Immunotherapy in ovarian cancer

Gina M. Mantia-Smaldone,¹ Bradley Corr¹ and Christina S. Chu^{1,*}

¹Division of Gynecologic Oncology, Hospital of the University of Pennsylvania; Philadelphia, PA USA

Keywords: ovarian cancer, immunotherapy, vaccine, adoptive transfer, tumor antigen

Abbreviations: TILs, tumor infiltrating lymphocytes; Tregs, T regulatory cells; TGF- β , transforming growth factor-beta; FR- α , folate receptor-alpha; IFN- α , Interferon-alpha; IFN- γ , interferon-gamma; IL-1, Interleukin-1; NK, natural killer; DCs, dendritic cells; MDSCs, myeloid-derived suppressor cells; TNF- α , tumor necrosis factor-alpha; GM-CSF, granulocyte-macrophage colony-stimulating factor; IP, intraperitoneal; MHC, major histocompatibility complex; APCs, antigen presenting cells; r IFN- γ 1b, recombinant interferon gamma 1b; VEGF, vascular endothelial growth factor; WT-1, Wilms tumor-1; CTLs, cytotoxic T lymphocytes; SLP, synthetic long-peptide; FBP, folate binding protein; STn, sialyl-Tn; HAMAs, human anti-mouse antibodies; EpCAM, epithelial cell adhesion molecule; HER2, human epidermal growth factor receptor 2; TCR, T cell receptor; CARs, chimeric antigen receptors; COACTs, anti-CD3/anti-CD28 monoclonal antibody-coactivated T cells; TRANCE, tumor necrosis factor-related activation-induced cytokine; PD-1, programmed death 1; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; OS, overall survival; PFS, progression-free survival

Ovarian cancer is the most deadly gynecologic malignancy, with more than 15,000 deaths anticipated in 2012.¹ While approximately 80% of patients will respond to frontline chemotherapy, more than 60% of patients will experience disease recurrence and only 44% will be alive at 5 y.^{1,2} Host anti-tumor immune responses are associated with a significant improvement in overall survival for women with ovarian cancer.^{3,4} By bolstering these responses, it may therefore be possible to significantly influence the prognosis of women with this lethal disease. In this review, we will focus on innovative immune-based strategies which are currently being investigated in the treatment of ovarian cancer.

Immunotherapy in Ovarian Cancer

Immunotherapeutic strategies in epithelial ovarian cancer have been a rising area of interest over the past two decades largely due to significant advancements in the knowledge of tumor antigens and antibody responses as well progression in the fields of cancer vaccines, lymphocyte transfer, and immunomodulatory therapy. It is now commonly believed that ovarian cancers are immunogenic tumors. A large stepping stone in the advancement of anti-tumor immune responses in ovarian carcinomas has been the characterization of tumor infiltrating lymphocytes (TILs).⁵ Correlation between the presence of TILs and prolonged progression-free (PFS) and overall (OS) survival has been demonstrated in patients with advanced stage ovarian carcinoma,^{4,6} and the prognostic value of TILs was demonstrated to persist among all populations regardless of stage or grade of disease.⁷ Specifically, the presence of CD8⁺ TILs has been demonstrated to correlate with increased survival.⁶⁻⁹ Confirmed by systematic review, CD8⁺ TILs are a superior marker for prognosis, as their presence

correlates across all stages and histologies of ovarian carcinoma while CD3⁺ T cells only seem to show prognostic significance in serous ovarian carcinomas.⁶ Adams, et al. reported that patients with more abundant CD8⁺ T cells demonstrated increased survival independent of tumor debulking, while patients with low CD8⁺ T cells showed significantly better prognosis if optimally debulked compared with those with suboptimal debulking.³ These studies have resulted in an emerging consensus that, in the future, personalized therapy based on an individual's immune prolife may alter outcome.

Conversely, the presence of immunosuppressive regulatory T cells (Tregs), classified as CD4⁺/CD25⁺/FoxP3⁺ T cells, have been associated with decreased survival in ovarian carcinoma.^{10,11} Woo, et al. were among the first to demonstrate increased proportions of CD4⁺CD25⁺ tumor associated Tregs, which secrete immunosuppressive TGF- β , in patients with advanced ovarian cancer.¹² Tregs have been found to inhibit nonspecific T cell activation in vitro and suppress endogenous tumor-associated antigen (TAA) specific T cell immunity. Curiel, et al. demonstrated an inverse correlation between the presence of Tregs and patient survival in ovarian cancers.¹⁰ Sato, et al. further demonstrated that decreased survival occurs in patients with low ratios of CD8⁺/Tregs while high ratios of CD8⁺/Tregs are associated with increased survival. These data suggest that Tregs may have an adverse effect on the beneficial prognostic factors conferred by CD8⁺ TILs. Immune strategies targeting TILs are currently under investigation and will be discussed in detail below.

Additionally, ovarian cancers express tumor antigens, and patients have demonstrated spontaneous anti-tumor responses which are specific to these antigens.⁸ A number of potential tumor antigens have been described in ovarian cancer with varying potential for vaccination strategies.¹³ These antigens are separately classified as tumor-associated antigens (TAAs) and universal tumor antigens. TAAs can be sequestered from ascites or whole tumor collected during cytoreductive surgery. While TAAs can be specific to a patient and tumor, they are

*Correspondence to: Christina Chu; Email: christina.chu@uphs.upenn.edu
Submitted: 03/01/12; Revised: 05/03/12; Accepted: 05/13/12
<http://dx.doi.org/10.4161/hv.20738>

Table 1. Immunotherapeutic strategies under investigation in Ovarian Cancer

Innate Immunity	Adaptive Immunity	Immunomodulation
Cytokines	Peptide vaccines	Treg blockade
Adoptive transfer of NK cells	Viral-based peptide vaccines	CTLA-4 blockade
	Whole tumor antigen vaccine	Pegylated liposomal doxorubicin
	Monoclonal antibodies	
	Adoptive transfer of T cells	
	Dendritic cell-based vaccines	

often also expressed by normal cells, creating limitations for their use. Currently several TAAs associated with ovarian cancer have been described and include HER2/neu, p53, CA125, STn, FR- α , mesothelin, NY-ESO-1, and cdr-2. Universal tumor antigens, including hTERT and survivin, are those expressed in a variety of tumors and are not found in most normal human cells. Immunotherapeutic regimens strengthening tumor antigen-specific anti-tumor responses have great potential in treating women with both recurrent and microscopic residual disease.

Despite promise for success, to date no advancement in the knowledge of tumor immunology has yielded a significant change in the standard therapy for ovarian carcinomas. The gold standard approach for these tumors is still a combination of cytoreductive surgery with carboplatin and paclitaxel. However, the immunogenicity of ovarian cancer yields great promise for future therapies.

