Review Article

Immunotherapy: An Evolving Paradigm in the Treatment of Advanced Cervical Cancer

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ABSTRACT

Purpose: In 2014, the US Food and Drug Administration approved the first targeted agent, bevacizumab, in the treatment of advanced stage, persistent, or recurrent cervical cancer. This oncologic milestone has catalyzed interest in the investigation of alternate therapies, including immunotherapy, in an effort to extend life and possibly cure patients with advanced stage disease.

Methods: This review article focuses on the evolving paradigm of immunotherapy in the treatment of cervical cancer, describing the biologic basis of this treatment modality and discussing applicable Phase I to II clinical trials.

Findings: To date several trials have been conducted exploring vaccine-based therapies, adoptive T-cell therapy, and immune-modulating agents in patients with cervical cancer with promising results.

Implications: Immunotherapy represents a promising therapeutic paradigm in the treatment of advanced cervical cancer. Additional investigation is warranted to try and identify alternate immune targets and predictors of response, allowing for the selection of patients most likely to benefit from immune-based treatments. (*Clin Ther*. 2015;37:20–38)

Key words: Cervical cancer, Immunotherapy, Human papillomavirus, Therapeutic vaccine, Checkpoint inhibitor, Chimeric T cell receptor antigen.

INTRODUCTION

In 2011 an estimated 529,800 cases of cervical cancer were diagnosed worldwide, with 275,100 deaths.[1] This global burden is attributable to the disproportionately high incidence of cervical cancer in developing, resource-poor countries that lack adequate health care infrastructure and screening programs. In the United States, an estimated 12,360 cases of cervical cancer will be diagnosed in 2014, with 4020 deaths; it is anticipated that this number will continue to decrease as human papillomavirus (HPV) vaccination rates increase and the focus shifts to primary prevention.[2] Despite advances in screening, vaccination, and treatment of early-stage disease, a proportion of patients will be diagnosed as having advanced stage (stage IVB), recurrent, or persistent cervical cancer. For this subset of patients, systemic chemotherapy remains the cornerstone of treatment.[3,4]

Since the publication of the initial studies examining cisplatin in the treatment of cervical cancer, a number of effective single-agent and combination drug regimens have been identified that exhibited improved response rates, without a significant effect on overall survival.[5–21] The poor oncologic outcome in this patient population represents an unmet clinical need and has driven the exploration of new treatment paradigms.[3]

Most recently, the results of Gynecologic Oncology Group (GOG) protocol 240 were presented and

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published, illustrating a significant improvement in overall survival (17 vs 13.3 m) with the incorporation of the antiangiogenic agent bevacizumab to a chemotherapy backbone, without a significant deterioration in quality of life.[22–25] This oncologic milestone represents the first time a targeted agent has resulted in an overall survival advantage in the gynecologic cancer arena. Ultimately, on August 14, 2014, the US Food and Drug Administration (FDA), following priority review, expanded the indication of bevacizumab to include advanced cervical cancer based on the findings of GOG 240. These results have opened the door to the development and study of additional therapies, including immunotherapy, to be used solely or in conjunction with targeted agents and cytotoxic chemotherapy.[26]

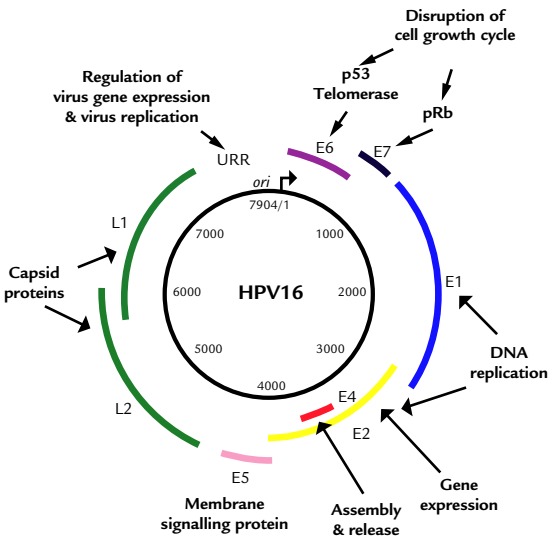

Figure 1. Schematic of human papillomavirus (HPV) genome. Adapted with permission from Elsevier.[76]

HPV PATHOGENICITY

Cervical cancer is unique among gynecologic malignant tumors because several risk factors have been well established and the causative agent, HPV, is known. HPV is a double-stranded, circular DNA virus (approximately 8 kilobase pairs) that exhibits unidirectional transcription. Approximately 170 HPV genotypes have been identified, with HPV-16 and HPV-18 accounting for >70% of invasive cervical cancer. The HPV genome is composed of 7 early proteins (E1, E2, E4, E5, E6, E7, and E8) and 2 late, structural proteins (L1 and L2) (Table I and Figure 1). Importantly, the complementary DNA for L1 represents the structural and immunogenic basis for the licensed prophylactic HPV vaccines currently available. To establish an infection, the HPV virus must infect the basal epithelial cells located in the cervical transformation zone, which are actively replicating and differentiating. In select cases, the viral DNA is incorporated into the host cell genome, resulting in interruption of several early genes, including E2, E4, and E5. Interruption of E2, which normally functions as a transcriptional regulator of E6 and E7, leads to up-regulation of E6 and E7, degradation of p53 and pRB, respectively, and ultimately malignant transformation.[27–29]

A BRIEF HISTORY OF CANCER IMMUNOTHERAPY

During the 1850s, noting that their patients' cancers would sometimes shrink when the tumor became

Table I. HPV genome.

E1	E2	E4	E5	E6	E7	L1	L2
ATPase	Regulator of E6 and E7	Disrupts cytokeratin matrix for release of virions	Potentiation of membrane bound EGF receptors	Bind and inacti-vate p53	Bind pRB leading to E2F activity	Major capsid (conserved)	Minor capsid (variable)

ATP = adenosine triphosphate; EGF = epidermal growth factor.

Figure 2. William B. Coley (1862–1936), Pioneer of cancer immunotherapy.

infected, German physicians were the first to suggest that the body's immune system could be reprogrammed to fight cancer. The field of immunotherapy was born during this period, when throughout Europe physicians encouraged by the success of Edward Jenner's smallpox vaccine attempted to design a cancer vaccine by injecting their patients with crude tumor extracts from other patients with cancer. Unfortunately, these early treatments lacked notable efficacy.

During the waning years of the 19th century, a young New York surgeon, William Bradley Coley (1862-1936) (**Figure 2**), was struck by the occasional spontaneous remission of sarcoma achieved by patients who had developed erysipelas.[30] In an effort to reproduce this phenomenon, in 1891 at Memorial Hospital in New York City, Coley began intratumoral injections of live or inactivated *Streptococcus pyogenes* and *Serratia marcescens*. Coley's first patient had an inoperable malignant tumor and is reported to have made a complete recovery, living another 26 years until a heart attack claimed his life. The mechanism of action of Coley's toxins (as they came to be known) involved stimulating antibacterial phagocytes to kill bystander tumor cells. This strategy was supported by Elie Metchnikoff, a contemporary of Coley's who discovered the immune system's ability to cause inflammation and destroy invading bacteria.

However, with the exception of intravesical injection of live BCG vaccine following surgical resection of superficial bladder cancer, the ability to prolong patient survival using Coley's toxins was sporadic and not reproducible. In addition, Coley's work drew significant criticism as essayists weighed the merits of administering infectious agents to weakened patients with cancer.[31] Finally, his work was overshadowed by the advent of x-ray and radium treatment, advances in surgery and supportive care, and, ultimately, chemotherapy.

