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Immunotherapy for Ovarian Cancer: What's Next?

Lana E. Kandalaft, Daniel J. Powell Jr, Nathan Singh, and George Coukos

From the Ovarian Cancer Research Center, University of Pennsylvania, Philadelphia, PA.

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Corresponding author: George Coukos, MD, PhD, University of Pennsylvania 1000 Courtyard Blg, 3400 Spruce St, Philadelphia, PA 19104; e-mail: gcks@mail.med.upenn.edu.

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ABSTRACT

In the past decade, we have witnessed important gains in the treatment of ovarian cancer; however, additional advances are required to reduce mortality. With compelling evidence that ovarian cancers are immunogenic tumors, immunotherapy should be further pursued and optimized. The dramatic advances in laboratory and clinical procedures in cellular immunotherapy, along with the development of powerful immunomodulatory antibodies, create new opportunities in ovarian cancer therapeutics. Herein, we review current progress and future prospects in vaccine and adoptive T-cell therapy development as well as immunomodulatory therapy tools available for immediate clinical testing.

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INTRODUCTION

Epithelial ovarian carcinoma is the fourth most common cancer in women, and the most lethal gynecologic malignancy in the United States, accounting for approximately 22,000 new cases and 15,000 deaths per year. Due to incremental improvements in surgery and chemotherapy, the 5-year survival rate has increased from 37% in the 1970s to 45% in the 1990s.[1] However, no substantial decrease has been seen in death rates, as the majority of patients relapse and die from their disease despite response to first-line therapy.[2] Based on large cooperative randomized clinical trials, the combination of carboplatin and paclitaxel still remains the best performing chemotherapy regimen. Thus, novel therapeutic approaches are direly needed. Although not traditionally considered responsive to immune therapy, increasing evidence indicates that ovarian cancers are, in fact, immunogenic tumors (Table 1).[3-35] This evidence comes from diverse epidemiologic and clinical data comprising: evidence of spontaneous antitumor immune response and its association with longer survival in a proportion of patients with ovarian cancer; evidence of tumor immune evasion mechanisms and their association with short survival in some patients with ovarian cancer; and pilot data supporting the efficacy of immune therapy.

SPONTANEOUS OR ELICITED IMMUNE RESPONSE AND CLINICAL IMPACT

A spontaneous antitumor immune response has been convincingly demonstrated in some patients with ovarian cancer. Tumor-reactive T cells and antibodies have been detected in peripheral blood of patients with advanced stage disease at diagnosis,[11,12] while oligoclonal tumor-reactive T cells have been isolated from tumors or ascites.[13-21] The tumor rejection antigens expressed by ovarian cancer have not been thoroughly characterized, but a number of known tumor-associated antigens recognized by peripheral blood or tumor-associated lymphocytes have been described to date. These comprise the cerebellar degeneration-related protein cdr2; p53; HER2/*neu*; mesothelin; folate receptor-α; cancer-testis antigens, such as NY-ESO-1, MAGE melanoma antigen family members, and sperm surface protein Sp17; mucins or glycoproteins such as Lewis(y); sialylated-Tn; cancer antigen CA-125 (MUC-16) and MUC-1; universal tumor antigens, such as survivin and the human telomerase reverse transcriptase; and others.[10] Importantly, the detection of antitumor immune response in the form of intraepithelial (also called intratumoral) tumor-infiltrating lymphocytes (TILs; ie, T cells infiltrating tumor islets) predicts significantly longer survival in ovarian cancer. We first reported in an Italian cohort that patients whose tumors had intraepithelial T cells experienced longer progression-free and overall survival as compared with patients whose tumors lacked intraepithelial T cells.[36] Survival at 5 years was substantial (38%) in patients whose tumors had intraepithelial T cells (n = 102) and negligible (4.5%) in patients lacking them (n = 72), even after complete response to chemotherapy. A signature of antitumor immune response activation was identified in tumors with intraepithelial T cells.[36] The impact

Table 1. Clinical Evidence That Ovarian Cancers Are Immunogenic

Finding	Reference
Spontaneous anti-tumor response	
Association between intraepithelial T-cell infiltration and patient survival	3-9
Tumor-associated antigen expression	Reviewed in 10
Antigen specific antibodies	11
Tumor-reactive T cells in blood, ascites, and tumor	11-21
Tumor immune evasion	
Inverse association between survival and intratumoral regulatory T cell	6-8
Inverse association between survival and B7-H4+ macrophages	22
PD-L1 expression by tumor predicts low T-cell infiltration	5
ETBR expression restricts T-cell infiltration and predicts poor survival	23, 24
Clinical responses to immunotherapy	
Interleukin-2 (T-cell growth factor)	25, 26
Anti-CTLA-4 antibody	27, 28
Vaccine responses	29-32
Adoptive TIL transfer	33-35

