Immunotherapy for Gynecologic Cancers: State of the Science

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Keywords: immune therapies, ovarian cancer, cervical cancer, uterine cancer

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Overview

In choosing the Breakthrough of the Year in 2013, the editors of *Science* recognized years of efforts toward the tantalizing goal of enlisting the body's own immune system to fight cancer.(1) Since then, we have seen the acceleration of multiple approaches from the laboratory to the clinic including immune checkpoint blockade, adoptive cellular therapy, and cancer vaccines. How these strategies fare in clinical trials may ultimately lead to paradigm shifts in therapy. This article will outline rationales for immunotherapy in gynecologic cancers and highlight but a few of the many recent developments in applying these technologies.

Ovarian Cancer

The relationship between the immune system and ovarian cancer has long been suspected.(2) T cell infiltration of tumors is an independent prognostic factor for overall survival by multivariate analysis in advanced ovarian cancer.(3-6) The phenotype of the immune response has been shown to be critical; CD8+ T cell infiltration of tumors are associated with improved prognosis, while both regulatory T cells and myeloid derived suppressor cells have been shown to negatively impact survival.(5, 7, 8) This has also been supported by analysis of The Cancer Genome Atlas project, where the immunoreactive-like subtype has the best survival profile amongst high-grade serous ovarian cancers.(9) Knowing ovarian cancer is immunogenic, how the immune response could be modulated to improve prognosis has been an important area of study. Understanding how chemotherapy and surgery influence or are influence by immune factors, and how immune therapies could be integrated into current treatment paradigms are increasingly important considerations. These issues have been examined further in the context of neoadjuvant chemotherapy and surgical outcome in high grade serous ovarian cancer. Tumor

infiltration by T cells did not continue to hold prognostic benefit in patients treated with neoadjuvant chemotherapy, whereas CD8+ T cells held prognostic benefit only in cytoreduction to no residual disease, but not in optimally (<1 cm) or incompletely cytoreduced patients.(10)

Efforts to stimulate immune responses against tumors through immune checkpoint blockade have yielded promising results with monoclonal antibodies targeting the PD-1/PD-L1 pathway. The PD1/PD-L1 interaction is a mechanism that normally prevents autoimmunity, but is exploited by tumors to evade the immune response. PD-L1 expression has been shown to be associated with improved survival in high grade serous ovarian cancers. Nivolumab, an anti-PD-1 monoclonal antibody demonstrated response rates of 15% with overall survival of 20 months in platinum resistant ovarian cancer with minimal toxicities.(11) Similar results have been reported for pembrolizumab, another monoclonal antibody targeting PD-1 and avelumab, a monoclonal antibody targeting PD-L1.(12, 13)

Therapeutic vaccines have also been under active study for ovarian cancer.(14) More recently, vaccines have been combined with cytotoxic chemotherapies to treat advanced ovarian cancer. This has been spurred by the recognition that cytotoxic chemotherapies active in ovarian cancer may also synergize with immune-based therapies.(15-17) Peptide vaccination targeting p53 in combination with gemcitabine and interferon- α has generated antigen-specific T cell responses and increased circulating CD4+ and CD8+ T cells without increasing regulatory T cells.(18) Peptide vaccination targeting survivin in combination with metronomic cyclophosphamide has also been shown to be immunogenic and capable of generating polyfunctional T cells in advanced ovarian cancer patients.(19)

Immunotherapies showing promise in other malignancies will be considered for ovarian cancer. Adoptive T cell therapy using chimeric antigen receptors (CAR-T cells) targeting mesothelin have been infused in ovarian cancer patients.(20) Combinations of chemotherapies with immunomodulatory antibodies are also being studied. Bringing these approaches to ovarian cancer may ultimately take immune therapies beyond the treatment of recurrent disease to the adjuvant, maintenance, or neoadjuvant settings.

Cervical Cancer

HPV related cancers have long had one of the strongest rationales for an immune-based approach to therapy. A number of factors work in favor of immunotherapy for cervical cancer: a well-characterized pathogen initiates defined precursor lesions and timeline for progression to invasive cancers. With a known viral cause for these cancers, prophylactic vaccines have been developed and deployed to prevent infection and subsequent dysplasia and invasive cancer.(21) Despite this avenue for prevention, cervical cancer remains a significant cause of mortality in many parts of the world where access to vaccination and screening services continues to be limited.