Coley's work would have been forgotten were it not for the efforts of his daughter, Helen Coley Nauts. While mourning the death of her father in 1936, Mrs. Nauts, a housewife and mother with no formal medical training, became inspired by her father's work and taught herself oncology, immunology, and record keeping to interpret and publish her father's work. Without any financial backing, she spent 3 years tracking down 896 cases of microscopically confirmed cancers that had been treated with her father's mixed bacterial toxins. Mrs. Nauts' groundbreaking publications rekindled the medical community's interest in exploring the link between cancer and the immune system, and her father, Dr William B. Coley, has since been regarded as the father of cancer immunotherapy. With a grant of $2000 from Nelson Rockefeller, Mrs Nauts and her devoted friend Oliver R. Grace founded New York's Cancer Research Institute in 1953.[32]

With the first quantitative descriptions of antibodies appearing in the 1880s, studies of the humoral arm of the immune system dominated immunology throughout most of the 20th century. In 1975, while working in Cambridge, Georges J.F. Köhler and César Milstein discovered how to make synthetic antibodies.[32] The first therapeutic monoclonal antibodies, rituximab and trastuzumab, were approved during the late 1990s to treat lymphoma and breast cancer, respectively.

Advances concerning the cellular arm of the immune system occurred during the 1940s, with Landsteiner and Chase reporting that delayed hypersensitivity could be transferred among mice using immune cells obtained from sensitized donors. Further advances emerged from Gross' work in which syngeneic mice immunized against tumors in the same inbred strain could reject subsequent tumor challenges. In 1983, the gene encoding interleukin

2 (IL-2) was sequenced, leading to its expression in *Escherichia coli* and the development of recombinant IL-2. On the basis of its ability to reproducibly cause tumor regression in humans, IL-2 was approved by the US FDA for patients with metastatic renal cell carcinoma in 1992 and for metastatic melanoma in 1998.[32]

MECHANISTICS OF THERAPEUTIC ANTITUMOR IMMUNITY

The immune system, whose principle function is protection against infection from microbes, can be broadly divided into the innate and adaptive components. The innate immune system, composed of cells and proteins that are perpetually ready to combat infection, is active and primed for response in a nonspecific manner. The principle components of innate immunity include (1) epithelial barriers (skin), (2) natural killer cells, (3) dendritic cells (DCs), and (4) phagocytic leukocytes. Although thought to play a role in the prevention of certain premalignant or early malignant lesions, the innate immune system is not alone capable of a response sufficient to overcome widespread metastatic disease. Conversely, the adaptive immune system, which is normally quiescent, is activated to target those foreign agents that successfully circumvent the policing of the innate pathway. The adaptive immune response is specific and can be further divided into humoral (antibody B-cell mediated) immunity and cell-mediated (T-cell specific) immunity. It is the adaptive immune system that is being explored as a potential therapeutic strategy in the treatment of malignant disease.

Initiation of tumor-specific immunity requires that DCs sample tumor-derived antigens delivered exogenously

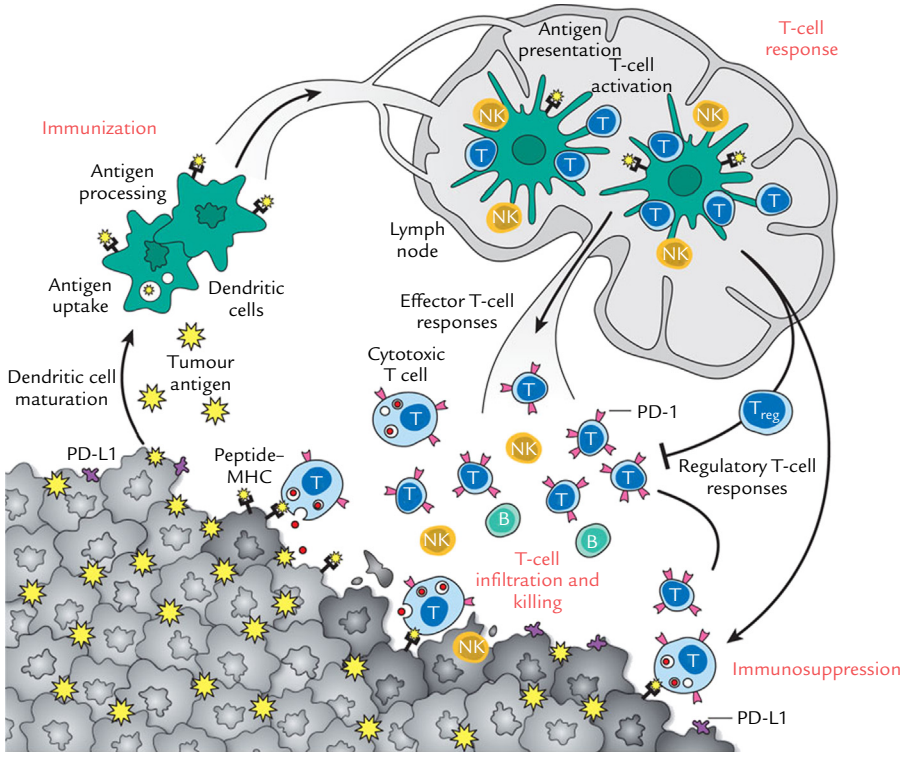

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Figure 3. Generation and regulation of antitumor immunity. MHC = major histocompatibility complex; PD-1 = programmed death receptor-1; PD-L1 = programmed death ligand-1. Reprinted with permission from Elsevier.[30]

via a therapeutic vaccine or directly recognized and processed from malignant cells.[30] A maturation signal allows DCs to differentiate extensively and promote immunity (Figure 3). Such activation signals can also be provided exogenously using Toll-like receptor ligands. In lymphoid organs, loaded DCs generate protective T-cell responses ($CD8^+$ effector T cells) and trigger antibody and natural killer T-cell responses.[30] Importantly, without maturation by a stimulatory adjuvant, DCs will present antigens at the steady state, promoting tolerance by regulatory T cells rather than immunity. Ultimately, cancer-specific T cells enter the tumor bed and must overcome immune suppression, which may be dependent on infiltration of regulatory T cells or down-regulation of major histocompatibility complex (MHC) class I molecules by the cancer itself.[30] In addition, vascular cells that comprise the cancer can suppress T-cell adhesion to the tumor endothelium, a process mediated by vascular endothelial growth factor. Thus, this molecular cascade provides 3 sites for therapeutic intervention via cancer immunotherapy: (1) promoting antigen-presenting functions of DCs, (2) enhancing the production of protective T-cell responses, and (3) circumventing immunosuppression in the tumor bed.

IMMUNOLOGIC DIFFERENCES BETWEEN PREVENTION AND TREATMENT

Despite advances in cervical cancer screening, vaccination, and early detection, a proportion of patients are diagnosed as having invasive disease. In contrast to prevention, treatment of established disease relies on the activation of cytotoxic T cells, moving beyond the formation of neutralizing antibodies, which has posed a therapeutic dilemma for researchers on many levels. Such an immune response would require the delivery of antigen to the cytosolic compartment of antigen-presenting cells (APCs) to allow for HLA class I presentation, inducing a $CD8^+$ cytotoxic T-cell response. This is fundamentally different than the memory B-cell–driven, antibody-mediated response that neutralizes viral particles and plays a pivotal role in vaccination and primary prevention.

The L1 and L2 viral capsid proteins are not appreciably expressed by infected basal epithelial cells, making them poor targets for a therapeutic HPV-based vaccine. Conversely, the HPV E6 and E7 oncoproteins are expressed in HPV-associated cancers and are ideal targets for a therapeutic HPV vaccine. Because of the intracellular localization of these antigens, therapies are directed at a cellular immune response. Various therapeutic HPV vaccines targeting HPV E6/E7 antigens have been tested in the clinical setting, including live vector–based vaccines, protein-based vaccines, peptide-based vaccines, nucleic acid–based vaccines, and whole cell–based vaccines.[33]

CERVICAL CANCER: THERAPEUTIC VACCINES

Bacterial Vectors

There are 2 primary categories of live vector-based vaccines: bacterial vectors and viral vectors (Table II). The primary advantages of live vector–based vaccines are their efficient delivery of antigen and their ability to replicate in the host, resulting in a potent immune response. Limitations include tolerability issues due to live vector infusion and neutralizing antibody formation against the live vector, limiting the efficacy of repeat immunization.

Table II. Immunologic vaccines examined in Phase II clinical trials in patients with cervical cancer.