Abbreviations: PD-L1, programmed death ligand 1; ETBR, endothelin B receptor; TIL, tumor-infiltrating lymphocytes.

of intraepithelial $CD3^+$ or $CD8^+$ T cells was confirmed by multiple independent studies on ethnically and geographically diverse populations.[3-7] Importantly, intraepithelial T cells were more prevalent in tumors with increased proliferation, indicating that improved outcome is not due to indolent tumor cell behavior.[3]

Significant progress has been made recently in our understanding of immune evasion mechanisms operating in some patients with ovarian cancer. $CD4^+$ $CD25^+$ $FoxP3^+$ T regulatory T cells (Treg) were first demonstrated in ovarian cancer,[8,9] where increased Treg frequency predicts poor patient survival.[6,9] Immunosuppressive B7-H4 expressing macrophages were recently found to correlate with survival in ovarian cancer.[22] In addition, ovarian cancer cells express programmed death ligand 1 (PD-L1 or B7-H1), a ligand for the immunosuppressive T-cell receptor PD1, which blocks T-cell responses. Expression of PD-L1 by tumor cells predicted paucity of intraepithelial TILs and short overall survival in ovarian cancer.[5] Finally, overexpression of the endothelin B receptor (ET_BR), which suppresses T-cell–endothelial adhesive interactions and T cell homing to tumor, correlated with absence of TIL and short survival in ovarian cancer.[23,37]

The association of antitumor immune response (intraepithelial T cells) with prolonged survival, and vice versa the association of immune escape mechanisms with poor survival, suggest that ovarian cancers are intrinsically immunogenic. Indeed, ovarian cancers should no longer be considered immunologically inert tumors, as pilot clinical data indicate that patients with ovarian cancer can in fact respond to the same immunotherapy approaches as patients with other immunogenic tumors, such as melanoma,[24] including interleukin-2 (IL-2), CTLA-4 antibody, and adoptive transfer of ex vivo expanded TIL. Notably, all of these therapies capitalize on preexisting endogenous antitumor immune response. Importantly, weekly intraperitoneal IL-2 infusion produced an approximately 17% complete pathologic response rate in patients with platinum-resistant ovarian cancer,[25,26] while objective responses and/or prolonged survival have been reported with CTLA-4 antibody[27,28] as well as with adoptive transfer of ex vivo expanded TIL.[34,38]

CANCER VACCINES

As with many other tumor types, vaccines have been the main approach to ovarian cancer immunotherapy.[10,33,39,40] Consistent with experience in other immunogenic tumors,[41] vaccines have shown limited efficacy as monotherapy in patients with advanced recurrent disease. Clearly, much work is required to improve their performance. Current efforts to improve vaccines are directed broadly toward optimizing the choice of antigens; improving vaccine delivery systems to maximize the magnitude and quality (phenotype and polarization) of T-cell response; and developing combinatorial approaches with adoptive T-cell or immune modulation therapy to maximize activation and function of vaccine-primed T cells in vivo.

Some results to date are noteworthy and provide encouragement for further vaccine development and optimization for use in combinations. In a retrospective review of patients treated in the adjuvant setting after secondary complete response, Sabbatini et al[42] noted that patients vaccinated with monovalent or heptavalent vaccines against carbohydrate epitopes experienced significantly longer time to progression and higher progression-free survival rates relative to controls from the same institutions treated with alternate consolidation therapies. In addition, vaccination with anti-idiotype ACA-125, an analog of CA-125, resulted in CA-125–specific antibodies and was associated with prolonged survival.[29] Another study was performed using carcinoembryonic antigen-MUC-1-TRICOM poxviral-based vaccines in 16 patients including three patients with ovarian cancer. Immune responses to MUC-1 and/or carcinoembryonic antigen were seen after vaccination in nine patients. A patient with clear-cell ovarian cancer and symptomatic ascites had a radiographically and biochemically durable (18-month) clinical response.[30] A major limitation in vaccine development in ovarian cancer stems from the lack of well-characterized rejection antigens and by the significant molecular heterogeneity of the disease. HER2 is a rejection antigen in breast cancer and it is possible that despite low expression, it may also serve as rejection antigen in ovarian cancer.[17,43] Vaccination against HER2 has resulted in sustained antigen-specific T-cell and humoral immunity as well as epitope-spreading in patients with ovarian cancer.[44] NY-ESO-1 is a bona fide ovarian cancer rejection antigen.[31,45] However, NY-ESO-1 is expressed in fewer than 30% of ovarian cancers, highlighting the limitations of a monovalent vaccine. In a recent study, one patient experienced complete objective response to NY-ESO-1 peptide vaccine, but later recurred with a NY-ESO-1–negative tumor, proving that single-target immunization can result in immune escape tumor variants after initial response.[45]