Cancer Immunotherapy

Immunotherapy has found particular success in the treatment of other immunogenic cancers, in particular melanoma and renal cell carcinoma,¹⁴ and successful strategies are being extrapolated into the treatment of ovarian cancer. Traditionally, immunotherapeutic strategies have focused on enhancing, inducing or suppressing innate or adaptive immune responses. Anti-tumor cytokines, including interferon- α (IFN- α), interferon-gamma (IFN- γ) and interleukin-1 (IL-1), as well as natural killer (NK) cells are targets for innate immune-based strategies. Adaptive-immune approaches aim to generate tumor antigen-specific cellular responses and include peptide vaccination, viral-based peptide vaccination, whole tumor antigen vaccination, anti-tumor monoclonal antibodies, and adoptive transfer of T lymphocytes and dendritic cells (DCs).¹⁵ In addition, more recent approaches have investigated immunomodulatory strategies aimed at removing immune inhibitory responses due to Tregs and CTLA-4.^{14,15} (Table 1)

Cytokine therapy. Anti-tumor immune responses have been generated in preclinical models with the administration of cytokines, including IL-2, -4, -7, -12 and -18, IFN- α , IFN- γ , tumor

necrosis factor α (TNF- α) and granulocyte-stimulating factor (GM-CSF).¹⁶ Cytokine therapy affords the opportunity for immune regulation via induction and amplification of favorable antitumor immune responses.¹⁷ While cytokines are easy to manufacture and administer, they lack specific immunomodulatory effects.¹⁷

IL-2, a T-cell growth factor, has demonstrated anti-tumor responses in chemotherapy-resistant cancers, in particular melanoma and renal cell carcinoma.¹⁸ Intraperitoneal (IP) IL-2 therapy was administered to women with platinum-resistant or -refractory ovarian cancer in a phase I/II trial.¹⁸ Twenty-five percent experienced a treatment response with a median survival time of 2.1 y.¹⁹ IL-2 treatment, which was well tolerated, resulted in a significant association between changes in CD3 T cells and IFN- γ producing CD8 T cell counts at early treatment time points and survival, suggesting that IP IL-2 should be further explored in the treatment of platinum-resistant ovarian cancer.

Interferon- α (IFN- α) administered at high doses can interfere with tumor cell replication, and at lower doses may activate T cells and upregulate MHC-I expression on ovarian cancer cells.²⁰ In a phase II trial, IP IFN- α alternating with cisplatin was administered to 14 women with minimal residual disease (≤ 5 mm) as salvage therapy.²¹ Half of the cohort experienced pathologic complete remissions and remained disease free over a median follow-up of 22 mo (range 11–30 mo). In a subsequent phase I/II trial, IP IFN- α and carboplatin demonstrated an objective response of 42.8% in 16 women who had previously received intravenous cisplatin-based chemotherapy for recurrent or refractory ovarian cancer.²²

Interferon-gamma (IFN- γ) also exhibits several anti-tumor immune mechanisms, including upregulation of MHC expression on antigen presenting cells (APCs) and activation of T cell mediated pathways.²³ Preclinical evaluation of a CD80 (B7-1)/IFN- γ modified vaccine demonstrated enhancement of tumor-specific cytotoxic activity, resulting in reduction of tumor growth.²⁴ However, this vaccine has not yet been attempted in clinical trials.

Granulocyte-macrophage colony-stimulating factor (GM-CSF), a cytokine which stimulates the proliferation and differentiation of granulocytes and monocytes, has reported anti-tumor activity in a subset of cancer patients.²⁵ In a phase II trial, GM-CSF was administered to 72 asymptomatic patients with recurrent Müllerian malignancies without an indication for immediate systemic chemotherapy.²⁵ GM-CSF, which was generally well-tolerated, resulted in a decline in serum CA-125 levels which correlated with leukocytosis, as well as in a clinical response (1 complete response and 20 stable disease) in a subset of women.²⁵ GM-CSF was also evaluated in combination with recombinant interferon gamma 1b (rIFN- γ 1b) in a phase II trial of women with recurrent, platinum-sensitive ovarian, fallopian tube and primary peritoneal cancer.²⁶ In this cohort of 59 patients, GM-CSF and rIFN- γ 1b in combination with carboplatin produced a response rate of 56%.²⁶

In summary, cytokine immunotherapy has demonstrated promising results in ovarian cancer patients. However, these results need to be interpreted with caution due to the heterogeneity

Table 2. Peptide-based Vaccines studied in Ovarian Cancer

Target	MHC-I or MHC-II restricted	Phase	Reference	Clinical Response	Immune Response
HER2/neu	II	I/II	27,28	NA	HER2/neu-specific IgG antibody and T cell responses
NY-ESO-1	both	I	29	NA	NY-ESO-1 specific CD4 and CD8 T cell responses
	I	I	30	3 CR 6 PD	NY-ESO-1 specific CD8 T cell responses
p53	I	II	31	2 NED 12 RD	p53-specific CD8 T cell responses
	n/a	II	32,33	2 SD 18 PD	p53-specific, Th2 dominant CD4 T cell responses
WT-1	I	Case report	34	SD x 1 y	Weak correlation between CA125 and the mononuclear phagocyte/lymphocyte ratio
	I	II	35	1 SD 4 PD 1 NE	NA
STn	II	I	36	3 CR 2 PD 2 NA	Anti-STn Th1 T cell responses
	NA	II	37	Median OS 12.7 mo	Anti-STnIgG and IgM antibody responses, Anti-OSM (ovine submaxillarymucin) antibody responses
Lewis ^y	NA	I	44	5 NED 19 RD	Anti- Lewis ^y antibody responses

NA, not available; CR, complete response; PD, progressive disease; n/a, not applicable; NED, no evidence of disease; RD, recurrent disease; SD, stable disease; NE, not evaluable

of the small study patient samples as well as the lack of standardization in measures of immunologic and clinical responses. Therefore, additional investigation is warranted to determine the clinical efficacy of these therapies.

Ovarian cancer vaccines. Peptide vaccines. Peptide vaccines target TAAs, including Her2/neu, NY-ESO-1, p53, VEGF and WT-1, which are expressed by ovarian cancer cells (Table 2). Peptide vaccines are generally well tolerated, easily produced, cost-effective, and have been shown to generate sustained immune responses.¹⁷ However, the immunogenicity of peptide vaccinations may require the co-administration of immune-boosting adjuvants, such as GM-CSF and/or Montanide to encourage induction of in vivo immune responses.³⁰ There are two subtypes of peptide vaccinations under investigation in ovarian cancer. Short-peptide vaccines aim to induce either T helper or cytotoxic T cell (CTL) responses via binding of epitopes specific for MHC I or II, respectively.³⁸ Long-peptide vaccines, unlike short-peptide vaccines, are not MHC restrictive and may contain epitopes to induce either T helper or CTL responses. These vaccines, however, have generally been less rigorously investigated in ovarian cancer patients.

Her2/neu is a tumor antigen expressed in both breast and ovarian cancer. A vaccine containing MHC-II restricted HER-2/neu peptides was administered to patients who had HER-2/neu-overexpressing cancers.²⁷ The majority of patients developed antigen-specific IgG antibody immunity²⁷ as well as antigen-specific T cell responses.²⁸ These responses appeared to correlate with improved clinical outcomes.²⁸

NY-ESO-1 is a cancer-testis antigen which is highly immunogenic and has high expression in ovarian cancer.³⁹ In a phase I trial, 18 women with HLA-DP4+ epithelial ovarian cancer with minimal disease burden were administered a NY-ESO-1 peptide vaccine utilizing the epitope EOS₁₅₇₋₁₇₀ with Montanide ISA51 adjuvant; this epitope is recognized by both HLA-DP4-restricted CD4+ T cells as well as HLA-A2 and HLA-A24 restricted CD8+ T cells.²⁹ This vaccine was well-tolerated and was able to generate both antigen specific CD4+ and CD8+ T cell responses. In a phase I trial, women with ovarian cancer in high-risk first remission were administered a HLA-A*0201-restricted NY-ESO-1b peptide vaccination with Montanide ISA-51 every 3 weeks for 5 vaccinations.³⁰ This vaccine was generally well-tolerated and capable of inducing specific CD8+ T-cell immunity in patients with or without NY-ESO-1 tumor expression. One third of patients experienced a complete clinical remission; however, these individuals had primary tumors which did not express NY-ESO-1, suggesting that NY-ESO-1 expression may be a dynamic process especially in patients with advanced disease.