Type	Vaccine	Target
Live (bacterial and viral) vector–based vaccine	ADXS11-001 (bacterial)	HPV-16 E7 fusion protein
	TA-HPV (viral)	HPV-16 E6 and E7 peptide
Peptide	HLA-A*201	HPV-16 E7 peptide
Protein	SGN-00101	Fusion protein of HPV-16 E7
Nucleic acid	ZYC101a	HPV-16 E7 HLA-A2 restricted peptide
	VGV-3100a	Plasmid targeting HPV-16 and HPV-18 E6 and E7

HPV = human papillomavirus.

Several bacterial-based vectors, including *Listeria monocytogenes*, *Lactobacillus plantarum,* and *Lactococcus lactis,* have been tested in therapeutic vaccines.[34–36] The *Listeria*-based vectors are unique and have been the most extensively studied. *Listeria monocytogenes* is a gram-positive facultative intracellular bacterium that has been extensively used to examine cell-mediated immunity.[37,38] *Listeria* preferentially infects APCs (macrophages) and, unlike other intracellular bacteria, such as salmonella, escapes into the host cell cytoplasm by disrupting the phagosomal membrane. This ability to evade the phagosome is dependent on the expression of listeriolysin O (LLO). Given *Listeria*'s presence in the cytoplasm and the phagolysosome, peptides derived from *L monocytogenes* can be presented by the MHC class I and class II molecules, inducing both $CD4^+$ and $CD8^+$ T-cell–mediated responses.[39,40]

The first clinical use of a *Listeria*-based HPV vaccine was reported by Maciag et al[41] in 2009. The authors studied an HPV-16 E7 antigen fused to a nonhemolytic fragment of the Lm protein LLO (Lm-LLO-E7). In this Phase I trial, the tolerability of Lm-LLO-E7 was assessed in 15 patients with previously treated metastatic, refractory, or recurrent cervical carcinoma. Patients received 1 of 3 dose levels of Lm-LLO-E7 (1×10^9 CFU, 3.3×10^9 CFU, or 1×10^{10} CFU) as an intravenous infusion, followed by a second dose 3 weeks later. All patients experienced a flulike syndrome, which responded to nonprescription symptomatic treatment.[41] Severe (grade 3) adverse events related to Lm-LLO-E7 were reported in 6 patients (40%), but no grade 4 adverse events were observed. At the highest dose, some patients had severe fever and dose-limiting hypotension. By the end of the study protocol, 2 patients had died, 5 had progressed, 7 had stable disease and 1 qualified as a partial responder.

In addition to the above, preliminary results from a separate cervical cancer immunotherapy trial using a *Lm*-based vector (ADXS11-001) were presented at the American Society of Clinical Oncology (ASCO) meeting in June 2013.[42] ADXS11-001 is a live attenuated *L monocytogenes* bioengineered to secrete an HPV-16 E7 fusion protein targeting HPV transformed cells (**Figure 4**).[43] In this prospective Phase II study conducted in India, a total of 110 patients with recurrent or refractory cervical cancer previously treated with chemotherapy, radiotherapy, or both were randomized to either 3 doses of ADXS11-001 at 1×10^9 CFU or 4 doses of ADXS11-001 at 1×10^9 CFU with cisplatin chemotherapy. The primary end point was 12-month survival. As of May 2013, 110 patients had received 264 doses of ADXS11-001. Final 12-month overall survival was 36% (n = 39/110) with an 18-month survival of 22% (n = 16/73). Activity was observed against all high-risk HPV strains detected, including HPV-16, -18, -31, -33, and -45. A total of 45 of 110 patients (46%) experienced 104 mild-moderate grade 1 to 2 adverse events, and 2 patients (2%) experienced a serious grade 3 adverse event.

Updated survival results for the above study were presented at the 2014 ASCO meeting in June 2014.[44] The final 12-month survival was 36% (n = 39/110), and the 18-month survival was 28% (n = 31/110). The response rate was 11% (6 complete responders and 6 partial responses of 110 patients) with tumor responses observed in both treatment arms; 35 additional patients had stable disease for >3 months, for a total disease control rate of 43% (n = 47/110).[44] The mean duration of response in both treatment groups was 10.5 months, with activity against different high-risk HPV strains observed. The incidence of serious grade 3 adverse events possibly related or related to ADXS11-001 was 2%.

On behalf of the NRG GOG, Dr. Warner Huh presented GOG protocol 265, a United States–based Phase II evaluation of ADXS11-001 in the treatment of persistent or recurrent squamous or nonsquamous cell carcinoma of the cervix.[45] A previous Phase I dose escalation study evaluated the safety profile of ADXS11-001 in patients with advanced cervical cancer.[41] The primary objectives of this study were to evaluate the tolerability and safety profile of ADXS11-001 and to assess the activity of ADXS11-001 in patients with persistent or recurrent cancer of the cervix. Secondary objectives included progression-free survival, overall survival, and objective tumor response. Eligible patients were treated with ADXS11-001 at a dose of 1×10^9 CFU on day 1, repeated every 28 days for 3 total doses in the absence of disease progression or unacceptable toxic effects. Tumor tissue and serum samples were collected periodically for translational research. Outcome results are pending, and as of May 5, 2014, the first stage of the study was closed to patient accrual. After an evaluation of survival, the study is anticipated to reopen in the second stage.

The survival end point in a Phase II trial reflects the cell-based corridor through which active immunotherapies must traverse to gain regulatory approval with the US FDA. Importantly, the manner in which