A viable alternative to vaccines directed toward specific antigens are whole tumor antigen vaccines created using tumor cells, autologous tumor lysate, or tumor-derived RNA.[46-48] Advantages of these vaccines include the opportunity to induce immunity to a personalized and broad range of antigens, which could minimize the development of tumor escape variants; the inclusion of yet unidentified tumor rejection antigens; no HLA haplotype restriction; and the simultaneous administration of MHC class I and class II epitopes, which could prove beneficial for immunologic memory. A meta-analytic review of

173 published peer-reviewed immunotherapy trials (of melanoma, renal cell, and hepatocellular carcinomas, lung, prostate, breast, colorectal, cervical, pancreatic, and ovarian cancers) that used either molecular defined synthetic antigens (1,711 patients) or whole-tumor antigen (autologous or allogeneic tumor cells; dendritic cells pulsed with tumor extracts or mRNA, 1,733 patients) found that 8.1% of patients vaccinated with whole-tumor antigen had objective clinical responses, while 3.6% of patients vaccinated with molecularly defined tumor antigens (synthetic peptides or proteins, and viral or plasmid vectors encoding peptides or proteins) had objective clinical responses ($P < .001$, χ^2 test).[37]

Several groups have used viruses to increase tumor cell immunogenicity for whole-tumor cell vaccination. Objective responses have been seen after intracavitary delivery of a viral oncolysate vaccine generated with ovarian cancer cell lines infected with influenza-A virus[49,50] or with autologous tumor cells infected with Newcastle disease virus.[51] We recently investigated preclinically the use of replication-restricted herpes simplex virus (HSV) 1 to infect autologous tumor cells for vaccine preparation. HSV-infected tumor cells used directly or pulsed on dendritic cells elicited potent antitumor immune response in the mouse, which was superior to the use of ultraviolet-irradiated tumor cells.[32,52,53] Thus, whole-tumor antigen vaccines can produce objective response if immunogenicity is increased through the use of pathogens.

An alternative approach to deliver effectively whole-tumor antigen is by using dendritic cells (DCs). In a pilot study using mature DCs pulsed with whole autologous tumor lysate, three of six subjects demonstrated remission inversion (ie, their progression-free survival postvaccination was longer than the interval between prevaccine recurrence and prior chemotherapy treatment).[54] The use of DC/tumor cell fusion approach is a viable alternative whereby autologous DCs are fused with tumor cells, which allows DCs to express the entire antigen repertoire of the tumor cells to $CD4^+$ and $CD8^+$ T cells. DC/ovarian tumor cell fusions have been generated and demonstrated to be able to induce antitumor cytotoxic T-lymphocyte activity in vitro.[55]

Although whole tumor vaccines offer distinct advantages, some drawbacks warrant consideration. First, surgical procurement of large number of autologous tumor cells may not be possible in many patients. Alternatives to this limitation exist, including use of allogeneic cell lines or the use of tumor mRNA. RNA electroporation of DCs is a convenient approach to generate a potent tumor vaccine.[53] An additional concern with whole tumor vaccination relates to the inclusion of a large number of self-antigens, which could potentially drive tolerogenic responses (ie, expand Treg) rather than cytotoxic lymphocyte responses. Recent work has demonstrated that DCs can be polarized ex vivo with the use of interferons, Toll-like receptor agonists, or p38 mitogen-activated protein kinase inhibitors to drive cytotoxic lymphocytes and Th17 effector cells at the expense of Treg.[56] In contrast, if immunization is successful, there may be increased concern for breaking tolerance to self-antigens, leading to immunopathology. To date, pilot studies with whole tumor vaccines have reported no autoimmunity in patients with ovarian cancer.

A major limitation of cancer vaccines presently stems from the inability to elicit a rapid and overwhelming T-cell response, which is required to reject established tumors. A potential solution to this limitation is provided by combinations with immune modulation therapy aiming at breaking peripheral tolerance mechanisms, which may reduce the number of tumor reactive T cells required to reject tumors. For example, we have recently shown that immune modulation through blockade of the endothelin B receptor, a vascular endothelial growth factor (*VEGF*) –regulated gene, markedly increases the efficacy of weak vaccines by reversing the inhibitory function of tumor endothelium and enabling homing of tumor-reactive T cells.[23,37] Furthermore, depletion of Treg is a critical maneuver to enhance vaccine therapy.[57] A pilot study at the University of Pennsylvania is testing this hypothesis by administering partially mature DCs pulsed with autologous tumor cell lysate to subjects with recurrent ovarian cancer in combination with immune modulation with oral metronomic cyclophosphamide (to deplete Treg)[58] and bevacizumab (to disrupt the blood-tumor endothelial barrier).[23] Despite weak vaccine immunogenicity, as assessed by interferon-γ ELISpot, partial objective responses have been observed by RECIST (Response Evaluation Criteria in Solid Tumors Group), which can be attributed in part to the addition of vaccine.[58a] An additional approach to enhance the efficacy of vaccines may be provided by combination with postvaccine adoptive transfer of ex vivo expanded T cells. At the University of Pennsylvania, we are testing adoptive transfer of ex vivo CD3/CD28-costimulated, vaccine-primed T cells (after high-dose outpatient cyclophosphamide and fludarabine) to rapidly achieve expansion and activation of tumor-specific T cells postvaccine (Fig 1). This approach could not only result in increased survival, engraftment, and function of tumor-reactive T cells, but also in durable reduction of $CD4^+FoxP3^+$ T regulatory cells.

