

New Strategies in Advanced Cervical Cancer: From Angiogenesis Blockade to Immunotherapy

Krishnansu S. Tewari¹ and Bradley J. Monk²

Abstract

Cervical cancer remains unique among solid tumor malignancies. Persistent infection with oncogenic subtypes of the human papillomavirus (HPV) results in carcinogenesis, predominantly occurring at the cervical transformation zone where endocervical columnar cells undergo metaplasia to a stratified squamous epithelium. The molecular cascade involving viral oncoproteins, E6 and E7 and their degradative interactions with cellular tumor suppressor gene products, p53 and pRb, respectively, has been precisely delineated. The precursor state of cervical neoplasia may last for years allowing for ready detection through successful screening programs in developed countries using cervical cytology and/or high-risk HPV DNA testing. Prophylactic HPV L1 capsid protein vaccines using virus-like-particle technology have been developed to prevent primary infection by the most common high-risk HPVs (16 and 18). Women who lack access to health care and those who undergo sporadic screening remain at risk. Although radical surgery (including fertility-sparing surgery) is available for patients with early-stage cancers, and chemoradiation plus high-dose-rate brachytherapy can cure the majority of those with locally advanced disease, patients with metastatic and nonoperable recurrent cervical cancer constitute a high-risk population with an unmet clinical need. On August 14, 2014, the FDA approved the antiangiogenesis drug bevacizumab for women with advanced cervical cancer. This review will highlight advances in translational science, antiangiogenesis therapy and immunotherapy for advanced disease. *Clin Cancer Res*; 20(21); 5349–58. ©2014 AACR.

Background

Clinical review

Cervical cancer is the third most common malignancy in women worldwide with a global incidence of 500,000 and mortality 250,000 (1). In the United States, approximately 12,360 women will be diagnosed with invasive cervical cancer in 2014 and nearly 4,020 women are expected to die from this disease (2, 3). Unlike many other solid tumors, cervical cancer has a proclivity for younger women with a median age of diagnosis of 47 to 49 years in the United States.

Cervical cancer is unique among malignant diseases because risk factors are very well established, it has a prolonged preinvasive state that can be detected through screening, the etiology of the disease is known, and pro-

phylactic vaccines are available (4). The single most common risk factor for cervical cancer is not ever having undergone cytologic screening with a Papanicolaou (Pap) test (5). Most other risk factors are related to sexual behavior (e.g., early age at intercourse, multiple partners, promiscuous partner) as they increase the likelihood of infection with one or more of the 14 high-risk subtypes of the human papillomavirus (HPV), which Harald zur Hausen identified as the cause of cervical cancer in the 1980s and for which he was awarded the Nobel Prize in Physiology/Medicine in 2008 (6).

As noted above, oncogenic HPV infection can be prevented using prophylactic HPV vaccines that are made from virus-like particles (VLP) derived from the highly antigenic viral L1 capsid protein of HPV 16 and HPV 18, which are responsible for 70% of invasive cervical cancer and 50% of the high-grade precursor lesion, cervical intraepithelial neoplasia III (CIN3; refs. 7 and 8). These VLPs are devoid of DNA, and bivalent and quadrivalent vaccines are available with the latter, including protection from anogenital warts caused by HPV 6 and HPV 11 (9–11). Regulatory approval for a 9-valent prophylactic HPV vaccine is currently being sought (Table 1; ref. 12). Unfortunately, HPV vaccine uptake in the United States among adolescent females and males has not been robust in the 8 years since the first vaccine was approved by the FDA.

Screening for cervical cancer has been successful in developed countries when performed repeatedly (13, 14). Early

¹Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Chao Family Comprehensive Cancer Center, University of California, Irvine Medical Center, Orange, California. ²Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Arizona Cancer Center, Creighton University School of Medicine at Dignity Health St. Joseph's Hospital and Medical Center, Phoenix, Arizona.

Corresponding Author: Krishnansu S. Tewari, Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of California, Irvine, 101 The City Drive South, Building 56, Room 264, Orange, CA 92868. Phone: 714-456-7631; Fax: 714-456-6632; E-mail: ktewari@uci.edu

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Table 1. Recent advancements in prevention and screening of cervical cancer

Modality	Clinical trial	Principal findings
Prevention	V503-001 study (ref. 12; 9-valent VLP)	96.7% reduction in the combined incidence of high-grade cervical/vulvar/vaginal disease (CIN2/3+, VIN 2/3+, ValN2/3+) caused by HPV types 31,33,45,52,58: 1 case in those who received 9-valent HPV (6/11/16/18/31/33/45/52/58) vs. 30 cases in those who received quadrivalent HPV (6/11/16/18) 97.1% reduction (95% CI, 91.8–99.2) in combined incidence of cervical/vulvar/vaginal disease of any grade caused by HPV types 31, 33, 45, 52, 58 (3 cases in the 9-valent arm vs. 103 cases in the quadrivalent arm) 96.0% efficacy (95% CI, 94.4–97.2) against 6-month persistent HPV infection with 31, 33, 45, 52, 58 (35 cases in 9-valent arm vs. 810 cases in quadrivalent arm)
Screening	ATHENA study (ref. 16; cobas HPV test)	Cobas HPV test 15.63% more sensitive at detecting \geq CIN3 compared with liquid-based cytology: Sensitivity: cobas HPV test 58.26% (95% CI, 44.02–74.37) vs. cytology 42.63% (95% CI, 31.75–55.41) Positive predictive value \geq CIN3 among women referred to colposcopy: PPV: cobas HPV test 12.25% (95% CI, 10.69–13.91) vs. 6.47% cytology (95% CI, 5.54–7.50) False-positive rate \geq CIN3: cobas HPV test 4.09% (95% CI, 3.89–4.28) vs. cytology 6.04% (95% CI, 5.81–6.27)