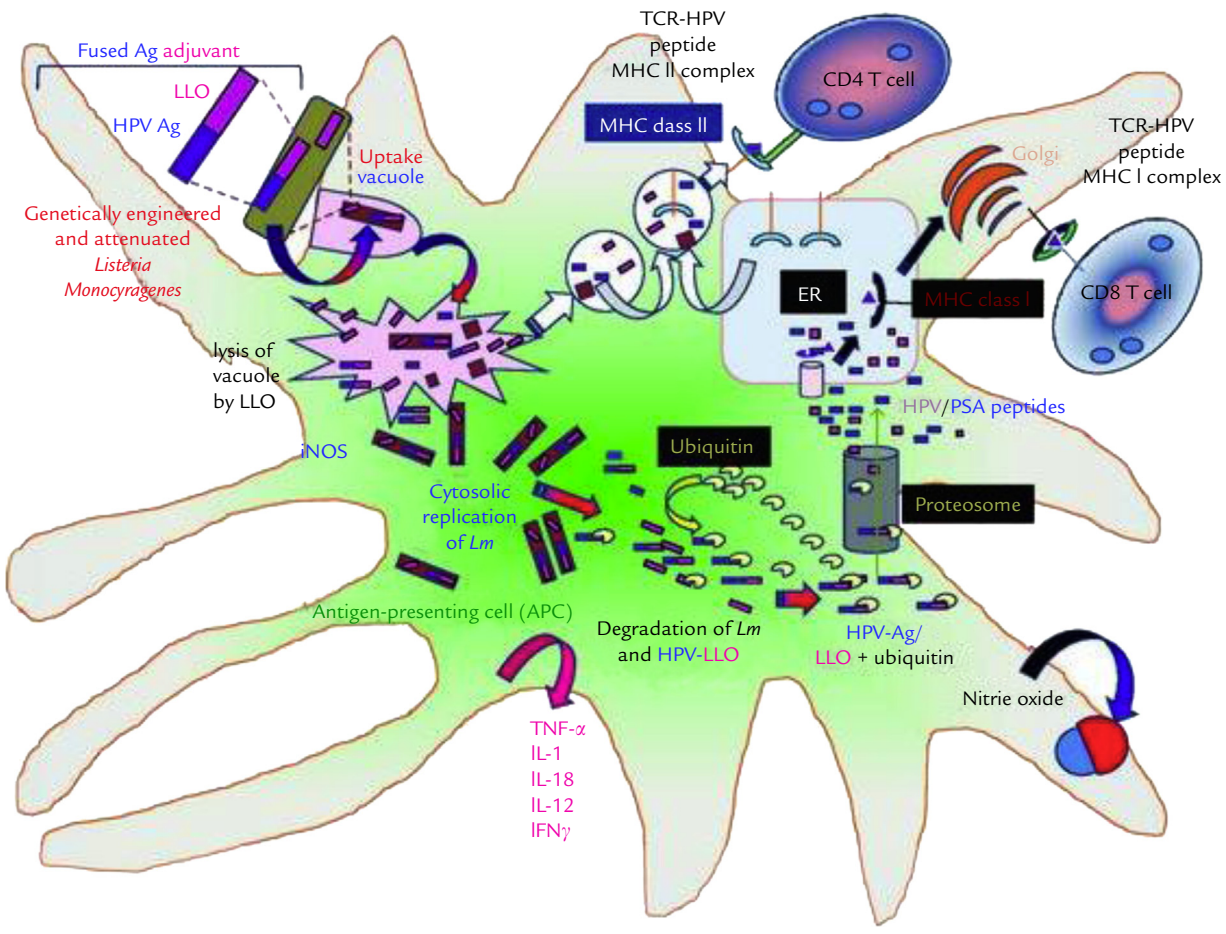

Figure 4. **ADXS11-011. Schematic presentation of LLO-Ag (HPV) fusion protein processing and presentation in antigen presenting cells by Listeria monocytogenes.** Adapted with permission from: Anu Wallecha, Chris French, Robert Petit, Reshma Singh, Ashok Amin, and John Rothman, "Lm-LLO-Based Immunotherapies and HPV-Associated Disease," Journal of Oncology, vol. 2012, Article ID 542851, 10 pages, 2012. doi:10.1155/2012/542851.[43]

immune therapies exert their anticancer effects is often not captured by traditional Response Evaluation Criteria in Solid Tumors or World Health Organization criteria. For example, the inflammatory response generated by immunotherapies may make a target lesion appear larger than on prior imaging studies. For these reasons, overall survival represents a more uniform end point than objective response rate in Phase II trials studying immunotherapy.

Viral Vectors

Viral vectors are advantageous given their high infection efficiency and superb expression of antigen encoded by the virus in infected cells. To date, several viral vectors, including adenoviruses, adeno-associated viruses, alphaviruses, vesicular stomatitis viruses, vaccinia viruses, and fowlpox viruses have been examined in the synthesis of therapeutic HPV vaccines. The bulk of current clinical data are based on studies that use vaccinia virus–based vaccines.[46,47] In one study, a live recombinant vaccinia virus expressing modified forms of the HPV-16 and -18 E6 and E7 proteins was administered to 29 patients with International Federation of Gynecology and Obstetrics stage IB or IIA cervical cancer starting 2 weeks before radical hysterectomy. Patients were monitored closely for adverse effects of the vaccination. Serial blood samples were examined for HPV-specific cytotoxic T lymphocytes (CTLs) or changes in levels of antibodies to HPV-16 or -18 E6 and E7 proteins and to vaccinia virus. After a

single vaccination, HPV-specific CTLs were found in 4 patients (HLA A1, A3, 3 patients; HLA A1, A24, 1 patient). Eight patients developed HPV-specific serologic responses.[46]

Peptide and Protein-Based Vaccines

The primary benefits of peptide-based vaccines rest on their relative tolerability, stability, and ease of production when compared with protein-based and live vector–based vaccines. In general, however, peptide-based vaccines have poor immunogenicity and require use of adjuvants to enhance vaccine potency.

Peptide-based vaccines using the E7 peptide generate CTLs that were protective against HPV-16 E7 positive tumors.[48] In a Phase I/II clinical trial, 19 terminally ill cervical cancer patients were immunized with HLA-A*0201–restricted HPV-16 E7 peptide, with confirmed tolerability but no detectable CTL response.[49] Another Phase II clinical trial in patients with end-stage cervical cancer used E6 or E7 HLA-A*0201–binding peptides pulsed onto cytokine-stimulated autologous peripheral blood mononuclear cells.

In an alternate, early Phase I study, Kenter et al[50] examined the toxicity, tolerability, and immunogenicity of an HPV-16 E6 and E7 long peptide vaccine.[50] Three groups of patients with end-stage cervical cancer (n = 35) were vaccinated with HPV-16 E6 combined with or separated from HPV-16 E7 overlapping long peptides with Montanide ISA-51 adjuvant 4 times at 3-week intervals. Coinjection of the E6 and E7 peptides resulted in a strong and broad T-cell response dominated by immunity against E6 and was capable of inducing a broad interferon γ–associated T-cell response even in patients with end-stage cervical cancer. The same vaccine regimen was studied in a subset of patients with resected HPV-16–positive cervical cancer.[51] Vaccine-induced T-cell responses against HPV-16 E6 were detected in 6 of 6 patients and against E7 in 5 of 6 patients. These responses were broad, involved both $CD4^+$ and $CD8^+$ T cells, and could be detected up to 12 months after the last vaccination.[51] Despite the above successes, peptide-based vaccines result in immune tolerance rather than activation and require the identification of epitopes associated with a particular MHC allele, limiting therapeutic generalizability.

In addition, ISA Pharmaceuticals is actively studying the safety profile, tolerability, and efficacy of ISA101, a HPV-16 E6/E7 long peptide vaccine at different doses, with or without interferon alfa, as combination therapy with carboplatin and paclitaxel in women with HPV-16–positive advanced stage, metastatic, or recurrent cervical cancer (NCT02128126). Patients will be treated with 6 cycles of carboplatin and paclitaxel with ISA101 vaccination beginning on day 15 of cycle 2 for a total of 3 vaccine rounds. Patients will also be randomized to receive interferon alfa-2b to assess the effect on immune response after vaccination.

When compared with peptide-based vaccines, protein-based vaccines can circumvent MHC specificity limitations because the antigens are processed in APCs, which contain all possible HLA epitopes of an antigen.[33] However, protein-based vaccines exhibit low immunogenicity, require combined use of adjuvants, and have additionally been associated with a limited CTL response. In the cervical cancer arena, protein-based vaccines have been tested in the treatment of preinvasive disease, revealing tolerability and antigen-specific T-cell responses.[52–56] Currently, additional studies are under way in an attempt to identify the most effective adjuvant to fuse with protein-based vaccines, including *Bordetella pertussis* adenylyl cyclase, *Pseudomonas aeruginosa* exotoxin A, a heat shock protein derived from *Mycobacteria,* Toll-like receptor agonists, and a penetrating peptide polyphemus protein.

Among the protein-based vaccine candidates, SGN-00101, a fusion protein consisting of heat shock protein from *Mycobacterium bovis* and HPV-16 E7, has generated a significant amount of clinical interest.[57,58] In a Phase II clinical trial, 21 women with biopsy-proven high-grade cervical intraepithelial neoplasia and visible colposcopic lesions received 4 injections of HPV-16 heat shock protein E7 fusion protein, followed by a loop electrosurgical excision of the transformation zone (LLETZ).[52] Immune parameters were evaluated before vaccination and at the time of LLETZ, and HPV testing was performed at intervals before and after LLETZ. Seven of 20 women (35%) evaluable for response had complete regression of their intraepithelial neoplasia at the time of LLETZ, 1 (5%) had regression to cervical intraepithelial neoplasia I, 11 (55%) had stable disease, and 1 (5%) had progression due to enlargement of her lesion.[52]

Nucleic Acid–Based Vaccines

Nucleic acid vaccines may be DNA based or created using naked RNA replicons and have been used in the clinical arena to promote an antigen-specific immune response.[57,59] The primary benefits of a DNA-based

vaccine rest on the fact that naked DNA is stabile, safe, and cost-effective. In addition, DNA-based vaccines do not elicit neutralizing antibodies in the vaccinated patient and can therefore be administered repeatedly with similar efficacy.[57] In an effort to enhance antigen presentation to APCs and improve antigen processing and presentation, a significant amount of research has been directed at identifying the most effective adjuvant to use in DNA-based vaccines. The E7 gene of HPV-16 has been fused to both a plant virus coat protein and mutated immunotoxin to improve immunomodulant activity. In addition, enhanced delivery methods, including electroporation, microencapsulation, and gene gun, have led to improved targeting of DNA to APCs.[57]

One of the rare nucleic acid vaccine therapies to a Phase II/III study incudes ZYC101a, an HPV-16 E7 HLA-A2 restricted peptide, which also encodes segments of HPV-16 and HPV-18 E6 and E7 viral proteins.[60,61] In a Phase II study of 21 patients receiving the vaccine, 11 had elevated $CD8^+$ T-cell responses to HPV-16 and/or HPV-18 peptides, and 7 of these also had increases to corresponding HPV-6 and/or HPV-11 peptides. In addition, T cells primed and expanded in vitro with an HPV-18 peptide had cross-reactivity to the corresponding HPV-11 peptide.