ADOPTIVE T-CELL THERAPY

Effective cancer immunotherapy is dependent on the presence of large numbers of antitumor lymphocytes with appropriate homing and effector functions that enable them to seek out and destroy cancer cells in vivo. The adoptive transfer of ex vivo expanded tumor-reactive T cells holds the potential of achieving this condition in a short period of time (Fig 1). Clinical trials testing spontaneous or induced polyclonal or oligoclonal T cells conducted in the past two decades have provided crucial lessons that can guide further optimization. The use of ex vivo expanded TILs has yielded promising clinical results to date. The advantages of TIL-based adoptive therapy include the presence of spontaneously occurring T cells with natural avidity against tumor which have escaped thymic deletion; the use of a polyclonal population of T cells, which can limit immunologic escape of tumors; and the natural selection of patients whose tumor microenvironment is already conducive to T-cell homing. Initial studies using TILs in the treatment of metastatic melanoma during the late 1980s and early 1990s demonstrated objective antitumor responses, which however were short lived. Based on animal studies showing that host lymphodepletion before T-cell transfer enhances persistence of T cells and antitumor response, a scheme of incremental lymphodepletion through high-dose nonmyeloablating chemotherapy and added whole body radiation was tested. Infused cells were both long lived, and highly penetrating, showing regression of voluminous metastatic tumors, with up to 16% complete response and 72% overall objective response rates in recent reports with maximal lymphodepletion and radiation. T-cell persistence correlated with long lasting responses.[41,59] Although these are phase I studies accruing a highly selected cohort of patients with metastatic melanoma with pre-existing antitumor immunity, whose tumors yield tumor-reactive TILs, the results

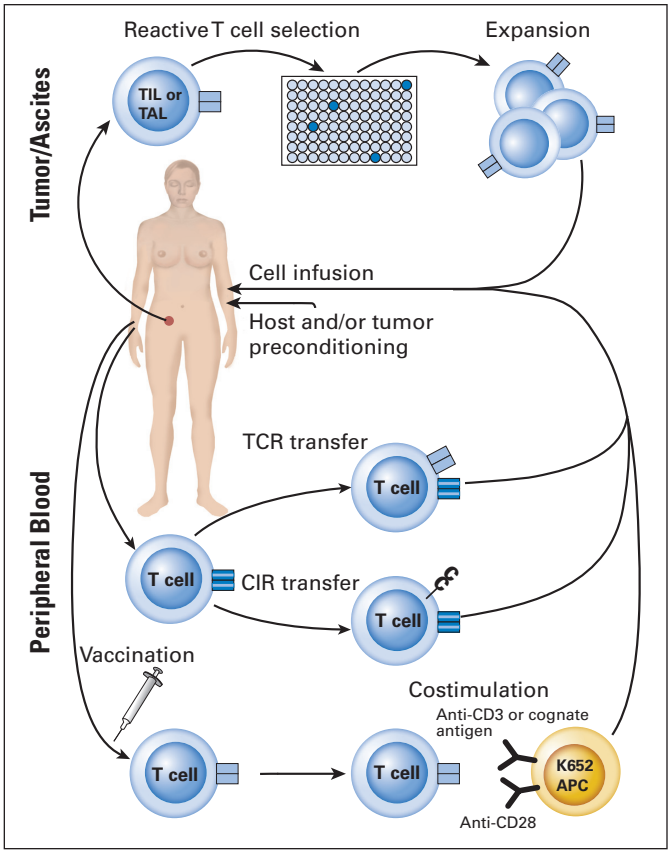

Fig 1. Adoptive transfer of T cells is a powerful approach for the treatment of patients with advanced malignancies. It can be accomplished by the adoptive transfer of previously isolated and expanded tumor-specific tumor-infiltrating lymphocytes (TILs) or the adoptive transfer of ex vivo CD3/CD28-costimulated, vaccine-primed T cells (after high-dose outpatient cyclophosphamide and fludarabine) to rapidly achieve expansion and activation of tumor-specific T cells postvaccine. Alternatively adoptive T-cell transfer using genetically modified T cells, which express exogenous tumor antigen-specific T-cell receptors (TCRs), to mediate objective cancer regression. TAL, tumor-associated lymphocytes; CIR, chimeric immune receptor.