Vaccination with subcutaneous short p53 peptide vs. intravenous p53 peptide pulsed DCs was recently compared in a phase II trial of women with stage III, IV or recurrent ovarian cancer.³¹ Montanide and GM-CSF adjuvants were administered along with the subcutaneously injected peptide, while IL-2 was administered in both study groups. Both vaccination approaches resulted in comparable p53-specific immune responses with similar tolerability and safety, suggesting that the less demanding

peptide vaccination strategy may be as effective as a cell-based approach.

A synthetic long-peptide (SLP) vaccine targeting p53 was evaluated in a phase II trial involving 20 patients with recurrent ovarian cancer after primary standard treatment.³² The vaccine was generally well-tolerated and was successful in inducing p53-specific, Th2-dominant T cell responses in patients who received all four vaccinations. However, clinical response did not correlate with vaccine-mediated immunity. Further, p53-SLP vaccination did not have long-term impact on responses to secondary chemotherapy or survival in this cohort of patients.³³ In a second phase II trial, the authors combined the p53-SLP vaccine with cyclophosphamide with the aim of targeting Tregs and thereby improving the immunogenicity of the vaccine.⁴⁰ Combination therapy with cyclophosphamide and the p53-SLP vaccine induced higher p53-specific responses compared with p53-SLP monotherapy and produced a clinical response in 20% of patients, suggesting that further investigation is warranted.

Preclinical data suggests that immunization with a VEGF peptide generates a VEGF-specific antibody response which interferes with VEGF-dependent angiogenesis in an ovarian cancer xenograft nude mouse model.⁴¹ Further investigation in humans is necessary to determine the role of this vaccine in treating or preventing ovarian cancer. A preliminary report has also suggested a possible role for WT1 peptide for treatment of recurrent ovarian cancer.³⁴

Multi-peptide vaccines have also been examined in ovarian cancer patients. A vaccine combining multiple MHC I restricted peptides present on ovarian cancer cells, including adenomatous polyposis coli (APC), ubiquitin conjugating enzyme E2, BAP31, replication protein A, Abl-binding protein 3c, Cyclin I, topoisomerase II α , integrin β 8 subunit precursor, cell division control protein 2 (CDC2), TACE/ADAM17, g-catenin, and EDDR1, administered along with Montanide ISA-51 and GM-CSF was successful in generating peptide-specific T cell responses in patients with breast and ovarian cancer without evidence of disease.⁴² However, 50% of the ovarian cancer patients experienced recurrence over a median follow-up of 492 d. An additional phase I trial examined the safety and immunogenicity of a vaccination with five MHC-1 restricted peptides and one MHC-2 restricted peptide along with Montanide ISA-51 and GM-CSF in nine women with epithelial ovarian, fallopian or primary peritoneal cancer who were HLA-A1, HLA-A2 or HLA-A3 positive.⁴³ Following in vitro stimulation, CD8+ T-cell responses were reported to the peptides in this vaccine, including folate binding protein (FBP₁₉₁₋₁₉₉) and Her-2/neu₇₅₄₋₇₆₂. While vaccine-related toxicities were low-grade, the vaccine lacked potency, did not demonstrate ex vivo immune responses, and failed to produce a substantial clinical effect.

In addition to peptide-based strategies, vaccines targeting sialyl-Tn (STn), a disaccharide molecule associated with MUC1, have been evaluated in phase I and II trials which included ovarian cancer patients.^{36,37} These vaccines were capable of inducing STn-specific immune responses but did not demonstrate a clinical benefit. Another carbohydrate epitope associated with MUC1, Lewis^x, was examined in a phase I trial which included

25 ovarian cancer patients with complete clinical response to chemotherapy following primary therapy for residual or recurrent disease.⁴⁴ While this vaccine was generally well tolerated and was capable of inducing anti-Lewis^x antibodies, a clinical benefit has yet to be determined.

Viral-based peptide vaccines. Recombinant viruses expressing human antigens have also been employed as vaccines in ovarian cancer patients. Antigens can be easily produced at high concentrations in vivo and these proteins are not HLA-restricted.³⁸ Intradermally injected recombinant vaccinia and fowlpox viruses expressing NY-ESO-1 were used to vaccinate 19 women with NY-ESO-1 expressing ovarian cancers who had complete responses to primary therapy.⁴⁵ This protocol was capable of inducing NY-ESO-1 specific immune responses in half of the patients, translating into a mean disease-free interval of 19.9 mo. Details of this trial have yet to be published. Recombinant vaccinia (PANVAC-V) and fowlpox (PANVAC-F) viruses producing both CEA and MUC1 as well as three T cell co-stimulatory molecules (B7.1, ICAM-1 and LFA-3) were administered along with GM-CSF in a phase I/II trial of 25 patients with progressive CEA or MUC1 overexpressing metastatic cancers (including three ovarian cancers).⁴⁶ This vaccination protocol was well-tolerated and was capable of inducing antigen-specific T cell responses. One of the three ovarian cancer patients achieved a durable clinical response lasting 18 mo following vaccination. Further investigation is necessary to determine the role of virus-based peptide vaccines in the treatment of ovarian cancer.

Whole tumor antigen vaccines. Unlike the above immunotherapies, whole tumor antigen vaccines have the potential to provide an individualized broad range of tumor antigens which may enhance host antigen-specific anti-tumor immune responses.⁸ Whole tumor antigen vaccines can be created using tumor cells, autologous tumor lysates or tumor-derived RNA.⁸ Given that they are generally poorly immunogenic due to a lack of stimulatory immune signals, whole tumor cell vaccines must be administered with adjuvants, such as GM-CSF, Montanide ISA-51 or Toll-like receptor agonists.⁴⁷ A recent meta-analysis suggests that individuals may have better clinical responses following whole tumor antigen vaccination compared with synthetic peptide vaccination.⁴⁸ A phase I/II randomized trial is currently underway at our institution which aims to determine the feasibility, safety and immunogenicity of an autologous oxidized tumor cell lysate vaccine (OC-L) in combination with Ampligen, a Toll-like receptor 3 agonist (NCT01312389).

In summary, ovarian cancer vaccines have been examined in several phase I and II clinical trials. Peptide vaccines, the most studied subtype, have resulted in encouraging clinical responses in ovarian cancer patients and are generally well tolerated and safe. However, peptide vaccines have poor immunogenicity requiring administration of immune adjuvants and have generally failed to demonstrate a clear clinical benefit. In contrast to peptide vaccines, viral-based peptide vaccines result in proteins which are not HLA restricted. Yet, preliminary studies have failed to report clinical responses with this type of vaccine. While offering the possibility of individualized treatment, the role of whole tumor antigen vaccines in ovarian cancer has not yet been

determined. Further, this strategy relies on the availability of fresh tumor samples for vaccine development, and given the possibility of a broad range of antigens, it will likely be difficult to standardize vaccines among all ovarian cancer patients. However with continued investigation, vaccines may offer hope to ovarian cancer patients in both the therapeutic and preventive settings.