VGX-3100, a DNA vaccine composed of plasmids targeting HPV-16 and HPV-18 E6 and E7 proteins, was found to be safe in Phase I studies using electroporation, producing $CD8^+$ T cells exhibiting full cytolytic functionality in all cohorts.[62] On the basis of these findings, the vaccine is currently being examined in a randomized, double-blind, placebo-controlled Phase II clinical trial in patients with high-grade cervical intraepithelial neoplasia (NCT01304524).

The alternate class of nucleic acid vaccines is RNA based, and as such these vaccines are self-replicating and self-limited. The RNA-based vaccines may be administered as either RNA or DNA, which is then intracellularly transcribed into RNA replicons (naked RNA molecules). The replicon-based vectors can subsequently replicate in a wide range of cell types, resulting in sustained expression of antigen. Despite the preclinical success of RNA replicons, RNA replicon–based vaccines have undergone limited clinical testing.

WHOLE CELL–BASED VACCINE THERAPIES

DC Vaccines

The nonlytic nature of HPV infections results in a delayed induction of inflammatory responses through Toll-like receptors and inflammasomes. The absence of such an inflammatory signal subsequently results in negative immune regulation, changing the state of APCs and inhibiting cytotoxic effector T-cell induction.[57] The most potent APCs are the DCs, which express high levels of MHC and require costimulatory molecules, in essence functioning as their own adjuvants. As such, researchers have actively explored use of DCs for antigen loading and therapeutic administration to affected patients in an effort to exogenously prime the immune system against the offending agent, circumventing the relative immune tolerance seen after infection.

The greatest advances in DC-based vaccine therapy have been made in patients with prostate cancer.[63,64] Sipuleucel-T, a DC-based vaccine, was the first therapeutic cancer vaccine to be approved by the US FDA. In a prospective, randomized Phase III clinical trial, patients with metastatic castration-resistant prostate cancer with no or minimal symptoms, receiving the vaccine had a 4.1-month improvement in overall survival.[63,64]

In the cervical cancer arena, 2 studies have been conducted examining DC-based vaccine therapy.[65,66] In 2003, Ferrara et al[65] evaluated HPV E7 antigen-loaded autologous DCs as a cellular tumor vaccine in a case series of patients with cervical cancer patients. Autologous monocyte-derived DCs were pulsed with recombinant HPV-16 E7 or HPV-18 E7 oncoprotein and administered to 15 patients with stage IV cervical cancer. Tolerability, toxicity, and induction of serologic and cellular immune responses were monitored. The vaccine was well tolerated, and no local or systemic adverse effects or toxic effect were recorded. A specific serologic response was seen in 3 of 11 evaluated patients. Specific E7 $CD8^+$ T-cell immune responses were detected in 4 of the 11 patients with late stage cancer, although no objective clinical response was observed.

This study was followed by another study that examined the safety profile and immunogenicity of an HPV-16/18 E7 antigen-pulsed mature DC vaccine in patients with stage IB or IIA cervical cancer.[66] Escalating doses of autologous DC (5, 10, and 15×10^6 cells for injection) were pulsed with recombinant HPV-16/18 E7 antigens and keyhole limpet hemocyanin (an immunologic tracer molecule) and delivered in 5 subcutaneous injections at 21-day intervals to 10 patients with cervical cancer with no evidence of disease after they underwent radical surgery. The DC

vaccinations were well tolerated without grade 3 toxic effects. All patients developed $CD4^+$ T-cell and antibody responses after vaccination, and 8 of 10 patients had increased levels of E7-specific $CD8^+$ T-cell counts when compared with prevaccination baseline levels.

Despite the promising clinical results, significant hurdles need to be overcome before the implementation of widespread DC-based therapy. Specifically, treatment requires the use of autologous DCs, limiting large-scale production. Furthermore, DC harvesting, ex vivo culturing, and identifying a route of administration that ensures the DC vaccine reaches the T-cell targets in lymphoid tissues represent ongoing limitations to the therapeutic expansion of this paradigm.

CHIMERIC T CELL RECEPTOR ANTIGENS

In an effort to circumvent the above limitations, and to broaden the application of adoptive cellular therapy, gene therapy approaches for the redirection of T-cells to defined tumor-associated antigens (TAAs) have been developed.[67–70] The most recent strategies have involved the engineering of autologous T-cells with chimeric antigen receptors (CAR) (Figure 5).[71,72] These CARs are composed of an antigen-binding moiety, derived from the variable region of a monoclonal antibody, linked via a transmembrane motif to a lymphocyte-signaling moiety located in the cytoplasm. Variable, extracellular bindings motifs allow for recognition of tumor-associated antigens, including cell surface specific molecules. Currently, CD19 is the most widely utilized target of CAR-modified T-cells, used therapeutically in the treatment of acute lymphoblastic leukemia (ALL). CD19 is universally expressed by the leukemic cells, while expression is limited to B-cells and their progenitors in no-tumor tissues.[68] This allows for acceptable toxicity, and a tolerable side effect profile. Grupp et al[73] reported on the outcomes and longer follow up of the first 30 patients with relapsed, refractory ALL treated with anti-CD19 CAR.[73] Remarkably, 92% of patients treated with this novel therapy have experiences complete remissions, many of which are durable. At a median follow up of 6 months, sustained remissions were achieved of up to 1 year or more, with a 6-month event-free survival of 70%, and overall survival of 76%. The major toxicity experienced in all responders was cytokine-release syndrome, characterized by fever, nausea, muscle pain, and in rare cases respiratory distress. Use of the IL-6 receptor antagonist, tocilizumab, in addition to corticosteroids, led to resolution of these symptoms in 1–2 days for the majority of patients.

The elimination of the need to identify and harvest tumor-specific lymphocytes from patients represents the principle benefit of CAR T cell-based therapy.[69] In CAR therapy, lymphocytes are isolated following

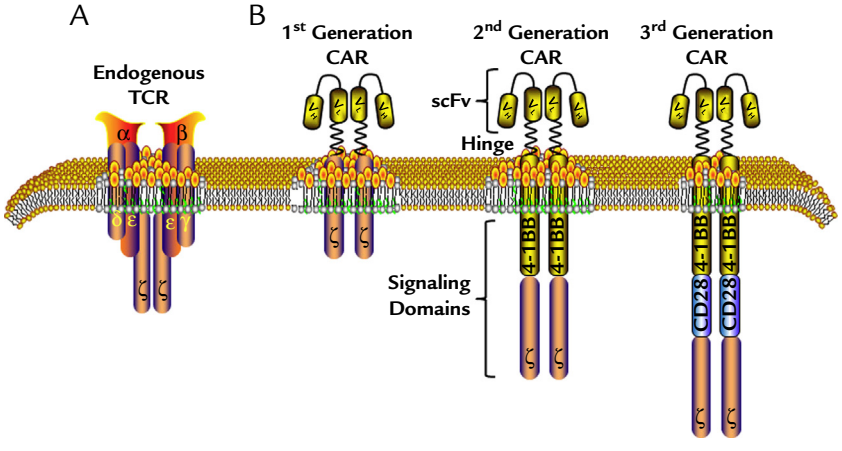

Figure 5. T cells redirected to possess specificity for tumor antigens (A). T cells express a single endogenous TCR. (B) Alternatively, T cells can be engineered to express chimeric antigen receptors or "CARs" that recognize surface antigens in an MHC-unrestricted manner. CAR T cells are composed of an extracellular portion that is usually derived from an antibody, a hinge region, and intracellular signaling modules derived from T cell signaling proteins. Adapted with permission from: Garfall AL, Fraietta JA, Maus MV. "Immunotherapy with Chimeric Antigen Receptors for Multiple Myeloma," Discovery Medicine 17(91):37-46, January 2014.72

apheresis, viral CAR transduction is performed, followed by ex vivo expansion and re-infusion. This provides for an efficient mechanism for tumor-specific T-cell expansion. Exploration of CAR based therapies in patients with cervical carcinoma is warranted, and will require the identification of appropriate ligand binding domains (TAA), transmembrane linkers and intracellular signaling elements to optimize tumor cell recognition and limit off-target toxicity.

