
<tr>
<td>III</td>
<td>Topotecan, 0.75 mg/m² days 1–3, plus paclitaxel, 175 mg/m²</td>
</tr>
<tr>
<td>IV</td>
<td>Topotecan, 0.75 mg/m² days 1–3, plus paclitaxel, 175 mg/m², plus bevacizumab, 15 mg/kg</td>
</tr>
</tbody>
</table>

*Schema for GOG 240, a trial designed to evaluate 4 regimens against cervical cancer in a head-to-head fashion. This includes paclitaxel plus cisplatin, topotecan plus cisplatin, and both regimens with bevacizumab.*
was performed with erlotinib (an oral tyrosine kinase inhibitor) in patients with recurrent squamous cell carcinoma of the cervix. The objectives of the study were to evaluate the proportion of patients with tumor response, PFS for at least 6 months, and the frequency and severity of toxicities. The study enrolled 28 patients, of whom 25 were evaluable. No objective responses were seen, 4 patients (16%) had stable disease, and only 1 patient had a PFS of 6 months or more. The one-sided 90% CI for response was 0% to 8.8%. The drug was well tolerated, with the most common adverse events being gastrointestinal, fatigue, and rash. The authors concluded that erlotinib was inactive as monotherapy in patients with recurrent squamous cell carcinoma of the uterine cervix.

Cetuximab is another drug targeting EGFR that has been evaluated in cervical cancer. Cetuximab (C225) is a human/murine chimeric monoclonal antibody formed through cloning the light and heavy chains of the murine antibody (M225) and adapting them for expression with the constant regions of the human immunoglobulin kappa light chain and gamma 1 heavy chain. Preclinical studies examining this drug have shown that cetuximab binds to EGFR with high affinity and is able to compete with EGF and transcription growth factor-alpha (TGF-α), which inhibits subsequent receptor activation and signaling. Binding of cetuximab to EGFR also induces receptor dimerization, internalization, and down regulation. After these receptor alterations, downstream cellular effects are witnessed, including potentiation of apoptosis and cell cycle arrest through upregulation of a cyclin-dependent kinase inhibitor. Other downstream effects include inhibition of tumor VEGF production, which subsequently leads to reduced tumor microvessel density and inhibition of metastases, and invasion through matrix metalloprotease inhibition.

Bellone et al. evaluated EGFR-1 expression in both short-term and established cervical cancer cell lines that were generated from primary and metastatic/recurrent disease sites. The authors evaluated the sensitivity of cervical cancer cell lines to treatment with cetuximab and found that 14 of 14 (100%) of primary tumors and 7 of 8 (87.5%) of established cervical cancer cell lines expressed EGFR-1. The cell lines derived from recurrent or metastatic sites of disease expressed higher levels of EGFR-1 than those from primary sites. Tumor proliferation was significantly inhibited by cetuximab in all cervical tumors studied.

The GOG evaluated the use of cetuximab as monotherapy in a phase II trial of the treatment of persistent or recurrent squamous or non–squamous cell carcinoma of the cervix. This study schema used weekly cetuximab until disease progression or adverse events prevented further therapy (GOG protocol 227-E). The trial closed to accrual in July 2009 and the results are eagerly anticipated. However, the addition of cetuximab to cisplatin in the treatment of persistent or recurrent disease (GOG 76-DD) did not indicate additional benefit beyond cisplatin therapy.
First Randomized Trial of Targeted Agents in Cervical Cancer

Finally, a recently reported head-to-head randomized phase II trial compared EGF-based therapy with antiangiogenesis therapy in women with advanced or recurrent cervical cancer. This study evaluated lapatinib, an oral EGFR-TKI with HER2 activity, versus another oral tyrosine kinase inhibitor, pazopanib, which targets VEGFR, platelet-derived growth factor receptor (PDGFR), and c-Kit. This study convincingly suggested the superiority of antiangiogenesis therapy. Pazopanib improved PFS (hazard ratio, 0.66; 90% CI, 0.48–0.91; P = .013) and was well tolerated (Figure 3). The only grade 3 adverse event that occurred in greater than 10% of subjects was diarrhea (11% pazopanib and 13% lapatinib). Pazopanib offers great promise in treating cervical cancer because of its oral administration and the low incidence of serious toxicity. This is especially important because most cervical cancer cases occur in the developing world, and therapy for advanced and recurrent disease is heavily focused on palliation, which renders treatment-related severe side effects unacceptable. However, although some therapeutics may be feasible in the developing world, perhaps the greatest strides in the third world could be made through better research into and implementation of widespread, inexpensive screening programs and administration of HPV-associated vaccinations.

Conclusions

Angiogenesis is central to cervical cancer development and progression. The dominant role of angiogenesis in cervical cancer seems to be directly related to HPV inhibition of p53 and stabilization of HIF-1α, both of which increase VEGF. The binding and inactivation of VEGF by bevacizumab seem to shrink cervical tumors and delay progression without appreciable toxicity, and this is being studied in a GOG phase III trial. Some trials of EGFR targeted agents have not shown good promise as monotherapy or combined therapy with traditional chemotherapeutics, but more recent results from a GOG trial of cetuximab are pending. Other intracellular tyrosine kinase inhibitors of angiogenesis, such as pazopanib, are also encouraging, especially in lieu of their oral administration. Further study of angiogenesis and its inhibition are ongoing and represent one of the highest priorities in therapeutic gynecologic oncology.

References