Monoclonal antibody treatment. Monoclonal antibody treatment aims to target antigens present on ovarian cancer cells. Traditionally, monoclonal antibodies have been used to facilitate an anti-tumor immune response via the neutralization of the tumor antigen, opsonization and activation of complement-mediated cytotoxicity, T cell-mediated cytotoxicity or antibody-dependent cell-mediated cytotoxicity.^{14,38} However, monoclonal antibodies can also be used to target molecules which are critical to tumor growth or survival pathways without eliciting an immune response.²⁰ Monoclonal antibody treatments have the advantages of being antigen-specific and generally easily to produce; however, in general, they are weakly immunogenic.¹⁷ Monoclonal antibodies targeting CA-125, MUC1, EpCAM, Her2/Neu, membrane folate receptor and VEGF have been examined in women with ovarian cancer. (Table 3)

Immune-mediated monoclonal antibody treatment. CA125 (MUC-16) is a logical target for monoclonal antibody treatment given that 80% of ovarian cancers express this mucin.⁴⁹ Oregovomab (B43.13, OvaRex) is a monoclonal antibody to CA125 which has been examined in ovarian cancer patients in both phase II and III trials.^{49,50} In a prospective, open-label, pilot phase II trial involving heavily pretreated patients with ovarian cancer, antibody and T cell responses specific to both oregovomab and CA125 were generated in the majority of patients.⁴⁹ In three patients, these immune responses corresponded to stabilization of disease with survival greater than 2 y. In a subsequent trial of 20 women with advanced recurrent ovarian cancer, oregovomab was administered alone and then with optionally concurrent second-line chemotherapy.⁵⁰ Treatment-induced antibodies were generated, including human anti-mouse antibodies (HAMAs) and anti-oregovomab antibodies (Ab2) in 79% of patients and anti-CA125 antibodies in 11% of patients. T cell responses to CA125, generated in 39% of patients, and to autologous tumor (63% of patients) were significantly associated with improved survival ($p = 0.002$). Given that therapy was well tolerated and induced antigen-specific responses, subsequent trials examined the role of oregovomab in both frontline⁵¹ and maintenance therapy.⁵² Preliminary results suggest that incorporation of oregovomab into frontline platinum/taxane chemotherapy may be more effective.⁵¹

Abagovomab (ACA125) is an anti-idiotypic antibody targeting CA125 which has also been examined in ovarian cancer patients.^{53,54} Anti-idiotypic antibodies target primary anti-CA125 antibodies and boost the host immune response by mimicking the antigen of interest, producing anti-anti-idiotypic antibodies which also recognize this antigen.¹⁵ In a phase Ib/II trial, abagovomab generated anti-anti-idiotypic antibodies and anti-CA125 antibodies in 68% and 50%, respectively, of 119 patients with advanced ovarian cancer.⁵⁴ Antibody-dependent cell-mediated cytotoxicity of CA125 positive cancer cells was observed in

26.9% of patients. Individuals who generated anti-anti-idiotypic antibodies experienced a significant survival advantage when compared with those who did not ($p < 0.0001$). Two other phase I trials also demonstrated robust anti-anti-idiotypic antibody responses to abagovomab but were unable to demonstrate similar clinical responses due to differences in study design.^{53,55}

Given its overexpression in 90% of ovarian cancers, MUC1 is another promising target for monoclonal antibody therapy.⁵⁶ In a phase I trial of 26 women with persistent or recurrent ovarian cancer following platinum therapy, HMFG1, a murine anti-MUC1 antibody, resulted in a small but statistically significant rise in anti-HMFG1 and anti-MUC1 antibody responses in 38% of those completing the vaccination regimen.⁵⁶ However, while generally well-tolerated, HMFG1 lacked clinical efficacy in this trial. A radiolabeled form of this antibody, yttrium-90-muHMFG1, was also examined in subsequent phase I/II and III trials.^{57,58} In the phase I/II trial, yttrium-90-muHMFG1 administered intraperitoneally resulted in improved median survival in the women who received this antibody following traditional surgery and platinum-based chemotherapy.⁵⁷ However, despite a significant decrease in intraperitoneal disease following treatment, the phase III study failed to demonstrate a survival advantage due to increased extraperitoneal recurrences.⁵⁸

EpCAM is an antigen overexpressed in ovarian cancer, especially in patients with metastatic and recurrent/chemotherapy-resistant disease, and is therefore another attractive target for antibody-mediated immunotherapy.⁵⁹ Catumaxomab is a tri-functional antibody, targeting EpCAM on epithelial tumor cells and CD3 on T cells and is capable of recruiting and activating immune effector cells in the tumor microenvironment.⁶⁰ Preliminary results from recent phase II/III trials suggest that paracentesis plus intraperitoneal administration of catumaxomab may be beneficial in treating recurrent ovarian cancer patients with malignant ascites,^{60,61} with small but statistically significant increases in puncture-free survival and time to next paracentesis compared with patients undergoing paracentesis alone.

Non-immune-mediated monoclonal antibody treatment. Monoclonal antibodies which block tumor growth or survival pathways have also been examined in ovarian cancer patients. Recent data suggest that survival in ovarian cancer patients is inversely proportional to human epidermal growth factor receptor 2 (HER2) expression.⁶² A monoclonal antibody targeting HER2, trastuzumab, is an available adjuvant therapy in HER2-positive breast cancer patients which has been recently examined in ovarian cancer patients. However, preliminary results in women with HER2-positive, recurrent or persistent ovarian or primary peritoneal cancers suggest a limited clinical benefit with an overall response rate of only 7.3%.⁶³ Monoclonal antibodies targeting FR α , including MOv18 and MORAb-003, have the potential of interrupting ovarian cancer cell growth by interfering with DNA synthesis.⁶⁴ Phase I trials in ovarian cancer patients have suggested that these antibodies are well tolerated but have limited clinical benefit in ovarian cancer patients.^{65,66}

Bevacizumab, a humanized monoclonal antibody targeting VEGF-A, blocks angiogenesis which is a key pathway in tumorigenesis. Bevacizumab has been examined in ovarian cancer

Table 3. Monoclonal Antibodies studied in Ovarian Cancer

Antibody	Target	Reference	Phase	Clinical Response	Immune Response
Oregovomab	CA125	49	II	3 SD 10 PD	Oregovomab and CA125-specific antibody and T cell responses
		50	I	4 NED 2 CR 7 PD	HAMA, anti-oregovomab and anti-CA125 antibodies
Abagovomab	CA125	53	I	NA	HAMA, anti-anti-idiotypic and antiCA125 antibodies, CA125-specific CD4 and CD8 T cells
		54	Ib/II	4 CR 30 SD 62 PD 23 NA	Anti-anti-idiotypic and antiCA125 antibodies, Antibody dependent cell-mediated cytotoxicity
		55	I	12 SD 21 PD	HAMA and anti-anti-idiotypic antibodies, CA125-specific IFN- γ producing T cells
HMFG1	MUC1	56	I	1 SD 25 RD	Anti-HMFG1 and anti-MUC1 antibodies
Catumaxomab	EpCAM, CD3	60,61	II/III	Longer puncture-free survival and time to next paracentesis	Human anti-mouse antibodies
Trastuzumab	HER2/neu	63	II	1 CR 2 PR 16 SD 22 PD	No anti-trastuzumab antibody formation
MOv18	FR- α	65	I	NA	No anti-c-MOV18 antibodies
MORAb-003	FR- α	66	I	9 SD 16 PD 1 NE	Limited anti-MORAb-003 antibodies
Bevacizumab	VEGF	69	II	2 CR 11 PR 32 SD 17 PD	NA
		70	II	0 CR 7 PR 27 SD 5 PD 5 NE	NA
		67	III	168 Responders (CR/PR) 82 Non-responders (SD/PD) 7 unknown	NA
		68	III	No difference in OS; significant improvement in PFS	NA