Abbreviations: ValN, vaginal intraepithelial neoplasia; VIN, vulvar intraepithelial neoplasia.

preinvasive lesions can be monitored conservatively whereas high-grade precursors can often be treated with local ablative or excisional therapies that do not result in significant cervical trauma that may lead to cervical incompetence during subsequent pregnancy. The application of reflex high-risk HPV DNA testing to minimally abnormal cytologic smears has significantly reduced the numbers of unnecessary colposcopies and biopsies performed each year, and cotesting with cytology and HPV DNA in women over 30 years old has allowed for the annual screening interval to be safely extended in many low-risk populations (15). Based on results from the ATHENA study, in April 2014 the FDA approved the cobas HPV test as a first-line screening tool for cervical cancer in women 25 years and older, making it the first-ever approved alternative to Pap smear (Table 1; ref. 16).

Cervical cancer is staged clinically according to the criteria established by the International Federation of Gynecology and Obstetrics (FIGO; ref. 17). Preinvasive disease (i.e., cervical dysplasia or CIN) is asymptomatic. Early invasive disease (FIGO 1A-IB1) may manifest with abnormal vaginal bleeding or discharge and dyspareunia (4). These lesions can be successfully cleared with a satisfactory surgical margin via radical hysterectomy with bilateral pelvic lymphadenectomy. Postoperative adjuvant therapy is based on intermediate-high risk (e.g., lymphovascular space invasion, depth of stromal invasion, and tumor diameter) and high-risk factors (disease at the surgical margin, parametrial tissues, and/or lymph nodes; ref. 4). In some cases of early invasive disease, radical trachelectomy with lymphadenectomy may be considered to preserve future childbearing

potential when this is a priority (4). In many instances, the surgical procedures for invasive cervical carcinoma can be performed using minimally invasive techniques (laparoscopy, robotics).

Symptoms reported by women with locally advanced disease (FIGO IB2-IVA) include flank pain, lower extremity swelling, hematuria, and/or rectal bleeding. Cisplatin-based chemoradiation plus high-dose rate intracavitary brachytherapy can prevent locoregional relapse and effect cure in 40% to 75% of patients (18). The administration of adjuvant chemotherapy following completion of radiotherapy in these populations is currently being studied in the OUTBACK intergroup study of the Australian, New Zealand, and NRG Oncology cooperative groups (NCT01414608).

Women with recurrent cervical cancer constitute a high-risk population for whom treatment options have been highly limited. Those with central recurrences with a negative metastatic work-up may be candidates for total pelvic exenteration (4). Otherwise, palliative chemotherapy has been the rule for recurrent disease with short-lived responses and rapid deterioration of quality of life and early death. A median overall survival (OS) of 7 months in most populations can be expected. Many women with recurrent disease have acquired resistance to platinum as a result of prior exposure during radiotherapy for locally advanced disease (4).

State of the science

Somatic mutations in cervical carcinoma. Early reports have implicated the following somatic mutations in cervical

carcinoma: PIK3CA, PTEN, TP53, STK11, and KRAS. Using a high-throughput genotyping platform, Wright and colleagues interrogated 80 cervical cancers for 1,250 known mutations in 139 cancer genes and reported the highest mutation rates were for PIK3CA (31.3%), KRAS (8.8%), and EGFR (3.8%; ref. 19). Although PIK3CA mutation rates did not significantly differ between squamous cell carcinomas and adenocarcinomas, KRAS mutations were identified only in adenocarcinomas. Importantly, PIK3CA mutations were associated with shorter survival (67.1 vs. 90.3 months; HR 9.1; 95% CI, 2.8–29.5; $P < 0.001$). In an analysis of 79 primary squamous cell carcinomas, Ojesina and colleagues identified several previously unknown somatic mutations, including E322K substitutions in the MAPK1 gene (8%); inactivating mutations in the HLA-B gene (9%); and mutations in EP300 (16%), FBXW7 (15%), NFE2L2 (4%), TP53 (5%), and ERBB2 (6%; Table 2; ref. 20). This team also observed somatic ELF3 (13%) and CBFβ (8%) mutations in 24 adenocarcinomas of the cervix.

Oncogenic HPV subtypes. Based on pooled data from 11 case–control studies from nine countries involving 1,918 women with histologically confirmed squamous cell carcinoma of the cervix and 1,928 control subjects, in addition to HPV types 16 and 18, types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 72, and 82 are considered to be carcinogenic (i.e., high-risk subtypes), and types 26, 63, and 66 have been classified as probably being carcinogenic (21). Low-risk HPV subtypes include 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108; ref. 21).