HPV oncoproteins have the characteristics of ideal tumor specific antigens, present only in malignant or dysplastic cells and required for maintaining the malignant phenotype. Therapeutic vaccination has successfully targeted well-defined preinvasive dysplastic lesions. This has been done successfully in another HPV driven entity, vulvar intraepithelial neoplasia (VIN) . A synthetic long peptide vaccine yields a 79% clinical response rate in vaccinated VIN patients after 12 months.(22) This approach has been taken to cervical intraepithelial neoplasia (CIN) 2/3 with VGX-3100, a synthetic DNA vaccine, in a randomized double-blind, placebo

controlled trial. Nearly half of patients receiving the vaccine had regression of CIN 2/3 lesions compared to only 30.6% of those who received placebo ($p=0.034$).⁽²³⁾

For invasive cervical cancer, the biologic timeline may be less favorable for vaccine monotherapy given the kinetics of T cell expansion *in vivo* after vaccination. Additionally the presence of tumor cell intrinsic factors that support immune evasion and features of the tumor microenvironment such as increased numbers of regulatory T cells may make it even less favorable.^(24, 25) While antigen specific interferon- γ producing T cells have been induced in patients with therapeutic vaccination, there have been few clinical responses.⁽²⁶⁾

For locoregionally advanced disease, reliance on platinum-based chemotherapy with radiation therapy presents potential synergies with immunotherapies in development. Cisplatin augments peptide vaccination targeting HPV type 16 to increase HPV specific T cells in preclinical models.⁽²⁷⁾ Radiation therapy has been shown to activate mechanisms seen with immune checkpoint inhibitors.⁽²⁸⁾ That 67% of squamous cell cancers of the cervix and 43% of vulvar cancers will have co-gain or co-amplification of CD274 and PDCD1LG2, genes that encode PD-L1 and PD-L2, also supports the use of the anti-PD-1 and anti-PD-L1 agents in these cancers.⁽²⁹⁾

The infusion of autologous tumor reactive T cells, adoptive T cell therapy, has been successful in other malignancies. In a trial conducted at the National Cancer Institute, patients with metastatic, locally advanced and resistant or refractory, or recurrent cervical cancer received a single infusion of T cells harvested and expanded *ex vivo* from fragments of metastatic tumor. Three of 9 patients with metastatic cervical cancer treated with adoptive T cell therapy had objective tumor responses, 2 complete responses, and 1 partial response. Clinical responses were

also found to correlate with the frequency of T cells recognizing HPV in the peripheral blood.(30)

Uterine Cancers

The uterus in normal reproductive physiology has the ability convert to an immune privileged site to isolate a pregnancy from the host immune system. This immunologic plasticity is not completely understood, but may result in unique immunomodulatory elements available to cancers. These factors may also present opportunities for immunotherapies to augment current treatments.

Twenty to thirty percent of endometrial cancers may have high microsatellite instability(MSI) due to genetic or epigenetic factors leading to accumulation of somatic mutations. MSI is noteworthy when considering agents targeting PD-1/PD-L1.(31) The successful application of anti-PD-1/PD-L1 therapies may arrive at the intersection of cancer genetics and cancer immunology. In lung cancers, a higher nonsynonymous mutation burden in tumor correlates with improved response to anti-PD-1 therapy.(32) Because somatic mutations have the potential to encode immunogenic neoantigens, tumors with DNA mismatch repair deficiencies may be especially susceptible to immune checkpoint blockade. Pembrolizumab, an anti-PD-1 antibody, was tested in 41 patients with progressive metastatic cancer, including 2 patients with endometrial cancer. High somatic mutation load was significantly associated with prolonged progression free survival ($p=0.02$). (33)

Endometrial cancers can also be rendered immunogenic through mutations in replicative DNA polymerase epsilon (POLE) leading to impaired DNA proofreading function.(34, 35) This is thought to make up about 5% of endometrial cancers.(36) Loss of DNA proofreading may

lead to the development of immunogenic neoantigens and susceptibility to immune checkpoint blockade. When 63 endometrial cancer patients were evaluated for tumor infiltrating lymphocytes and PD1/PD-L1 expression, PD-1 was found overexpressed in TIL and peritumoral lymphocytes in POLE mutated and MSI tumor. PD-L1 expression was also found to be more common in POLE mutated and MSI tumors.(37)

Future Directions

Development of immunotherapies for gynecologic cancers continues to accelerate. Tumor biology studies and genomic analysis will elucidate better targets for vaccine and T cell therapy. By clarifying the mechanisms of current immunotherapies and the immunologic effects of radiation and cytotoxic chemotherapy, rational combinations of therapies to augment responses can be developed. Biomarkers associated with response will also allow new immunotherapies to target patients most likely to benefit. With agents now poised to combat mechanisms long exploited by cancers to subvert anti-tumor responses, immunotherapy for gynecologic cancers may soon realize its potential.

Acknowledgments

Dr. Liao is supported by the Department of Defense, Ovarian Cancer Research Program, Ovarian Cancer Academy.

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