SD, stable disease; PD, progressive disease; NED, no evidence of disease; CR, complete response; HAMA, human anti-mouse antibody; NA, not available; RD, recurrent disease; NE, not evaluable

patients as a part of frontline therapy^{67,68} as well as secondary therapy for recurrent disease.^{69,70} Bevacizumab monotherapy demonstrated clinical response rates of 15–21% in two separate phase II trials of women with persistent or recurrent epithelial ovarian cancer, including those with platinum-resistant disease.^{69,70} Median PFS and OS ranged from 4.4 to 4.7 mo and 10.7 to 17 mo, respectively, and treatment was generally well-tolerated. However, Cannistra et al., reported a higher than expected rate of

gastrointestinal perforation (11.4%), resulting in early termination of subject enrollment.

Recently, two large phase III randomized trials of bevacizumab in the frontline therapy of advanced ovarian cancer have been reported. ICON7 compared carboplatin/paclitaxel alone vs. carboplatin/paclitaxel/bevacizumab plus bevacizumab maintenance, and at 42 mo follow up, noted a 2 mo improvement in PFS (24.1 vs. 22.4 mo, $p = 0.04$), with benefit greatest for those

at high risk for disease progression.⁶⁷ Similarly, the Gynecologic Oncology Group conducted a three arm randomized, double-blinded, placebo-controlled trial of carboplatin/paclitaxel with and without concurrent bevacizumab, with and without bevacizumab maintenance. The investigators noted a 4 mo improvement in PFS in those women receiving bevacizumab concurrently with primary chemotherapy as well as for maintenance (14.1 vs. 10.3 mo for combination therapy plus maintenance compared with carboplatin/paclitaxel alone, $p < 0.001$).⁶⁸ Given the high cost of bevacizumab, and the relatively modest influence on PFS, the role of its utility as a primary treatment modality in patients with advanced ovarian cancer remains unclear.

In summary, monoclonal antibody treatment is capable of producing antigen-specific immune responses in ovarian cancer patients. While results of phase I and II trials are promising, few randomized clinical trials have been conducted using this strategy, and the results of initial RCTs using anti-CA125 antibody therapy, the most studied target, have failed to demonstrate any clinical benefit despite inducing antigen specific immune responses.⁷¹ Monoclonal antibodies targeting tumor growth and survival pathways, especially bevacizumab, have offered promising results in patients with recurrent disease. However, additional studies are needed to determine the role of these antibodies in frontline therapy.

Adoptive transfer of immune cells. Immune cells, including T cells, NK cells, DCs and macrophages, can be removed from a patient, manipulated ex vivo and then infused back into the same patient in order to boost anti-tumor cellular immune responses.⁷²

T cells. Adoptive transfer of autologous TILs has been met with success in treating metastatic melanoma patients, with objective response rates ranging up to 50%.⁷³ In a pilot trial, adoptive transfer of ex vivo IL-2 expanded TILs following a single dose of intravenous cyclophosphamide resulted in high response rates in a sample of women with advanced or recurrent ovarian cancer. Of the 7 patients treated with adoptive transfer of TILs alone, 1 achieved a complete response and 4 achieved partial responses.⁷⁴ In comparison, adoptive transfer of TILs in combination with cisplatin-based chemotherapy resulted in objective responses in 9 out of 10 patients (7 complete responses, 2 partial responses).

Since this study established the feasibility of adoptive T cell transfer, attention has been placed on optimizing the anti-tumor efficacy of this therapy. Given that the availability of tumor-reactive TILs is limited, investigators are looking into generating tumor-specific T cells via the ex vivo CD3/CD28-costimulation of vaccine-primed peripheral blood T cells or by genetically modifying peripheral blood T cells to express high affinity cloned T-cell receptors (TCRs) or chimeric antigen receptors (CARs).⁸ In a phase I trial which included two patients with ovarian cancer, anti-CD3/anti-CD28 monoclonal antibody-co-activated T cells (COACTs) were capable of inducing cytokine production.⁷⁵ While this treatment was felt to be safe, the clinical benefit of this therapy has yet to be clearly defined. Our group is currently investigating the feasibility and safety of adoptive transfer using ex vivo CD3/CD28 co-stimulated autologous peripheral blood T cells in combination with a whole tumor lysates-pulsed dendritic

cell vaccine (DCVax®-L) in patients with recurrent ovarian or primary peritoneal cancer (NCT00603460).

In a phase I trial of women with metastatic ovarian cancer, autologous T cells were genetically modified to express anti-FR α CAR.⁷⁶ While this therapy was generally well-tolerated, no clinical responses were demonstrated, likely due to poor localization of T cells into tumor as well as a quick decline in circulating T cells. However, this was the first trial to explore the use of genetically modified T cells and additional studies are needed to further define the role in treating ovarian cancer patients.

Natural killer cells. NK cells are cytotoxic lymphocytes which are a part of the innate immune system and may play a role in tumor surveillance.⁷⁷ In a phase II trial which included 14 women with ovarian cancer, ex vivo activated haplo-identical related NK cells were adoptively transferred following lymphodepleting chemotherapy.⁷⁷ This protocol resulted in significant toxicities with reported transient donor chimerism and increased Treg responses. At a median of 36 d following transfer, 4 patients experienced a partial response while 12 had reported stable disease. Further investigation is warranted in order to determine the clinical benefit of this strategy in ovarian cancer.

Dendritic cells. DCs are specialized APCs which can be manipulated in order to boost tumor-antigen specific CTL responses via the recognition MHC-peptide complexes by peptide-specific TCRs on effector T cells.⁷⁸ DCs can present tumor antigens following exposure (or pulsing) to whole tumor cell lysates, tumor peptides or tumor cells.⁷⁹ A tumor cell lysate-pulsed dendritic cell vaccination was administered to women with advanced gynecologic malignancies in a recent phase I trial. Of the patients with progressive or recurrent ovarian cancer included in this trial, 50% demonstrated disease stabilization with PFS ranging 8–45 mo and lymphoproliferative responses were reported in two patients.⁸⁰ A phase I trial currently underway at our institution (NCT01132014) is examining the feasibility and immunogenicity of a DC vaccine loaded with autologous tumor lysate administered intranodally, alone or in combination with intravenous bevacizumab; results from this trial are still pending.