BISPECIFIC T-CELL ENGAGER (BITE) ANTIBODIES

In addition to the above advances, the developmental platform for immunotherapeutics has now come to include bispecific T-cell engager (BiTE) antibodies (Figure 6).[74] These novel molecules induce a transient cytolytic synapse between a cytotoxic T cell and the cancer target cell. This interaction results in discharge of cytotoxic T cell contents following perforin fusion with the T-cell membrane resulting in direct tumor cell lysis, while attempting to limit off-target toxicity.[75] In a phase 2 clinical trial conducted in patients with relapsed or refractory acute lymphoblastic leukemia (ALL), 36 patients were treated with blinatumomab (BiTE for CD 19 and CD3). The complete remission rate, inclusive of complete remission with partial hematologic recovery was 69%, with 88% of responders achieving a minimal residual disease response.[75] The most common adverse event noted on therapy was pyrexia.

These clinically impressive results resulted in United States FDA approval of this new leukemia treatment more than 5 months ahead of schedule, and only two months after initial filing, highlighting the potential promise these agents hold in the treatment of historically refractory malignancies. Blinatumomab's success marks the first approval for any CD19-tageting agent. Currently, BiTE antibodies directed against surface target antigens expressed on solid tumors are being evaluated in phase I clinical trials.[76]

TUMOR CELL–BASED VACCINES

An alternate area of active investigation involves the isolation and manipulation of tumor cells (harvested from the patient) or tumor-infiltrating lymphocytes followed by vaccination. The use of tumor cell vaccines is hypothesized to increase the antigen load, potentially resulting in a more robust immunogenic response, although concern for secondary malignant tumors has limited use.

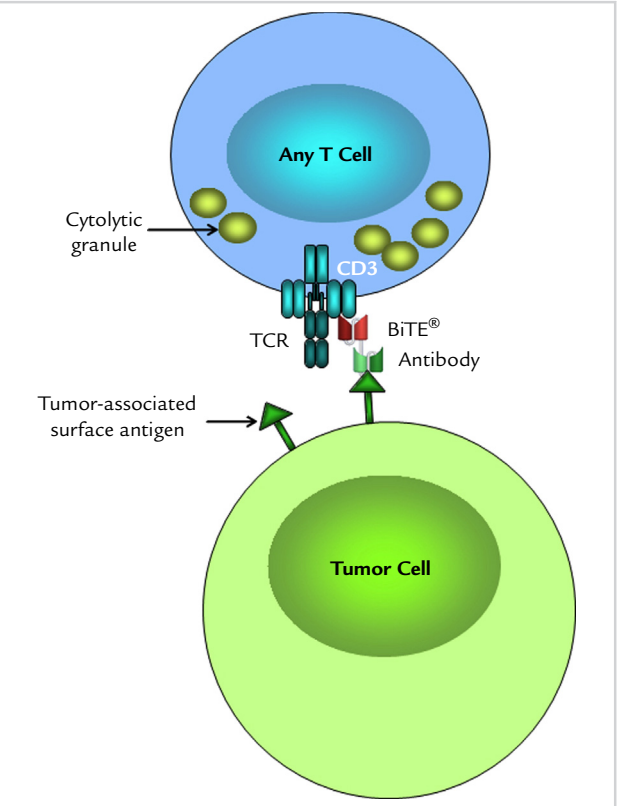

Figure 6. BiTE molecule forming cytolytic synapse between tumor cell expressing target of interest and any T cell through T cell receptor/CD3 interaction. Adapted with permission from: Wickramasinghe D., "Tumor and T Cell Engagement by BiTE," Discovery Medicine 16(88):149-152, October 2013.[74]

At the 2014 ASCO Annual Meeting, preliminary results regarding the use of adoptive T-cell therapy in the treatment of cervical cancer were presented in 9 patients with metastatic cervical cancer.[77] All patients underwent isolation and ex vivo expansion of tumor-specific infiltrating T cells (HPV-TIL) (Figure 7).[78] Infusion was preceded by nonmyeloablative conditioning and followed by high-dose bolus aldesleukin. The infused cells possessed reactivity against high-risk HPV E6 and/or E7 in 6 of 8 patients. The 2 patients with no HPV reactivity did not respond to treatment, and 3 of 6 patients with HPV reactivity had objective tumor responses.[59,77] One patient had a 39% partial response. and 2 patients with widespread disease (1 with chemotherapy-refractory HPV-16 squamous cell carcinoma and 1 with chemoradiation-

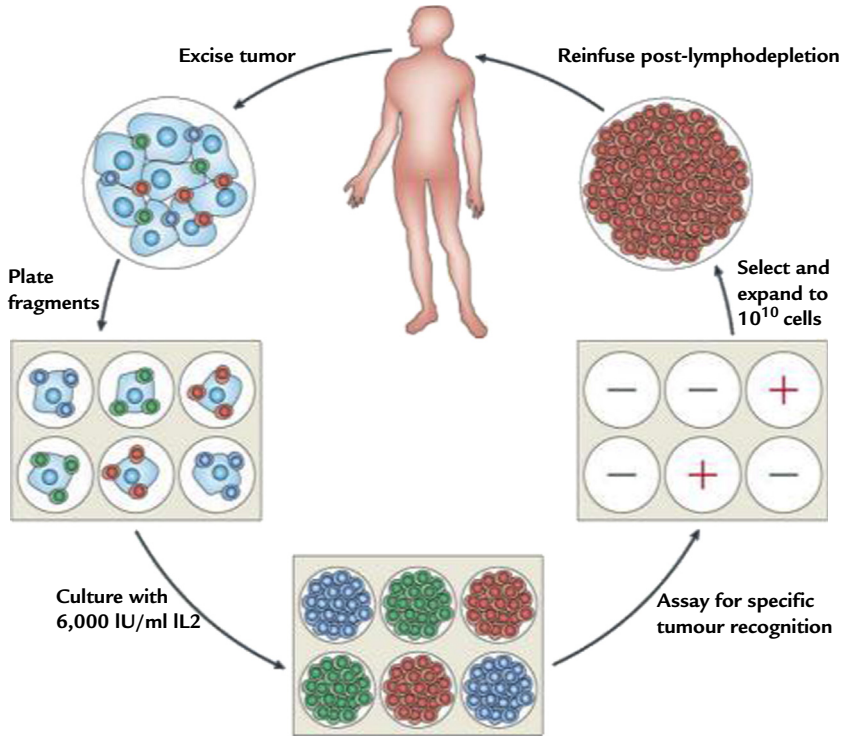

Figure 7. Adoptive T cell transfer using tumor-infiltrating lymphocytes. Adapted with permission from: Rosenberg SA, Restifo NP, Yang JC, et al., "Adoptive cell transfer: a clinical path to effective cancer immunotherapy," Nature Reviews Cancer 8, 299-308, April 2008.[78]

refractory HPV-18 adenocarcinoma) experienced complete tumor responses that were ongoing at the time of data presentation, 18 and 11 months after treatment.[59,77] Given the positive response in 3 of the 9 patients examined, 2 of whom exhibited a durable remission, the advanced cervical cancer cohort will be expanded to 35 patients. Importantly, the investigators are also studying the efficacy of adoptive T-cell therapy in HPV-related oropharyngeal, anal, vaginal, vulvar, and penile cancers.