DNA methylation. As discussed earlier, in screening programs high-risk HPV DNA testing offers improved sensitivity over cytology alone but is accompanied by a generally low specificity because a positive high-risk HPV DNA result does not discriminate between cancer-relevant lesions (e.g., CIN2-3) and transient, clinically irrelevant high-risk HPV infections (e.g., CIN1). Because the overall referral rates to colposcopy are high in a screening setting, there is a need for diagnostic tests that can distinguish

Table 2. Genes with significantly recurrent somatic mutations in cervical carcinomas

Gene	Description	Nonsilent mutations	Relative frequency (%)	Patients	Unique sites	Silent mutations	Indel + null	q
Squamous cell carcinoma (n = 79)								
FBXW7 ^a	F-box and WD repeat domain containing 7	12	15	12	8	0	2	4.03×10^{-12}
PIK3CA	Phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit α	11	14	10	5	0	1	$<9.08 \times 10^{-12}$
MAPK1 ^a	Mitogen-activated protein kinase 1	6	8	6	3	0	0	0.000671
HLA-B ^b	Major histocompatibility complex class I, B	7	9	6	7	1	3	0.00169
STK11	Serine/threonine kinase 11	3	4	2	2	0	1	0.012
EP300 ^b	E1A-binding protein p300	13	16	12	13	1	4	0.0354
NFE2L2 ^a	Nuclear factor, erythroid 2-like 2	3	4	3	2	0	0	0.0597
PTEN	Phosphatase and tensin homologue (mutated in multiple advanced cancers 1)	5	6	5	5	0	3	0.0693
Adenocarcinoma (n = 24)								
ELF3 ^c	E74-like factor 3 (ets domain transcription factor, epithelial-specific)	3	13	3	3	0	3	0.03
CBFB ^c	Core-binding factor, β subunit	2	8	2	2	0	1	0.0342

Abbreviations: Indel, insertions or deletions: null, nonsense, frame shift, or splice-site mutations; q , q value, false discovery rate (Benjamin–Hochberg procedure).

^aGenes with mutations observed in only squamous cell carcinomas.

^bGenes with a majority of mutations occurring in squamous cell carcinomas.

^cGenes with mutations observed in only adenocarcinomas.

Reprinted from ref. 20 by permission from Macmillan Publishers Ltd: Nature. Ojesina AI, Lichtenstein L, Freeman SS, Peadarullu CS, Imaz-Rosshandler I, Pugh TJ, et al. Landscape of genomic alterations in cervical carcinomas. *Nature* 2014;506:371–5. Copyright © 2014.

between women who are only transiently infected and those with cervical disease. Alternative strategies have been proposed, including double staining for p16 and the proliferation marker Ki67, measurement of type-specific viral load, and detection of viral integration, all of which have had mixed results (22). However, several studies have shown that abnormalities of the genome and epigenetic phenomena underlie neoplastic progression, with DNA hypermethylation of the promoter and 5' regions of tumor suppressor genes being an early event in carcinogenesis (23). Hypermethylated DNA enriched from cervical cancers have been studied by Hansel and colleagues using CpG island microarray hybridization (24). A methylation signature comprising the 5' regions of the genes *DLX1*, *ITGA4*, *RFXP3*, *SOX17*, and *ZNF671* specific for CIN3 and cervical cancer has been validated using quantitative methylation-specific PCR (24). Other investigators have separately identified *EpB41L3* methylation (25) and *CpG7091* methylation (26) as potential biomarkers for risk stratification and triage of women with high-risk HPV infections. Finally, in the randomized, controlled PROHTECT-3 trial, DNA methylation analysis of MAL and miR124-2 genes on HPV-positive self-samples was recently reported to be noninferior to cytology triage in the detection of CIN2 or worse, suggesting that full molecular screening should be feasible (27).

Viral oncogenes E6 and E7. Increasingly deregulated expression of the HPV E6 and E7 oncogenes is a recognized major transforming factor in the pathogenesis of high-grade dysplasia (CIN3) and invasive carcinoma (28). In the native double-stranded DNA episomal form of HPV, E6 and E7 are under subtotal transcriptional repression by the viral gene product E2. Deregulated viral oncogene expression results first in chromosomal instability, which induces chromosomal aneuploidy that favors integration of high-risk HPV genomes into cellular chromosomes (28). This leads to expression of viral cellular fusion transcripts, and because viral integration disrupts the E2 reading frame, enhanced expression of the E6 and E7 oncoproteins permit them to engage the cellular tumor suppressor gene products to cause p53 degradation and pRb inactivation, respectively. The Arg variant (Arg 72) of the p53 *Arg72Pro* polymorphism binds more ardently with oncogenic HPV E6 than the *Pro* variant and results in enhanced p53 degradation (29, 30). Individuals infected with oncogenic HPV who carry the *Arg72* variant are more likely to progress from CIN to invasive carcinoma than those harboring the *Pro72* variant (29, 30).

HPV E6 oncoproteins also recognize other target proteins, including the interferon regulatory factor (IRF)-3 and the Notch coactivator MAML1 through acidic leucine-rich motifs containing the LxxLL consensus sequence (31). Two zinc domains and a linker helix form a basic hydrophobic pocket through which E6 captures helical LxxLL motifs (31). Mutational inactivation of this binding pocket prevents hijacking of cellular LxxLL motifs and disrupts E6 oncogenic activities (31).