clearly demonstrate the power of adoptive immunotherapy and dispel the assumption that immunotherapy can only control small tumors.[24] Furthermore, although the role of $CD8^+$ T cells has been well established in adoptive immunotherapy,[41,59] $CD4^+$ cells can also produce objective responses.[60]

There is evidence that TIL-based adoptive therapy is an important opportunity in ovarian cancer. In the early 1990s, ovarian cancers were found to yield reactive TILs after IL-2 culturing in vitro that may be amenable to adoptive transfer.[61,62] Moreover, in pilot clinical trials, patients who received adjuvant therapy with adoptive transfer of tumor-derived lymphocytes expanded ex vivo with IL-2, after surgical debulking and first-line chemotherapy, showed a survival advantage.[34,38] Stage III epithelial ovarian cancer patients treated with consolidation adoptive transfer of expanded TILs after completion of cisplatin-based first-line chemotherapy ($n = 13$) had 3-year overall survival rate of 100%, while that of a control group of patients ($n = 10$) receiving only chemotherapy was 67.5% ($P < .01$). The 3-year disease-free survival rate of the patients in the TIL group and in the control group was 82.1% and 54.5%, respectively. While these results can be limited by the lack of random assignment, they nevertheless support the feasibility of adoptive therapy for ovarian cancer.[38]

Optimization of adoptive TIL therapy is a matter of intense investigation and currently directed at: optimizing methods to select tumor-reactive TIL and expand them under optimal costimulation conditions that allow preferential expansion of specific T-cell phenotypes; and optimizing host and/or tumor conditioning. As shown in the melanoma trials, although infused cells had an effector phenotype ($CD27^-$ $CD28^-$ $CD45RA^-$ $CD62L^-$ $CCR7^-$), TILs that persisted 2 months after infusion in patients who exhibited tumor regression were characterized by a less differentiated phenotype ($CD27^+$ $CD28^+$ $CD45RA^+$ but $CD62L^-$ $CCR7^-$) and longer telomeres.[35,63-66] These results argue that use of memory rather than effector cells may be more efficacious for adoptive transfer,[67] which has been confirmed by mouse models.[68] Because TILs comprise a large number of tumor-reactive effector cells, identification of culture conditions that preferentially expand memory phenotypes is a priority. Recent technological advances with the development of artificial antigen presenting cells (aAPCs) expressing a variable repertoire of costimulatory molecules and cytokines has generated new opportunities to provide the desired costimulatory molecules and cytokines to re-educate TILs, improving their potency and function in vivo. June et al[69] recently described the development of a next generation K562-based aAPC platform capable of expressing multiple gene inserts, including human lymphocyte antigen (HLA) -A2, CD64 (the high-affinity Fc receptor) CD80, CD83, CD86, CD137L (4-1BBL), and CD252 (Ox40L), and a variety of T-cell supporting cytokines. Cell-based aAPCs have proven to be more efficient at activating and expanding $CD8^+$ $CD28^-$ T cells, and antigen-specific T cells, than the magnetic bead-based aAPC.[69] Importantly, TILs from patients with ovarian cancer undergo robust expansion while maintaining their tumor reactivity after K562-based aAPC stimulation (Powell et al, unpublished).

A proportion of patients are not eligible for TIL adoptive therapy, because tumors are either unresectable or yield no tumor-reactive TILs. One strategy to overcome the daunting task of raising large numbers of tumor-reactive T cells is by engineering T cells to redirect their specificity. This can be accomplished by transducing lymphocytes to express a cloned T-cell receptor (TCR) with high affinity to tumor-associated epitopes. In this case, the cloned heterodimeric TCR is transduced to mixed peripheral blood T cells isolated from the patient, creating a large amount of bispecific T cells, which are polyclonal with respect to their original TCR, but potentially monoclonal for the cloned TCR (if recombination with endogenous TCR is minimized).[70] Alternatively, T cells can be transduced with a chimeric immunoreceptor.