Our institution recently reported results of a randomized phase I/II trial of autologous DCs pulsed with Her-2/neu, hTERT, and PADRE peptides administered with or without low dose cyclophosphamide for 11 patients with advanced ovarian cancer in remission.⁸¹ Of 9 patients receiving the full course of vaccinations, 3 recurred at 6, 17, and 6 mo, and 6 remain disease free at 36 mo of follow up. With no grade 3/4 vaccine-related toxicities noted, the 3-y overall survival was 90% with patients receiving cyclophosphamide demonstrating a non-significant survival advantage. Modest T cell responses to Her2/neu and hTERT were measured by IFN- γ ELISPOT, despite unexpected uniform baseline immunosuppression as demonstrated by virtually undetectable response to the diphtheria carrier protein component of the Prevnar™ vaccine. Brossart et al. conducted a trial of a HER-2/neu or MUC1-peptide pulsed autologous DC vaccine administered to ten patients with advanced breast or ovarian cancer.⁸² In this pilot study, peptide-specific CTL responses were demonstrated in 50% of the patients; however, these responses were not correlated with clinical responses.

Schlienger et al. demonstrated that immature dendritic cells combined with autologous tumor cells could be matured using TNF- α and tumor necrosis factor-related activation-induced cytokine (TRANCE), and then used to induce tumor specific T cells capable of secreting IFN- γ .⁵ A baseline T-cell response to autologous tumor associated antigens was measured in peripheral blood as well as the local tumor environment, indicating that tumor-specific T cells can be generated using autologous tumor cells as a source for antigens. Additional dendritic cell vaccines trials (NCT00703105, NCT00683241, and NCT01132014) are currently ongoing which may further elucidate a role for this technique in ovarian cancer patients.

In summary, cellular immunotherapy involving T cells, Natural Killer cells and Dendritic cells may be capable in boosting host anti-tumor cellular immune responses. However, this strategy is not well studied in ovarian cancer patients at this time, and therefore, results of these preliminary studies should be interpreted with caution.

Immunomodulation. Immunosuppressive responses have been reported in ovarian cancer patients which may limit the clinical effectiveness of the above proposed immunotherapeutic strategies.⁸³ Current investigations have focused on removing these immunologic brakes in order to facilitate host anti-tumor immune responses. As mentioned previously, Tregs are a subset of CD4⁺ T cells with immunosuppressive effects on host anti-tumor responses and are a poor prognostic indicator in ovarian cancer patients.^{10,11} Cyclophosphamide, an alkylating chemotherapeutic agent, has been used in an effort to reduce Treg responses. North and Berd demonstrated that cyclophosphamide eliminated CD8⁺ tumor suppressor cells, enhancing tumor immunotherapy and improving immune function.^{84,85} In animal models, cyclophosphamide administration has been linked to reduction in Tregs and enhancement of anti-tumor response.⁸⁶ However, initial studies in ovarian cancer patients demonstrated that cyclophosphamide failed to induce a reduction of Tregs or a qualitative difference in Treg function.^{40,81} Additional techniques to reduce Tregs focus on targeting CD25, the IL-2 receptor α chain, and include treatment with an anti-CD25 monoclonal antibody, Denileukindiftox and daclizumab.⁸ While there are some promising results from pilot studies with melanoma or breast patients, the impact of these CD25 targeting therapies have not been fully delineated in ovarian cancer patients.

T cell activation requires that TCRs on T cells recognize peptides presented by MHC-I or MHC-II complexes on APCs. This process is regulated by stimulatory or inhibitory receptors present on T cells. The latter, including CTLA-4 and programmed death 1 (PD-1), can significantly dampen effector T cell responses.⁸ By blocking these receptors, it may therefore be feasible to enhance the clinical benefit of spontaneous anti-tumor immune responses or those brought on by immunotherapeutic treatment.

Ipilimumab is a monoclonal antibody targeting CTLA-4 which has demonstrated promising clinical benefit in patients with melanoma and renal cell carcinoma.¹⁴ In a phase III trial, ipilimumab provided a significant survival advantage in

patients with previously treated metastatic melanoma albeit with some pronounced toxicity.⁸⁷ The same investigators then expanded their results to include the treatment of ovarian cancer patients.^{83,88} This group treated a total of eleven patients with advanced ovarian cancer using modified autologous tumor cells which secrete GM-CSF (GVAX) followed by ipilimumab.^{83,88} In several patients in this cohort, ipilimumab demonstrated significant anti-tumor effects corresponding with a drop in serum CA125 levels. These preliminary results suggest that CTLA-4 may be an appropriate target in ovarian cancer patients. PD-1 has been targeted in phase I trials with patients with hematologic malignancies and has yet to be examined in women with ovarian cancer.⁸ This inhibitory receptor seems like another justifiable target in ovarian cancer given that its receptor PD-L1 is expressed on ovarian cancer cells.⁸

Traditional cytotoxic agents, most notably pegylated liposomal doxorubicin (PLD), may also have immunomodulatory effects on ovarian cancer cells.⁸⁹ In addition to causing direct DNA damage, PLD alters the immunophenotype of surviving ovarian tumor cells and renders them more susceptible to anti-tumor T cell mediated cytotoxicity. Preliminary results demonstrated in preclinical studies may justify the combination of PLD with immunotherapeutic strategies aiming to enhance anti-tumor T cell responses. However, these findings have not yet been confirmed in patients with ovarian cancer.

Immunomodulation may prove to be the key to improving clinical responses in ovarian cancer patients undergoing immunotherapeutic treatment. While there are some promising results from pilot studies with melanoma or breast patients, the impact of these strategies have not been fully examined in ovarian cancer patients

Immunotherapy in Ovarian Cancer: Critical Commentary

Innovative immunotherapeutic strategies offer the promise of enhancing host anti-tumor responses which may improve clinical outcomes in women with ovarian cancer. While many preliminary phase I/II studies have demonstrated induction of anti-tumor responses, there is current no clinically effective antigen-specific active immunotherapy available for women with ovarian cancer.⁷¹

One major pitfall highlighted by this review is the examination of immunotherapeutics in women with recurrent and widely metastatic disease. These individuals likely have heavy tumor burden as a byproduct of tumor immune-evading responses, and this may account for the general lack of clinical benefit seen thus far with investigated immunotherapeutic strategies in ovarian cancer.³⁸ Immunomodulatory techniques combined with previously examined immunotherapies offer the promise of improving clinical responses in those with recurrent disease, and we believe strategies targeting Tregs and inhibitory signals such as CTLA-4 are most likely to be fruitful. Further, immunotherapy has been rarely studied in women with primary disease. While we appreciate that immunotherapy is unlikely to replace standard adjuvant chemotherapy, we believe that further investigation is warranted

to determine whether immunotherapy could be combined with frontline therapy or administered as maintenance or consolidation therapy.

Additionally, we believe that it is important to select appropriate candidates for clinical trials examining immunotherapy in ovarian cancer. Candidates for immunotherapy would ideally have documented baseline immune responses which would render them more responsive to these strategies. For example, ovarian cancer patients with naturally occurring tumor antigen-specific antibody responses and/or those whose tumors express tumor-associated antigens or contain abundant TILs may be more likely to demonstrate a clinical response to immunotherapies. Conversely, patients who have underlying autoimmunity may have aberrant immune responses interfering with the generation of appropriate anti-tumor responses, making them less appropriate candidates for immunotherapy. In the future, we expect that immunotherapeutic regimens will be offered to individuals with

favorable immune biomarker profiles which may translate into improved prognostic outcomes.