PI3K/Akt/mTOR pathway. The mammalian target of rapamycin (mTOR) plays an integral role in angiogenesis,

cell growth, proliferation, and survival. In the absence of PTEN inhibition, AKT phosphorylates and inhibits the tuberous sclerosis complex (TSC), which leads to mTOR activation and formation of two different multiprotein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2; ref. 32). Oncogenic HPV E6 also causes rapid degradation of TSC, resulting in TORC1 activation and downstream mTOR signaling (32). In a study of cervical cancer cell lines and an immunodeficient mouse xenograft model, mTOR pathway activation was demonstrable in most lineages. In addition, HeLa cells are defective in the tumor suppressor LKB1, which inhibits mTOR via TSC2 stimulation. Preclinical efficacy of mTOR inhibition by rapamycin and RAD001 was evident by virtue of decreased mTOR activity *in vivo* and a significant decrease in xenograft tumor burden (33).

Notch signaling. The Notch signaling pathway is an evolutionarily conserved binary transmembrane signaling pathway in higher eukaryotes that is involved in cell fate determination during development (34). Notch expression is associated with cell populations undergoing cell fate changes (e.g., columnar to squamous metaplasia at the transformation zone of the uterine cervix; ref. 35). The mammalian Notch genes (Notch-1 to -4) encode 300-kDa single pass transmembrane receptors. Binding of one of the Notch ligands (e.g., Delta1, Jagged-1) leads to a complex cleavage and activation of Notch proteins (36). The released and activated COOH-terminal fragment (i.e., intracellular Notch1) translocates to the nucleus and acts as a transcriptional modulator (36). In cervical carcinoma, Notch 1 regulates the small GTPase, RhoC, which is necessary for neoplastic transformation and tumor progression (37).

VEGF-dependent tumor angiogenesis. Tumor neovascularization has been recognized to impart an aggressive course in cervical cancer. Vascular markings seen colposcopically in women with abnormal Pap tests are hallmarks for invasive disease, and increased microvessel density and strong immunostaining for the endothelial cell marker, CD31, heralds a poor prognosis in cervical cancers (38). Vascular endothelial growth factor (VEGF) has emerged as an important therapeutic target to inhibit angiogenesis in many solid tumors (39–42). Overexpression of oncogenic HPV subtypes has been shown to enhance HIF1 α protein accumulation and VEGF expression (43). Conceptually, viral oncogenes E6 and E7 lead to altered p53 and retinoblastoma protein function, ultimately resulting in increased VEGF expression. Sequestration of VEGF using the monoclonal antibody bevacizumab prevents tumor angiogenesis. The activity of bevacizumab in recurrent cervical cancer was demonstrated in a phase II study by the Gynecologic Oncology Group (GOG; protocol 227C; ref. 44).

Immunologic considerations. HPV infection invokes a cellular immune response with regulatory T cells involved in local immune suppression in HPV-associated malignancies (4). Active immunity may be further suppressed by host factors, including T regulatory cells (Treg), monocyte/macrophages, plasmacytoid dendritic cells, regulatory natural killer cells, and release of inhibitory cytokines (e.g., IL6,

IL10, tumor growth factor- β ; ref. 45). Finally, prior chemoradiation among patients with recurrent disease can result in months of immune underperformance. Each of these immunologic factors needs to be considered for circumvention through the development of active immunotherapies. HPV vaccines have induced tumor regression in animal models, and a number of novel approaches to vaccine design and construction are available (4). In addition, cytotoxic T-lymphocyte-associated molecule-4 (CTLA-4) and the programmed cell death 1 (PD-1) receptor are integral to two coinhibitory pathways on activated T cells (46). The CTLA-4 receptor on T lymphocytes is a negative regulator of T-cell activation that outcompetes CD28 for binding to B7 on antigen-presenting cells, thereby functioning as an immune checkpoint molecule (46). When PD-1 binds to its ligand PD-L1, present on tumor cells, the ability of the activated T cell to produce a robust immune response is diminished (46).