Recently, Rosenberg et al[71] at the National Cancer Institute demonstrated the clinical feasibility, safety, and preliminary efficacy of redirecting T cells of patients with melanoma using a TCR-specific to MART-1, a melanoma antigen. The genes encoding the α and β chains of the TCR were cloned from a TIL clone derived from a patient demonstrating a near-complete regression of metastatic melanoma after adoptive cell transfer of TILs. Gene transfer resulted in transfection of 30% of $CD8^+$ cells. Adoptive transfer of TCR-transduced cells in 15 patients resulted in durable engraftment at levels exceeding 10% of peripheral blood lymphocytes for at least 2 months after the infusion. High sustained levels of circulating engineered cells at 1 year after infusion was observed in two patients who demonstrated objective regression of metastatic melanoma lesions.[71] TCR-based engineering represents a potentially powerful strategy for ovarian cancer therapy as TCRs that recognize HLA-A2 restricted epitopes from

known ovarian cancer, antigens such as NY-ESO-1, p53, and others are available for clinically testing as well.[72-75] Optimization through selection of naturally occurring or recombinant high affinity receptors, engineering to prevent recombination with endogenous TCR, and the use of lentiviral vectors developed in the June lab with transfection efficiency above 90% are poised to improve this approach significantly.[76]

An alternative strategy to engineer T cells with redirected specificity is through genetic modification to recognize antigens in an MHC-unrestricted fashion through the use of chimeric antigen receptors (CARs), fusion genes encoding an extracellular domain that specifically binds to tumor epitopes through a single-chain variable fragment (scFv) antibody, linked to intracellular signaling modules that mediate T-cell activation.[70,77,78] The tumor binding function of CARs is usually accomplished by the inclusion of a scFv antibody, containing the V_H and V_L chains joined by a peptide linker of about 15 residues in length. In principle, universal targeting vectors can be constructed, because the scFvs bind to native cell surface epitopes and bypass the requirement for MHC restriction.[79,80] Thus, in comparison to TCRs, CARs have two major advantages: their HLA-independent recognition of antigen, which makes them broadly applicable regardless of the subject's HLA and regardless of the level of HLA expression on tumor cells, and their signaling, which redirects T-cell cytotoxicity and permits T-cell proliferation and survival on repeat antigen exposure. A potential drawback stems from their potential immunogenicity, if scFv are nonhuman. This can be averted by using human scFv. CARs will then be the tools of choice for T-cell engineering for cancer immunotherapies.

A large number of CARs targeting diverse tumors have been developed[70,81]; however, clinical pilot tests are just beginning. Some of the ovarian tumor antigens and CAR investigated in vitro and in vivo in T lymphocytes are FBP,[82,83] MUC-1,[84] HER-2, and mesothelin.[85] There has been a single study of adoptive transfer of CAR T cells in ovarian cancer.[86] While this study demonstrated safety, the results were disappointing, with no clinically evident tumor responses, most likely due to low expression of the transgenic CAR, and poor persistence of the transferred T cells.[86] Persistence can be dramatically improved by using human scFv and by adding costimulatory signaling capabilities to the intracytoplasmic domain of CARs. Indeed, one issue that needs to be addressed with CARs is that signaling through the cytosolic domain of the usual scFv-TCRz single chain construct does not fully replicate the multichain TCR signaling complex. This is solved by incorporating additional signaling modules in the cytoplasmic domain of the chimeric receptor. Efficient lentiviral and tissue culture technology now enables highly efficient transduction of primary T cells.[76]

ANTIBODY-BASED IMMUNOMODULATION

Given the limitations of immunotherapy, modulating immune check points by activation of effector cells, depletion of Tregs, or activation of professional APCs could substantially improve the therapeutic efficacy of vaccines or adoptively transferred T cells. The development of functional antibodies is now enabling effective immune modulation (Fig 2).

Dendritic Cell Activation

The main mechanism of immune stimulation by CD40 ligands is activation of DCs resulting in increased survival, upregulation of costimulatory molecules, and secretion of critical cytokines for T-cell priming, such as IL-12. This promotes antigen presentation, priming,