IMMUNE CHECKPOINT INHIBITORS

In addition to the vaccine-based therapies reviewed above, recent interest in immune checkpoint inhibition has emerged in an effort to reverse the immune-privileged state often encountered in the malignant microenvironment. This paradigm has been described as inhibiting the inhibitors responsible for the facilitation of an immune-tolerant landscape.[59] To date, the primary therapeutic targets of immune modulation include CTL antigen-4 (CTLA-4) (**Figure 8**)[79,80] and programmed death receptor-1 (PD-1) (**Figure 9**)[81], which play a nonredundant role as negative regulators of immune function. CTLA-4, through engagement with its ligands CD80 and CD86, plays a pivotal role in attenuating early activation of naive and memory T cells. Conversely, PD-1 is involved in modulating T-cell activity in peripheral tissues.

PD-1 is normally expressed on T cells after T-cell receptor activation, and binding of this receptor to its cognate ligands, programmed death ligand-1 (PD-L1) and PD-L2, down-regulates T-cell receptor signaling, resulting in T-cell anergy and apoptosis and leading to immune suppression.[57] Recently, Lyford-Pike et al[82] established the role of the PD-1: PD-L1 pathway in HPV-associated head and neck squamous cell cancer immune resistance, suggesting a rationale for therapeutic blockade of this pathway in patients with HPV-associated head and neck cancer. In September 2014, the US FDA approved the first immune modulator that acts as a PD inhibitor (pembrolizumab*) for use

*Trademark: Keytruda (Merck & Co, Inc, Whitehouse Station, New Jersey).

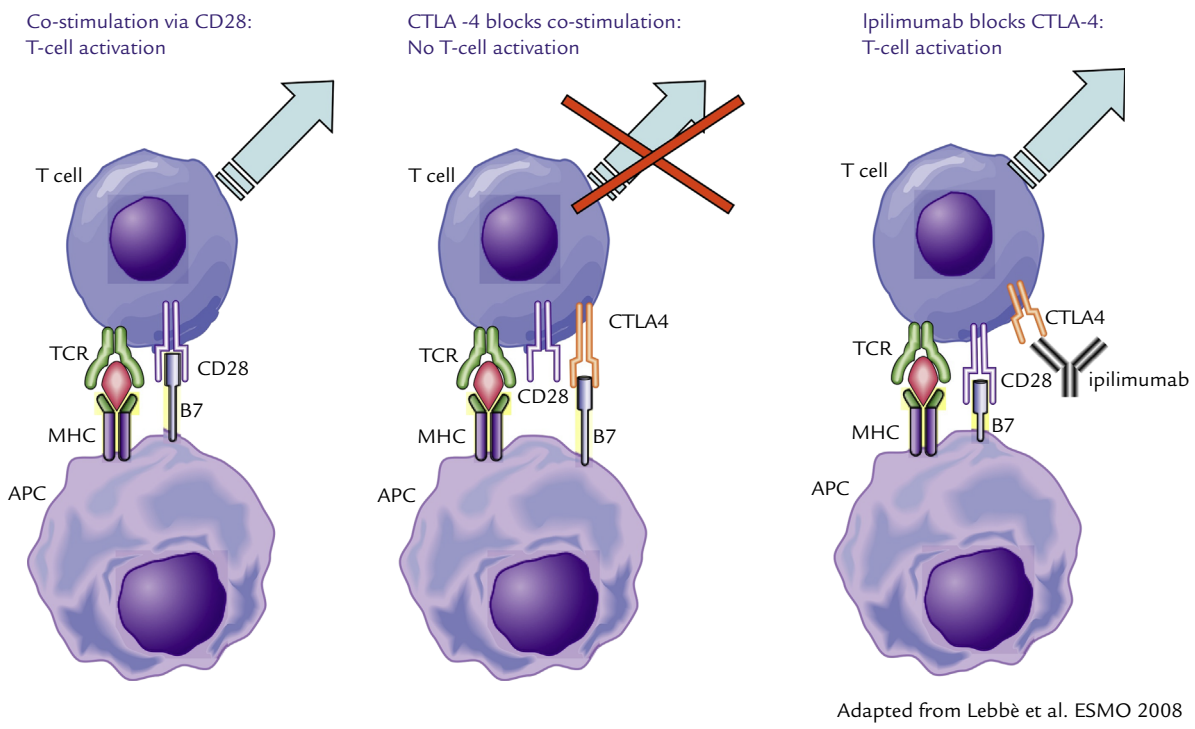

Figure 8. Ipilimumab blocks negative signaling from CTLA-4 resulting in T cell potentiation. Image adapted with permission from Lebbe C, et al. Oral presentation 7690 at ESMO 2008 et al.79 Oral presentation 7690 at ESMO 2008. Image also reused and adapted from e-Grand-Round, "State-of-the-art treatment for advanced melanoma; Mayor S.[80]

in patients with advanced or unresectable melanoma who no longer exhibited response to alternate agents. The drug was granted priority review by the FDA on October 28, 2014, and given the orphan drug designation. Ultimately, accelerated approval was granted based on a Phase I clinical trial showing efficacy in 173 patients with ipilimumab-refractory melanoma.[83] Patients were treated at either the 2-mg/kg dose or at the higher 10-mg/kg dose level. At both dose levels, 24% of patients exhibited a radiographic response to therapy, and this effect lasted 1.4 to 8.5 months and continued beyond this point in most patients. The most commonly reported adverse events included fatigue, cough, nausea, pruritus, rash, decreased appetite, constipation, arthralgia, and diarrhea.[83] Importantly, PD-1 blockade was effective in patients who had previously progressed with ipilimumab therapy, reflecting the complementary effects of the various checkpoint inhibitors. Currently, a protocol concept is under consideration by Merck & Co, examining the use of pembrolizumab in patients with advanced stage, recurrent, or persistent cervical cancer.

Alternate immune modulatory strategies have explored the use of monoclonal antibodies directed against CTLA-4. Ipilimumab is a fully human monoclonal antibody directed against CTLA-4, an immune inhibitory molecule expressed in activated T cells and suppressor T-regulatory cells. Through CTLA-4 blockade, there is stimulation of the immune response, breaking immune tolerance and overcoming immune suppression, with oncologic success seen in patients with advanced stage melanoma, renal cell cancer, and non–small cell lung cancer.[84] Ipilimumab was approved on March 25, 2011, by the US FDA for the treatment of unresectable metastatic melanoma based on the overall survival benefit in a randomized Phase III clinical trial.[85] The addition of ipilimumab