On the Horizon

Interruption of signal transduction pathways

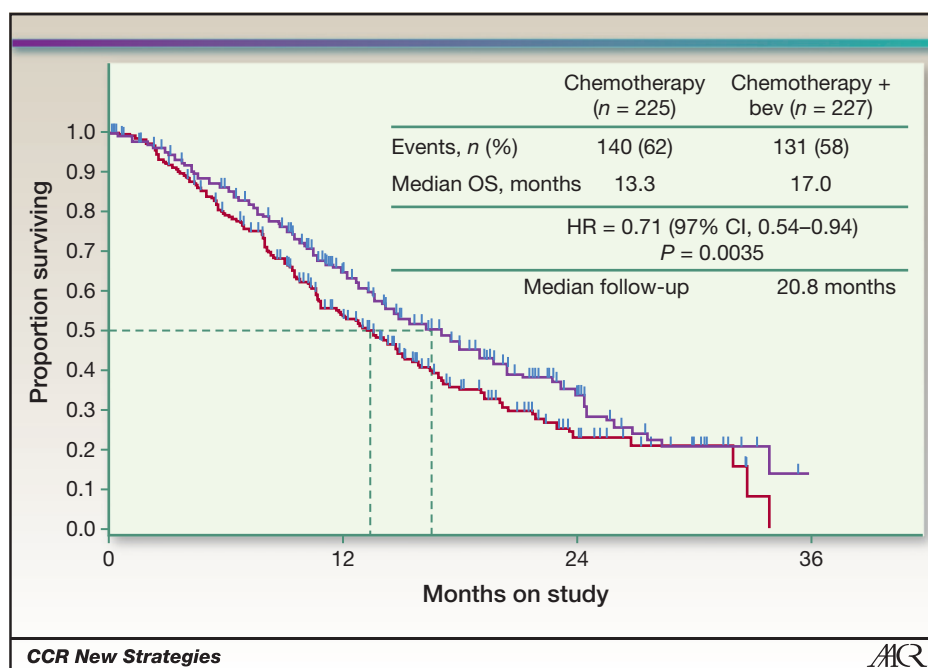
Antiangiogenesis therapy. Based on single-agent activity of bevacizumab in heavily pretreated patients with recurrent disease (44) and superiority of the antiangiogenesis oral tyrosine kinase inhibitor pazopanib over the anti-EGF therapy lapatinib in a randomized phase II study (47), GOG 240 was developed to study antiangiogenesis therapy using bevacizumab (ref. 48; and a nonplatinum chemotherapy doublet not discussed in this review; refs. 48 and 49) in advanced cervical cancer. This phase III, open-label, randomized trial was activated in 2009 and enrolled patients in the United States and Europe (48). Using a 2×2 factorial design, the trial studied two chemotherapy backbones

(cisplatin–paclitaxel and topotecan–paclitaxel) with and without bevacizumab. The primary endpoints included OS and toxicity, and secondary endpoints were progression-free survival (PFS) and objective response by RECIST v1 (48).

In 2013, the National Cancer Institute and the GOG jointly announced that GOG 240 demonstrated that compared with chemotherapy alone, the incorporation of the antiangiogenesis agent bevacizumab led to significantly improved OS (17 m vs. 13.3 m; Fig. 1; refs. 48 and 50), PFS (8.2 m vs. 5.9 m), and response rate (48% vs. 36%) without a significant deterioration in health-related quality of life (48). Major treatment-related toxicities included fistula (3%), thromboembolism (8%), and easily managed hypertension (25%; ref. 48).

Within 1 month of public presentation of the data, the cisplatin–paclitaxel–bevacizumab triplet from GOG 240 was listed as Category 2A in the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines for Cervical Cancer (51). This resulted in significant uptake of bevacizumab for recurrent and metastatic cervical cancer, well ahead of publication of the primary manuscript in February 2014. In March 2014, the United Kingdom's Cancer Drug Fund approved bevacizumab for women with advanced cervical cancer (52). During the second quarter of 2014, both Genentech and Roche filed with the FDA and European Medicines Agency, respectively. On July 14, 2014, the FDA accepted the application for priority review to expand the indication of bevacizumab for advanced cervical cancer. Priority review is given when the magnitude of benefit to patients is anticipated to be significant. Although a final decision was set for October 24, 2014, the FDA approved bevacizumab for cervical cancer on

Figure 1. OS of patients with advanced cervical cancer treated with chemotherapy with and without bevacizumab in Gynecologic Oncology Group protocol 240. Top blue curve represents chemotherapy plus bevacizumab (bev), and bottom red curve represents chemotherapy alone. From ref. 48, New England Journal of Medicine, Tewari KS, Sill MW, Long III HJ, Penson RT, Huang H, Ramondetta LM, et al., Improved survival with bevacizumab in advanced cervical cancer, 370, 734–43. Copyright © 2014 Massachusetts Medical Society. Reprinted with permission.



August 14, 2014, underscoring the agency's commitment to bring promising therapies to patients expeditiously. Bevacizumab is the first targeted agent to demonstrate an OS advantage in a gynecologic malignancy.

These data open the doors to study other classes of anti-angiogenesis agents in recurrent and possibly even first-line therapy for cervical cancer (53, 54). Although the multi-targeted tyrosine kinase inhibitor sunitinib, which exerts its antiangiogenic effects via inhibition of VEGFR-1, -2, -3, platelet-derived growth factor α and β , and related receptor tyrosine kinases, failed to demonstrate activity in 19 unselected patients with advanced cervical cancer enrolled on a phase II study (54), it is possible that efficacy could be detected with an enriched population. The GOG has completed a phase II study using brivanib, a dual inhibitor of VEGFR-2 and fibroblast growth factor receptor-1 (FGFR1), and results will be forthcoming (NCT01267253). Non-VEGF-dependent antiangiogenesis strategies designed to disrupt signaling in the angiopoietin axis using molecules such as trebananib should also be explored (54). Finally, the ability to prune existing tumor vasculature using vascular disrupting agents (VDA), which target genetically stable cell populations represents another promising avenue for therapeutic exploration in advanced cervical cancer (54). The antivascular effects of combretastatin A-4 phosphate (CA4P) have been demonstrated in both *in vitro* and *in vivo* models, and seem to arise through binding β -tubulin subunits to prevent microtubule formation resulting in cytoskeletal changes and endothelial cell damage, and ultimately leading to increased vascular resistance, reduced tumor blood flow, and central tumor necrosis (54).