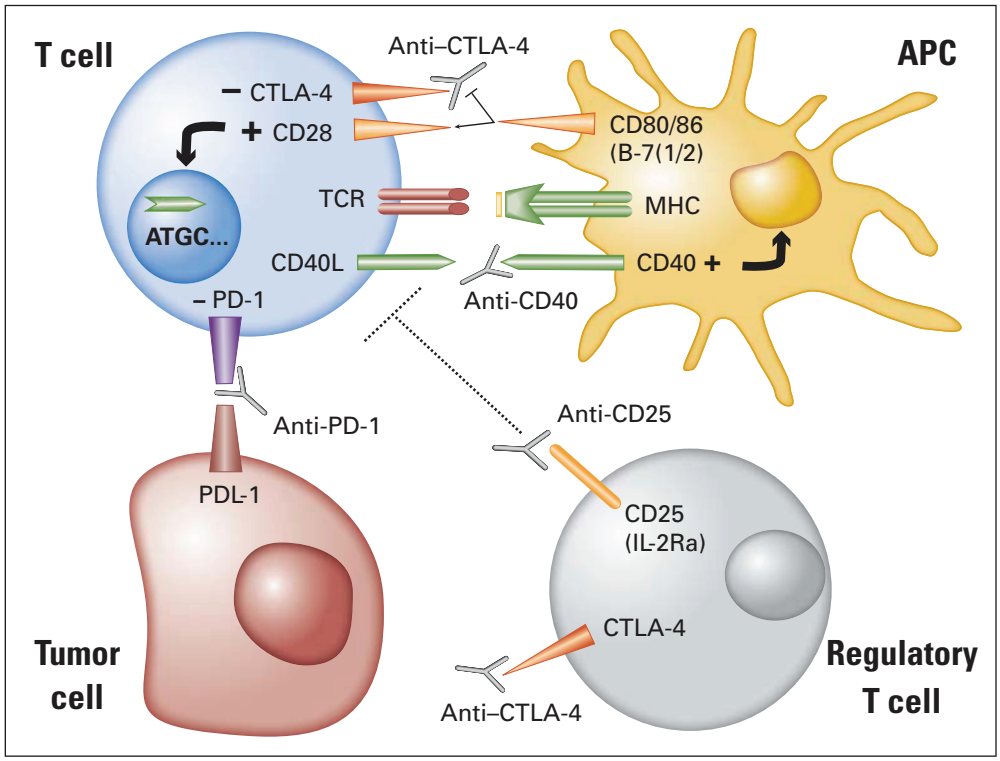

Fig 2. Improved therapeutic efficacy of vaccines or adoptively transferred T cells can be achieved by modulating immune check points, including activation of effector cells by blocking CTLA4 or PD-1; depletion of regulatory T cells by use of low-dose oral or intravenous cyclophosphamide or through targeting the interleukin-2 (IL-2) receptor α chain, also known as CD25; or activation of professional antigen presenting cells (APCs) by stimulation with CD40 ligands.

and cross-priming of $CD4^+$ and $CD8^+$ effector T cells.[87] However, agonistic anti-CD40 antibody is best used in combination with vaccines or Toll-like receptor agonists[87,88] because alone can accelerate the deletion of tumor-specific cytotoxic lymphocytes.[89] Additional value of CD40 ligation is provided by the fact that ovarian cancers, like many tumors, express the CD40 receptor[90-93] and respond to CD40 agonists with apoptosis and growth inhibition in vitro and in vivo.[92,94,95]

Effector T-Cell Activation

T-cell activation is triggered through the T-cell receptor by recognition of the cognate antigen complexed with MHC. This activation is regulated by complex signals downstream of CD28 family immune receptors, which includes costimulatory (CD28 and ICOS) and inhibitory receptors (CTLA-4, PD-1, and BTLA). PD-1 and CTLA-4 are induced on T cells after a TCR signal, and result in cell cycle arrest and termination of T-cell activation. The use of blocking CTLA-4 or PD-1 monoclonal antibodies can sustain the activation and proliferation of tumor-specific T cells, preventing anergy or exhaustion, and thereby allowing the development of an effective tumor-specific immune response.

CTLA-4 blockade activates directly CD4 and $CD8^+$ T effector cells by removing an inhibitory checkpoint on proliferation and function,[96] and combination of direct enhancement of Teff cell function and inhibition of Treg activity is essential for mediating the full therapeutic effects of anti-CTLA-4 antibodies during cancer immunotherapy.[97] The majority of clinical data to date have emerged from studies in patients with melanoma[98] where CTLA-4 blockade has yielded objective responses, while experience remains still anecdotal in ovarian cancer. Eleven patients with ovarian carcinoma, previously vaccinated with granulocyte-macrophage colony-stimulating factor modified, irradiated autologous tumor cells (GVAX), received ipilumimab (1 month to 3 years after GVAX).[27] In one patient, an objective radiographic response was noted and multiple infusions of anti-CTLA-4 antibody every 3 to 5 months have maintained disease control over 4 years.[27] Few patients showed manageable inflammatory toxicities. Tumor regression correlated with the $CD8^+$/Treg ratio, suggesting that other forms of therapy that target Treg depletion may provide a highly effective form of treatment when combined with the tumor vaccine and CTLA-4 antibody arsenal.[27]

PD-1 is a negative regulator of lymphocyte activation, which binds PD-L1 and PD-L2 ligands. PD-L2 is restricted to professional antigen-presenting cells, while PD-L1 is expressed on many tissues. Importantly, ovarian carcinoma cells as well as tumor-infiltrating tolerogenic DCs and myeloid derived suppressor cells express PD-L1,[99,100] and expression levels correlate with disease course. Constitutive expression of PD-L1 by tumors conferred resistance to immunotherapy in mice,[101] while antibodies blocking PD-L1 or PD-1 profoundly enhanced the efficacy of immune therapy.[101,102] A phase I study using PD-1 blocking antibody showed the antibody to be safe and well-tolerated in patients with hematologic malignancies. Clinical benefit was observed in 33% of the patients with one complete remission.[103]