Ongoing investigation may help to define the role of immunotherapy alone or in combination with synergistic treatment strategies in the treatment of ovarian cancer patients (Table 4). With careful selection of candidates as well as appropriate reporting of standardized treatment responses and adverse events, these trials may determine a role for immunotherapy in the treatment of ovarian cancer.

Disclosure of Potential Conflicts of Interest

The authors attest that there are no reportable conflicts of interest with the publication of the manuscript.

Acknowledgments

We would like to thank our colleagues for their thoughtful review of this manuscript.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; 62:10-29; PMID:22237781; <http://dx.doi.org/10.3322/caac.20138>.
2. Cannistra SA. Cancer of the ovary. *N Engl J Med* 2004; 351:2519-29; PMID:15590954; <http://dx.doi.org/10.1056/NEJMra041842>.
3. Adams SF, Levine DA, Cadungog MG, Hammond R, Facciabene A, Olvera N, et al. Intraepithelial T cells and tumor proliferation: impact on the benefit from surgical cytoreduction in advanced serous ovarian cancer. *Cancer* 2009; 115:2891-902; PMID:19472394; <http://dx.doi.org/10.1002/cncr.24317>.
4. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med* 2003; 348:203-13; PMID:12529460; <http://dx.doi.org/10.1056/NEJMoa020177>.
5. Schlienger K, Chu CS, Woo EY, Rivers PM, Toll AJ, Hudson B, et al. TRANCE- and CD40 ligand-matured dendritic cells reveal MHC class I-restricted T cells specific for autologous tumor in late-stage ovarian cancer patients. *Clin Cancer Res* 2003; 9:1517-27; PMID:12684428.
6. Clarke B, Tinker AV, Lee CH, Subramanian S, van de Rijn M, Turbin D, et al. Intraepithelial T cells and prognosis in ovarian carcinoma: novel associations with stage, tumor type, and BRCA1 loss. *Mod Pathol* 2009; 22:393-402; PMID:19060844; <http://dx.doi.org/10.1038/modpathol.2008.191>.
7. Hwang WT, Adams SF, Tahirovic E, Hagemann IS, Coukos G. Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis. *Gynecol Oncol* 2012; 124:192-8; PMID:22040834; <http://dx.doi.org/10.1016/j.ygyno.2011.09.039>.
8. Kandalaf LE, Powell DJ Jr., Singh N, Coukos G. Immunotherapy for ovarian cancer: what's next? *J Clin Oncol* 2011; 29:925-33; PMID:21079136; <http://dx.doi.org/10.1200/JCO.2009.27.2369>.
9. Leffers N, Fehrmann RS, Goeden MJ, Schulze UR, Ten Hoor KA, Hollema H, et al. Identification of genes and pathways associated with cytotoxic T lymphocyte infiltration of serous ovarian cancer. *Br J Cancer* 2010; 103:685-92; PMID:20664601; <http://dx.doi.org/10.1038/sj.bjc.6605820>.
10. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* 2004; 10:942-9; PMID:15322536; <http://dx.doi.org/10.1038/nm1093>.
11. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. *Proc Natl Acad Sci U S A* 2005; 102:18538-43; PMID:16344461; <http://dx.doi.org/10.1073/pnas.0509182102>.
12. Woo EY, Chu CS, Goletz TJ, Schlienger K, Yeh H, Coukos G, et al. Regulatory CD4(+)/CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer Res* 2001; 61:4766-72; PMID:11406550.
13. Chu CS, Kim SH, June CH, Coukos G. Immunotherapy opportunities in ovarian cancer. *Expert Rev Anticancer Ther* 2008; 8:243-57; PMID:18279065; <http://dx.doi.org/10.1586/14737140.8.2.243>.
14. Topalian SL, Weiner GJ, Pardoll DM. Cancer immunotherapy comes of age. *J Clin Oncol* 2011; 29:4828-36; PMID:22042955; <http://dx.doi.org/10.1200/JCO.2011.38.0899>.
15. Liu B, Nash J, Runowicz C, Swede H, Stevens R, Li Z. Ovarian cancer immunotherapy: opportunities, progresses and challenges. *J Hematol Oncol* 2010; 3:7; PMID:20146807; <http://dx.doi.org/10.1186/1756-8722-3-7>.
16. Connor J, Bannerji R, Saito S, Heston W, Fair W, Gilboa E. Regression of bladder tumors in mice treated with interleukin 2 gene-modified tumor cells. *J Exp Med* 1993; 177:1127-34; PMID:8459207; <http://dx.doi.org/10.1084/jem.177.4.1127>.
17. Liu B, Nash J, Runowicz C, Swede H, Stevens R, Li Z. Ovarian cancer immunotherapy: opportunities, progresses and challenges. *J Hematol Oncol* 2010; 3:7; PMID:20146807; <http://dx.doi.org/10.1186/1756-8722-3-7>.
18. Edwards RP, Gooding W, Lembersky BC, Colonello K, Hammond R, Paradise C, et al. Comparison of toxicity and survival following intraperitoneal recombinant interleukin-2 for persistent ovarian cancer after platinum: twenty-four-hour versus 7-day infusion. *J Clin Oncol* 1997; 15:3399-407; PMID:9363872.
19. Vlad AM, Budiu RA, Lenzner DE, Wang Y, Thaller JA, Colonello K, et al. A phase II trial of intraperitoneal interleukin-2 in patients with platinum-resistant or platinum-refractory ovarian cancer. *Cancer Immunol Immunother* 2010; 59:293-301; PMID:19690855; <http://dx.doi.org/10.1007/s00262-009-0750-3>.
20. Thibodeaux SR, Curiel TJ. Immune therapy for ovarian cancer: promise and pitfalls. *Int Rev Immunol* 2011; 30:102-19; PMID:21557637; <http://dx.doi.org/10.3109/08830185.2011.567361>.
21. Nardi M, Cognetti F, Pollera CF, Giulia MD, Lombardi A, Atlante G, et al. Intraperitoneal recombinant alpha-2-interferon alternating with cisplatin as salvage therapy for minimal residual-disease ovarian cancer: a phase II study. *J Clin Oncol* 1990; 8:1036-41; PMID:2189953.
22. Repetto L, Chiara S, Guido T, Bruzzone M, Oliva C, Ragni N, et al. Intraperitoneal chemotherapy with carboplatin and interferon alpha in the treatment of relapsed ovarian cancer: a pilot study. *Anticancer Res* 1991; 11:1641-3; PMID:1746921.
23. Kaur T, Slavcev RA, Wettig SD. Addressing the challenge: current and future directions in ovarian cancer therapy. *Curr Gene Ther* 2009; 9:434-58; PMID:20021329; <http://dx.doi.org/10.2174/156652309790031148>.
24. Qian HN, Liu GZ, Cao SJ, Feng J, Ye X. The experimental study of ovarian carcinoma vaccine modified by human B7-1 and IFN-gamma genes. *Int J Gynecol Cancer* 2002; 12:80-5; PMID:11860540; <http://dx.doi.org/10.1046/j.1525-1438.2002.01060.x>.
25. Roche MR, Rudd PJ, Krasner CN, Matulonis UA, Berlin ST, Lee H, et al. Phase II trial of GM-CSF in women with asymptomatic recurrent müllerian tumors. *Gynecol Oncol* 2010; 116:168-72; PMID:19922985; <http://dx.doi.org/10.1016/j.ygyno.2009.10.075>.
26. Schmeler KM, Vadhan-Raj S, Ramirez PT, Apte SM, Cohen L, Bassett RL, et al. A phase II study of GM-CSF and rIFN-gamma1b plus carboplatin for the treatment of recurrent, platinum-sensitive ovarian, fallopian tube and primary peritoneal cancer. *Gynecol Oncol* 2009; 113:210-5; PMID:19264351; <http://dx.doi.org/10.1016/j.ygyno.2009.02.007>.
27. Disis ML, Goodell V, Schiffman K, Knutson KL. Humoral epitope-spreading following immunization with a HER-2/neu peptide based vaccine in cancer patients. *J Clin Immunol* 2004; 24:571-8; PMID:15359116; <http://dx.doi.org/10.1023/B:JOCI.0000040928.67495.52>.
28. Disis ML, Gooley TA, Rinn K, Davis D, Piepkorn M, Cheever MA, et al. Generation of T-cell immunity to the HER-2/neu protein after active immunization with HER-2/neu peptide-based vaccines. *J Clin Oncol* 2002; 20:2624-32; PMID:12039923; <http://dx.doi.org/10.1200/JCO.2002.06.171>.
29. Odunsi K, Qian F, Matsuzaki J, Mhawech-Fauceglia P, Andrews C, Hoffman EW, et al. Vaccination with an NY-ESO-1 peptide of HLA class I/II specificities induces integrated humoral and T cell responses in ovarian cancer. *Proc Natl Acad Sci U S A* 2007; 104:12837-42; PMID:17652518; <http://dx.doi.org/10.1073/pnas.0703342104>.