Notch pathway disruption. The VEGF and Notch pathways (introduced in the preceding section) are tightly linked, with interendothelial signaling via delta-like 4 (DLL4) and Notch having emerged as a key regulator of endothelial heterogeneity (55). During sprouting angiogenesis, tip cell formation is the default response to VEGF whereas the stalk cell phenotype is acquired through notch-mediated lateral inhibition (55). Although Notch inhibition does not affect VEGFR2 expression, VEGFR3 is strongly modulated by Notch. Benedito and colleagues have shown that VEGFR3 kinase-activity inhibitors but not ligand-blocking antibodies are able to suppress sprouting of endothelial cells that had low Notch signaling activity (56). These data suggest that Notch signaling may function in the physiologic response to loss of VEGF signaling, and thus participate in tumor adaptation to VEGF inhibitors. In a neuroblastoma model, Hernandez and colleagues recently demonstrated that combined Notch and VEGF blockade led to blood vessel regression, increased endothelial cell apoptosis, and disruption of pericyte coverage of endothelial cells (57). A phase I study using a Notch inhibitor (NCT01158404) in patients with advanced solid tumors has recently closed to accrual, but moving forward, clinical trials designed to study concurrent blockade represent a promising antiangiogenesis strategy in advanced cervical cancer.

mTOR inhibitors. High-risk HPV-related E6 expression leads to rapid degradation of TSC2, resulting in TORC1

activation and downstream mTOR signaling. The high prevalence of PI3KCA mutations in cervical cancer suggests that mTOR-targeted agents should be explored in this disease. Fourteen cervical cancer tumors from a phase I program were analyzed for PIK3CA, KRAS, NRAS, and BRAF mutations (58). The 5 patients found to have PIK3CA mutations were treated with agents targeting the PI3K/AKT/mTOR pathway, and 2 had a partial response (58). In another phase I study involving 15 women with advanced gynecologic cancers treated with weekly temsirolimus (25 mg on days 1, 8, 15, and 22) and topotecan (1 mg/m² on days 1, 8, and 15), 1 of the 2 patients with advanced/recurrent squamous cell carcinoma of the cervix had stable disease with a median time to progression of 3 months (59). The dose-limiting toxicity was myelosuppression (59). This combined regimen, however, was not tolerated in women with a history of pelvic radiation therapy.

Single-agent temsirolimus (25 mg i.v. every week every 28 days) was studied in women with advanced cervical carcinoma in a phase II study of 38 patients (60). Among the 33 women evaluable for response, one experienced a partial response (3.0%). Nineteen patients had stable disease (57.6%), with a median duration of response of 6.5 months (range, 2.4–12.0 months; ref. 60). The 6-month PFS rate was 28% (95% CI, 14%–43%), and the median PFS was 3.52 months (95% CI, 1.81–4.7; ref. 60). No grade 4 to 5 adverse events were observed. Disease stability did not seem to be associated with PTEN and PIK3CA expression, copy number analyses, and PTEN promoter methylation (60).

Targeting other signal transduction pathways. The efficacy of antiangiogenesis therapy as demonstrated through inhibition of VEGF-mediated angiogenesis in GOG 240 also suggests that disruption of other pathways integral to cervical cancer carcinogenesis should be explored (61). Although a clinical signal for anti-EGF therapy has been lacking, future studies are likely to study biomarker-enriched populations (61). WEE1 targeting is predicated on a deficient G₁-S phase checkpoint, resulting in increased DNA damage at the G₂-M phase transition in cancer cells (61). Abrogation of G₂-M arrest releases cells with unrecognized and unrepaired DNA damage into premature mitosis, resulting in mitotic catastrophe and apoptotic cell death. A phase I/II clinical trial (NCT01076400) evaluating the WEE1 inhibitor, MK1775, in combination with topotecan and cisplatin in patients with advanced stage, metastatic, and recurrent cervical cancer is ongoing (61).

Preclinical data suggest that histone deacetylase and heat shock protein 90 may represent important targets in cervical cancer, and inhibitors of each class of proteins have been developed (61). Finally, the recognition that the activity of poly (ADP-ribose) polymerase-1 (PARP) inhibitors is not restricted to patients with BRCA mutations but that some activity may be seen in patients with the BRCAness phenotype (a possible surrogate for chemosensitivity) suggests that even PARP inhibitors may be considered for study in patients with advanced cervical carcinoma (61). Some cervical cancers may evade radiochemotherapeutic effect by overexpressing PARP.

Immunotherapy

Antivirus therapy (prophylaxis). At the 2013 Annual Meeting of the European Research Organisation on Genital Infections and Neoplasia (EUROGIN), Joura and colleagues reported results from the pivotal phase III randomized trial of the nine-valent HPV L1 VLP prophylactic vaccine V503 versus quadrivalent HPV L1 VLP (12). Although V503 prevented approximately 97% of high-grade cervical, vulvar, and vaginal disease caused by the five additional oncogenic HPV subtypes (refs. 31, 33, 46, 53, and 59; Table 1; ref. 12), preventive vaccines are unlikely to reduce the prevalence of HPV infections in the next few years because of their cost and limited availability in developing countries. In addition, prophylactic vaccines are not able to eradicate established HPV infections and their associated lesions.