Treg Depletion

$CD4^+$ $CD25^+$ $Foxp3^+$ Treg are responsible for maintaining peripheral tolerance by inhibiting T-cell activity. A number of Treg-depleting strategies have been investigated.[59,104-107] An example is the use of low-dose oral or intravenous cyclophosphamide.[108,109] Other strategies for Treg depletion are through targeting the IL-2 receptor α chain, also known as CD25. In mouse models, the use of anti-CD25 monoclonal antibody before vaccination led to complete tumor rejection and establishment of long-lasting tumor immunity with no autoimmune complications.[57,110] Administration of anti-CD25 antibody linked to a potent pro-inflammatory toxin showed significant but transient reduction in $CD4^+$ $CD25^+$ Treg in patients with metastatic melanoma.[111] Another clinical approach of targeting CD25 is through Denileukin diftitox, a fusion protein of IL-2 and diphtheria toxin that targets CD25-expressing cells used in patients with melanoma, ovarian cancer, and renal cell carcinoma.[112-115] Although effective in short-term infusions, these conjugates are quite immunogenic and induce neutralizing antibodies, which hamper their long-term application.

Another agent is daclizumab, which is a US Food and Drug Administration-approved humanized immunoglobulin G1-kappa monoclonal antibody that binds specifically to CD25.[116] It has been used in autoimmune disorders,[117,118] acute graft-versus-host disease,[119] and in patients with cancer with $CD25^+$ T-cell malignancies.[120] The advantage of daclizumab is that it is well-tolerated, and has a half-life of 20 days.[121] In a recent study, daclizumab was used in a single dose of 1 mg/m^2 before human telomerase reverse transcriptase peptide vaccine for metastatic breast cancer. Total $CD4^+CD25^+$ and $CD4^+CD25^+FoxP3^+$ cells remained suppressed for several weeks after a single infusion. Importantly, administration of CD25 antibody was compatible with effective vaccination.[122]

CONCLUSIONS

Evidence accumulated over the past two decades convincingly shows that ovarian cancers are immunogenic tumors. The dramatic advances in laboratory technology and clinical procedures in cellular immunotherapy, along with the development of powerful immunomodulatory antibodies, create new opportunities in ovarian cancer therapeutics. The challenge for the next decade will be to test rational combinations that offer maximal clinical benefit at the lowest cost. Selection of appropriate patients for clinical trial participation will be quite influential as evidence to date indicates that many patients with ovarian cancer display a spontaneous antitumor immune response. These patients may be best suited for vaccine therapy or TIL-based therapy as they are the most likely to harbor a natural repertoire of tumor-reactive T cells with tumor rejecting potential that can be expanded in vivo or ex vivo. In addition, patients whose tumors exhibit intraepithelial T cells may be most likely to respond to immune therapy as the tumor microenvironment is already conducive to T-cell homing and engraftment. Additional biomarkers are needed to maximize selection of patients who may benefit from immune therapy. Finally, more work will be necessary to develop strategies to integrate immune therapy with current standard of care. We have previously demonstrated that patients with advanced ovarian cancer whose tumors exhibit low frequency of intraepithelial $CD8^+$ T cells or high Ki67 expression are more likely to draw benefit from aggressive surgical cytoreduction, while debulking did not affect significantly the survival of patients with brisk CD8+ T cells or low Ki67 expression.[3] It

is possible that immunotherapy with adoptive transfer of TILs and/or vaccine plus immune modulation could be a rational adjuvant therapy for patients with intraepithelial T cells after conventional debulking surgery and chemotherapy. Based on the observation that VEGF antibody blockade enhances T-cell infiltration in tumors and that its efficacy depends on antitumor CD8 T-cell response,[123] it is possible that patients with intraepithelial T cells may also respond better to bevacizumab or other VEGF inhibitors. In contrast, our data suggest that maximal debulking efforts should be undertaken in tumors with low T cells and it is possible that these patients are not the best candidates for adjuvant immunotherapy that exploits natural antitumor immune response. Individualized adoptive therapy with engineered T cells redirected against known tumor epitopes might be the most efficient approach to adjuvant immunotherapy in this subset of patients. Careful preclinical evaluation in well-characterized animal models will be necessary to screen combinations before undertaking clinical studies.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Manuscript writing: Lana E. Kandalaft, Daniel J. Powell Jr, Nathan Singh, George Coukos
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