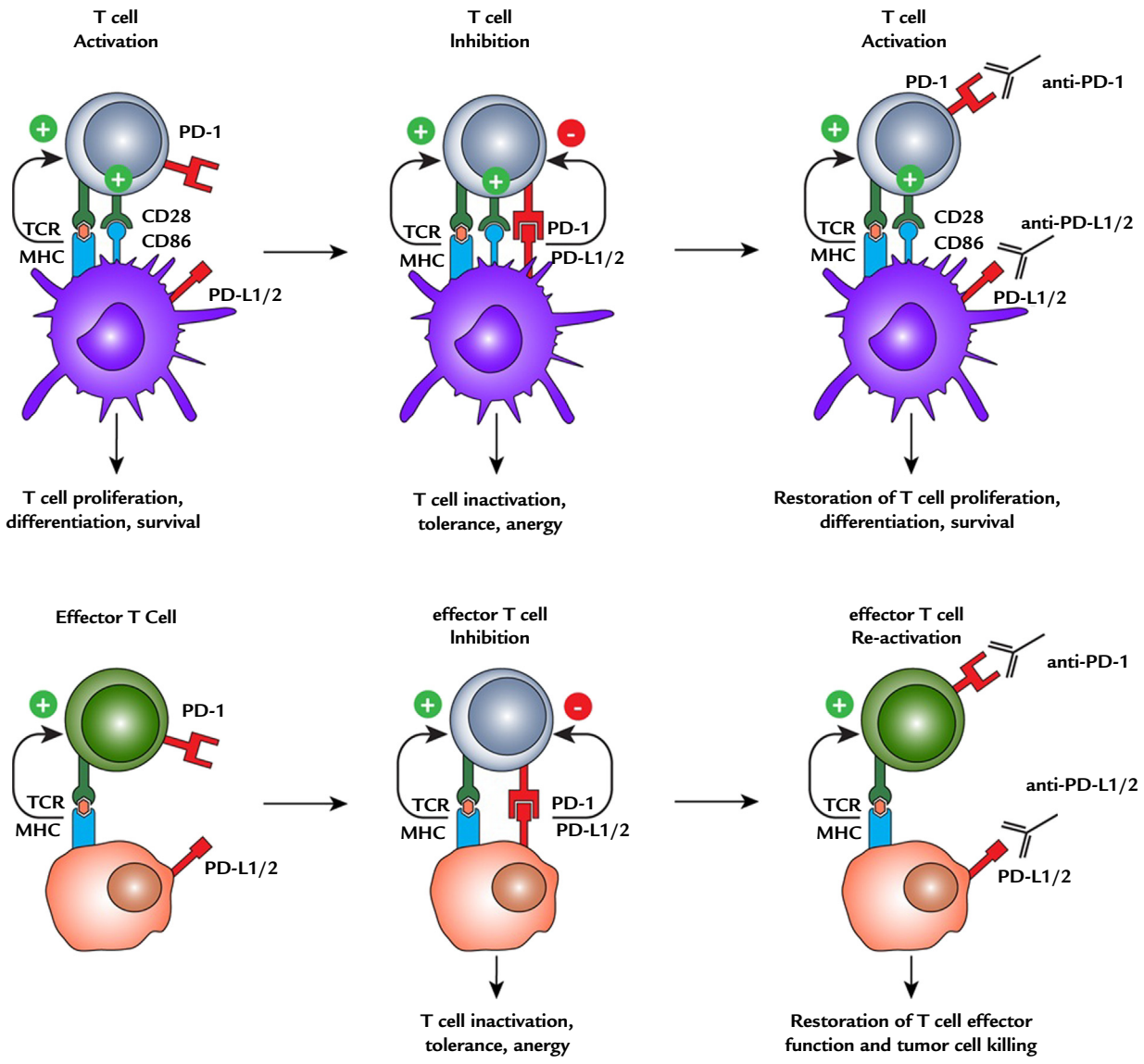

Figure 9. Effect of anti-PD1 (ie Nivolumab) and anti-PD-L1 (ie Pembrolizumab) on T cell function. The PD-1 pathway operates on two different levels, regulating both T cell activation by DCs and the effector function of antigen-specific T cells. PD-1 pathway blockade by monoclonal antibodies directed against PD-1, or its ligands, promotes T cell activation by shifting the balance of signals delivered by the DC from suppressive to activating. In the tumor microenvironment, tumor-specific T cells recognize tumor cells but are subsequently inactivated by the expression of PD-L1 or PD-L2 on the tumor cell, inducing tolerance and anergy. When rescued, by blocking the PD-1 pathway, T cells recognize antigen in the periphery and, in the absence of PD-1 engagement, they assume full effector function and eliminate tumor cells. Image adapted with permission from: Vasaturo A, Di Blasio S, Peeters DG, et al., "Clinical implications of co-inhibitory molecule expression in the tumor microenvironment for DC vaccination: a game of stop and go," Front. Immunol. Dec 2013. Doi 10.3389.[81]

resulted in a near doubling of the 1- and 2-year survival for study patients when compared with the peptide vaccine alone arm.[85]

In the cervical cancer arena, an ongoing Phase I clinical trial (NCT01711515) is examining the effect of ipilimumab administration after chemoradiation

in patients with locally advanced cervical cancer (stage 1B2/2A) with positive para-aortic lymph nodes only or those with stage 2B, 3B, or 4A disease with positive lymph nodes. The studies primary outcome includes assessment of dose-limiting toxic effects, whereas the secondary outcomes include response rate, progression-free survival, overall survival, location of recurrence, and chronic toxic effects experienced within 1 year of completion of therapy. Patients will receive cisplatin-based chemoradiation followed by brachytherapy. Within 2 weeks of completion of brachytherapy, patients will receive IV ipilimumab for 90 minutes once every 3 weeks for 12 weeks.

With the therapeutic efficacy of the immune checkpoint inhibitors ipilimumab and nivolumab established in several disease sites, the Cancer Therapy Evaluation Program of the National Cancer Institute distributed a mass solicitation for concepts that involved the PD-1 inhibitor nivolumab, with or without ipilimumab, for the treatment of advanced cervical cancer and ovarian cancer in the last quarter of 2013.[59] This resulted in the development of a proposed randomized, Phase II, placebo-controlled trial of nivolumab monotherapy with a crossover and run-in randomized Phase II trial of nivolumab with and without ipilimumab for advanced cervical carcinoma after failure of anti–vascular endothelial growth factor therapy (**Figure 3**).

More recently, an upstream immune checkpoint inhibitor has been identified and is actively being studied in metastatic, nonsquamous, non–small cell lung cancer. Bavituximab is a chimeric monoclonal antibody that targets phosphatidylserine via serum cofactor β_2-glycoprotein-1. Phosphatidylserine-targeting antibodies mediate multiple immunostimulatory changes in the tumor microenvironment, including a reduction of tumor-promoting immune cells and an increase in antitumor macrophages, DCs, and T cells.[86] Concepts are being discussed exploring the use of bavituximab in combination with PD-1 blocking agents in the treatment of recurrent, persistent, or metastatic cervical cancer.

ASSESSING RESPONSE AND MANAGING TOXIC EFFECTS

Understanding how to define response to treatment and identifying the unique toxic effects associated with immunotherapies are critical. Unlike traditional cytotoxic therapies, it takes time to establish an antitumor immune response, and target lesions may increase in size or appear to progress early in treatment.[87] With ipilimumab, as an example, 4 distinct patterns of response have been observed: (1) regression of baseline lesion with no new lesions; (2) stable disease followed by a slow, steady decline in tumor burden; (3) delayed response after initial increase in tumor burden; and (4) response after the appearance of new lesions (72). The last 3 patterns of response are not seen with traditional cytotoxic therapies and may be associated with improved immuno-oncologic outcomes.[88] These unique response patterns catalyzed the creation of exploratory immune-related response criteria, developed from modified World Health Organization criteria, which allow for a transient increase in tumor volume or the development of new lesions while receiving treatment.[88,89] Given the above, the FDA developed clinical considerations for immunotherapies, describing a series of clinical situations in which sponsors may elect to continue therapy despite evidence of disease progression.

The toxic effects associated with immunotherapy are also unique, with the most severe attributed to a breakdown of immune self-tolerance.[90] The most serious immune-related adverse events include enterocolitis, diarrhea, hepatitis, dermatitis, and endocrinopathies that require long-term hormone replacement therapy. As clinical experience increases, appropriate management algorithms have emerged, with the FDA detailing management of immune-related adverse events on their Risk Elimination and Management System website. Principally, these toxic effects are addressed by early identification, interruption of therapy, symptom management, possible use of corticosteroids, and potential termination of treatment with systemic immune suppression.

FUTURE OF IMMUNOLOGY IN CERVICAL CANCER

With the publication of the GOG protocol 240 and the FDA approval of bevacizumab for use in women with advanced stage, persistent, or recurrent cervical cancer, the efficacy of targeted biologic therapies in a patient populations with historically limited options has been established. Importantly, however, the response seen with antiangiogenic therapy is unlikely to result in durable cure for most treated patients, and

exploration into alternate therapies for this vulnerable population is implicit.

Immunotherapy represents a fifth therapeutic modality, joining surgery, radiation, chemotherapy, and antiangiogenesis therapy, in the treatment of patients with advanced stage cervical cancer. It is unclear whether, despite the HPV-associated origin of cervical carcinomas, immune therapy will result in a survival advantage. Preliminary results previously discussed show promise. As the role of immunotherapy for the treatment of cervical cancer continues to evolve, several clinical trials are in development, and active studies continue to accrue patients and report on results.

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Dr. Tewari designed the paper and mapped out which subjects should be addressed, wrote the history of immunology section, and obtained figures 4-9. Dr. Eskander performed the literature search, obtained figures 1-3, designed the tables, and wrote the first and second draft of the manuscript, each of which Dr. Tewari edited carefully.

CONFLICTS OF INTEREST

The authors have indicated that they have no conflicts of interest to report in regards to the content of this manuscript.

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