Therapeutic vaccines. Because appreciable levels of L1 and/or L2 capsid antigens are not expressed by infected basal epithelial cells and cervical cancer cells, therapeutic HPV vaccines targeting antigens other than L1 and L2 are needed. Both HPV oncoproteins, E6 and E7, represent ideal targets for therapeutic vaccine development. Technologies to create therapeutic HPV vaccines include live vector-

based vaccines, peptide/protein-based vaccines (62), nucleic acid-based vaccines, and whole cell vaccines (63). Live vectors can be bacterial (e.g., *Listeria monocytogenes*) or viral (e.g., Newcastle disease virus). One advantage of protein-based vaccines over those based on peptides derived from HPV antigenic proteins is that the former can circumvent the MHC specificity limitation associated with peptide vaccines. Nucleic acid vaccines may be DNA based or created from naked RNA replicons, whereas whole cell vaccines may be derived from dendritic cells or even tumor cells.

Two important immunotherapeutic studies in advanced cervical cancer were recently presented at the 2014 Annual Meeting of the American Society of Clinical Oncology. Basu and colleagues presented final results of a randomized phase II trial involving 110 women from India with recurrent cervical cancer that had progressed following treatment with chemotherapy and/or radiotherapy (64). Patients were randomized to receive live attenuated *L. monocytogenes* bioengineered to secrete a HPV 16 E7 fusion protein targeting HPV-transformed cells (ADXS11-001, 1×10^9 cfu \times 3 or 4 doses) with and without cisplatin (40 mg/m²). The final 12-month OS was 36% and 18-month OS was 28% (64).

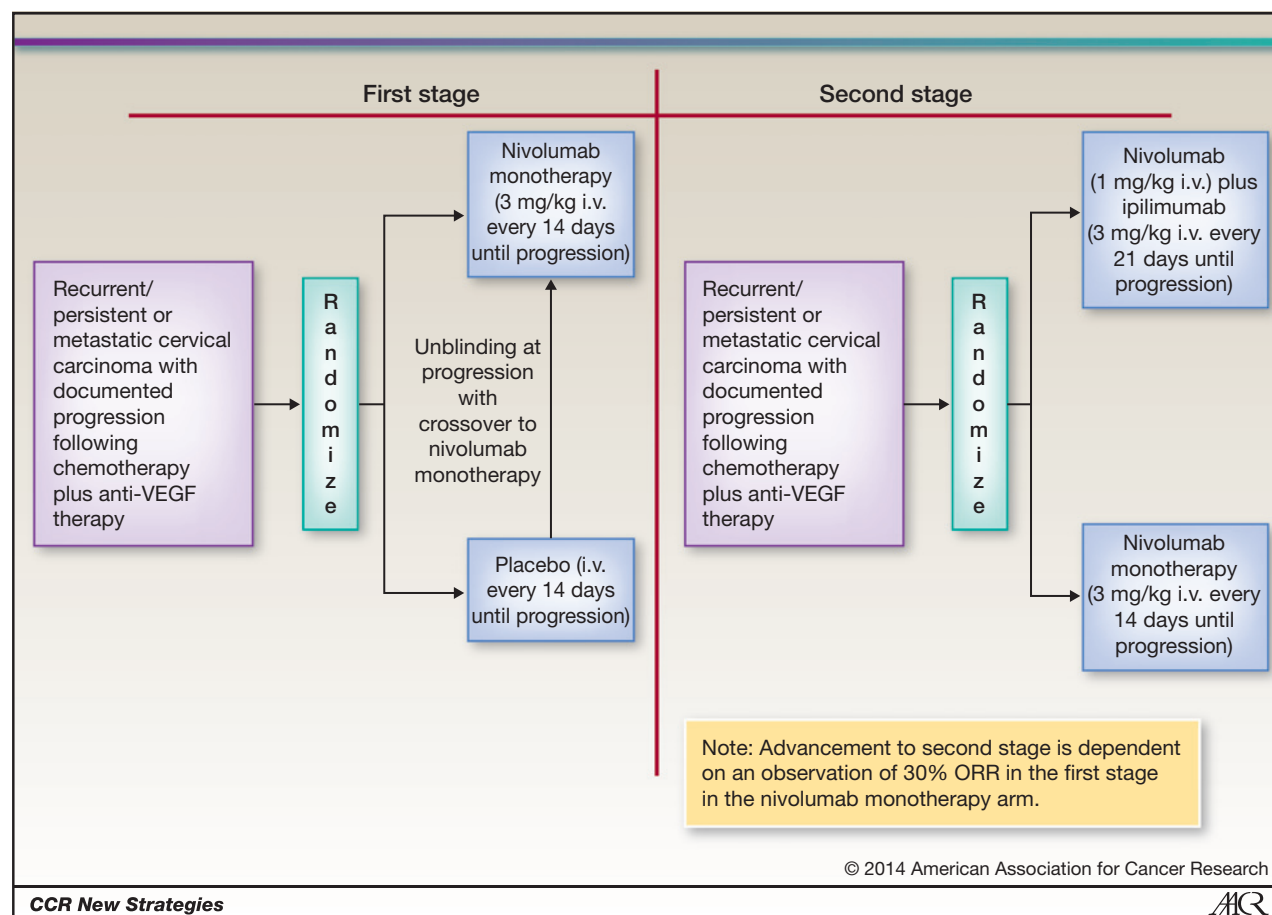


Figure 2. Proposed schema to study nivolumab in advanced cervical cancer: randomized phase II, placebo-controlled trial of nivolumab monotherapy with crossover and run-in randomized phase II trial of nivolumab with and without ipilimumab for advanced cervical carcinoma following failure of anti-VEGF therapy. ORR, objective response rate.

The overall response rate was 11% and included 6 complete responders and 6 partial responders (64). The average duration of response in both treatment groups was 10.5 months (64). The addition of cisplatin did not significantly improve OS or response rates. Grade 3 adverse events related to study drug occurred in 2% of study patients (64). ADXS11-001 has demonstrable activity in this population and a phase III randomized trial is being designed.

Adoptive T-cell therapy involves the isolation and *ex vivo* expansion of tumor-specific T cells. Hinrichs and colleagues reported on 9 patients with metastatic cervical cancer treated with a single infusion of tumor-infiltrating lymphocytes selected for HPV E6 and E7 reactivity (HPV-TIL; median 81×10^9 T cells, range $33\text{--}159 \times 10^9$; ref. 65). Infusion was preceded by non myeloablative conditioning and followed by high-dose bolus aldesleukin (65). Two patients with no HPV reactivity did not respond to treatment, and 3 of 6 patients with HPV reactivity demonstrated objective tumor responses (65). One patient had a 39% partial response and 2 patients with widespread metastases (one with chemotherapy-refractory HPV 16⁺ squamous cell carcinoma and one with chemoradiation-refractory HPV 18⁺ adenocarcinoma) experienced complete tumor responses that were ongoing at the time of presentation 18 and 11 months after treatment, respectively (65). These results are interesting and suggest that cellular therapy can mediate complete, durable regression of an epithelial malignancy.

Breaking immune tolerance. An emerging paradigm in the landscape of immunotherapy involves reawakening of silenced immune responses by inhibiting the inhibitors that are responsible for paralyzing T cells and creating a state of immune tolerance (46, 66, 67). Breaking of immune tolerance may represent a powerful strategy to overcome immune suppression and result in a more robust intervention than short-lived immune-activating approaches. This was the pathway through which regulatory approval of ipilimumab was granted for patients with advanced melanoma (68). Ipilimumab is a checkpoint blocking antibody directed against cytotoxic T-lymphocyte antigen 4 (CTLA-4; ref. 68). CTLA-4 attenuates the early activation of naïve and memory T cells (68). Another checkpoint blocking antibody, nivolumab, is directed against PD-1, which is primarily involved in modulating T-cell activity in peripheral tissues via interaction with its ligands, PD-L1 and PD-L2 (69, 70). Although both CTLA-4 and PD-1 function as negative regulators, their roles in immune modulation are nonredundant and do not overlap (71).

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During the last quarter of 2013, the Cancer Therapy Evaluation Program of the National Cancer Institute sent out a mass solicitation for concepts involving nivolumab with or without ipilimumab in the treatment of advanced cervical cancer and ovarian cancer. Following the July 2013 listing of the cisplatin–paclitaxel–bevacizumab triplet in the NCCN Cervical Cancer Treatment Guidelines, there has been nearly 40% uptake of bevacizumab in advanced disease in the United States. But GOG 240 and bevacizumab was never meant to be the end of the story. Rather, it represents a proof of concept of the efficacy of antiangiogenesis therapy in this disease. However, this efficacy is not expected to translate into durable cure for the majority of patients who comprise a population that has historically responded so poorly to salvage therapies. Now that bevacizumab has received regulatory approval for advanced cervical cancer, uptake will increase further and a new population of advanced cervical cancer patients will emerge, specifically those who have progressed on anti-VEGF therapy. This group will constitute yet another population with a high unmet clinical need who may be considered for participation in a clinical trial designed to break immune tolerance (Fig. 2). It is also important to consider breaking immune tolerance during the period when patients with advanced disease are deriving benefit from antiangiogenesis therapy rather than waiting for progression to occur.

Disclosure of Potential Conflicts of Interest

K.S. Tewari reports receiving speakers bureau honoraria from Vermillion and is a consultant/advisory board member for Advaxis, Caris, and Genentech/Roche. B.J. Monk reports receiving commercial research grants from Amgen, Array BioPharma, Eli Lilly, Genentech, Merck, Janssen/Johnson & Johnson, Novartis, and TESARO; speakers bureau honoraria from Johnson & Johnson and Roche/Genentech; and is a consultant/advisory board member for Arno, Array BioPharma, Astellas, AstraZeneca, Boehringer Ingelheim, Celgene, GlaxoSmithKline, Insys, Merck, Morphotek, Nektar, Qiagen, Roche/Genentech, and TESARO.

Authors' Contributions

Conception and design: K.S. Tewari

Development of methodology: K.S. Tewari, B.J. Monk

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.S. Tewari

Writing, review, and/or revision of the manuscript: K.S. Tewari, B.J. Monk

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.S. Tewari

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