Table 4. Current Clinical Trials investigating Immunotherapy in Ovarian Cancer

Trial Identifier	Phase	Objective
Cytokines		
NCT00003408	II	To study the effectiveness of biological therapy with sargramostim (GM-CSF), IL-2 and INF- α following high-dose chemotherapy and autologous stem cell transplantation in treating patient who have cancer (including ovarian cancer)
NCT00157573	II	To examine the ability of GM-CSF to alter disease progression in women who have recurrent but asymptomatic recurrence of their ovarian, fallopian tube or primary peritoneal cancer
NCT00659178	I	To assess the safety and biological activity of IL-18 (SB-485232) IV infusion in combination with Doxil in Advanced Stage Epithelial Ovarian Cancer
Vaccines		
NCT00001827	II	To determine the safety and immunogenicity of vaccination with a p53 peptide in ovarian cancer patients
NCT00017537	I	To study the effectiveness of MVF-HER-2(628–647) and CRL1005 Copolymer Adjuvant in Patients with metastatic cancer (including ovarian cancer)
NCT00088413	I	To evaluate the safety and tolerability of PANVAC-V priming vaccine and PANVAC-F boosting vaccine, which produce CEA and MUC-1, in combination with sargramostim (GM-CSF) in adults with metastatic carcinoma including ovarian cancer
NCT00423254	I	To evaluate the safety and immune response to DNA Vector pPRA-PSM With Synthetic Peptides E-PRA and E-PSM, a multi-component immune based therapy, in patients with advanced cancer, including ovarian cancer
NCT00437502	I	To evaluate the safety of a peptide vaccine (consisting of 12 different tumor-rejection antigens) plus GM-CSF and Adjuvant (Montanide ISA-51) as Consolidation Following Optimal Debulking and Systemic Chemotherapy for Women with Advanced Stage Ovarian, Tubal or Peritoneal Cancer
NCT00585845	I	To evaluate the safety and tolerability of CRS207, a live attenuated <i>Listeria monocytogenes</i> expressing human mesothelin, in patients with advanced cancer of the ovary or pancreas, non-small cell lung cancer or advanced malignant epithelial mesothelioma
NCT00660101	I/II	To determine the safety and efficacy of a DNP-Modified Autologous Ovarian Tumor Cell vaccine as therapy in ovarian cancer patients after relapse
NCT00948961	I/II	To examine the safety and tolerability of CDX-1401 in combination with Resiquimod and/or Poly-ICLC in patient with advanced cancers that are known to express the NY-ESO-1 protein (including ovarian cancer)
NCT01095848	I	To determine the safety and immunogenicity profile of DPX-0907 (consisting of 7 tumor-specific HLA-A2-restricted peptides, a universal T helper peptide, a polynucleotide adjuvant, a liposome and Montanide ISA51 VG) to treat breast, ovarian and prostate cancer
NCT01376505	I	To determine the safety and effectiveness of vaccination combining two chimeric (Trastuzumab-like and Pertuzumab-like) HER-2 B Cell Peptides emulsified in ISA 720 and Nor-MDP Adjuvant in Patients with Advanced Solid Tumors (including ovarian cancer)
NCT01416038	I/II	To determine the safety and immunogenicity profiles of DPX-Survivac, designed to target Survivin, with a regimen of low dose oral cyclophosphamide in the treatment of ovarian, fallopian tube and peritoneal cancers
NCT01522820	I	To determine the safety and immunogenicity of the DEC-205-NY-ESO-1 fusion protein vaccine, with and without sirolimus (an mTOR inhibitor), in patients with NY-ESO-1 expressing solid tumors (including ovarian)
NCT01526473	I	To determine the safety and immunogenicity of HER2 VRP (AVX901) in patients with advanced or metastatic malignancies that express HER2 (including ovarian)
NCT01312389	I/II	To determine the feasibility, safety and immunogenicity of OC-L, an autologous vaccine comprised of autologous Oxidized tumor Cell Lysates (OC-L) administered by intradermal/subcutaneous injection in combination with Ampligen, a Toll-like receptor 3 agonist
Monoclonal Antibody		
NCT00034138	I/II	To compare the safety, immunity and pharmacokinetic profile of OvaRex mAb-B43.13 (oregovomab) ascites fluid product and OvaRex mAb-B43.13 cell culture product in a patients with Stage III/IV epithelial ovarian cancer
NCT00034372	II	To compare the time to disease relapse of patients who demonstrate an immune response to OvaRex mAb-B43.13 (Oregovomab) with time to disease relapse of those who do not demonstrate an immune response to OvaRex mAb-B43.13
NCT00086632	II	To determine if oregovomab received during front-line chemotherapy for ovarian cancer can create anti-tumor immune responses and thus provide a survival benefit

Table 4. Current Clinical Trials investigating Immunotherapy in Ovarian Cancer

NCT00050375	III	To compare the time to disease relapse between post chemotherapy consolidation with oregovomab (OvaRex® mAb-B43.13) or placebo in women with ovarian, tubal or peritoneal cancer.
Cellular Immunotherapy		
NCT00003887	II	To study the effectiveness of donated white blood cells in treating patients who have relapsed cancer (including ovarian cancer) following allogeneic transplantation of donated bone marrow or peripheral stem cells
NCT00004604	I	To study the effectiveness of carcinoembryonic antigen (CEA) antigen RNA-pulsed autologous DC cancer vaccine in treating patients who have metastatic cancer (including ovarian cancer) that has not responded to prior treatment
NCT00005956	I	To study the effectiveness of HER2/Neu Intracellular Domain (ICD) Protein-pulsed, Autologous, Cultured DCs followed by autologous DC mixed with tetanus toxoid (TT) and autologous DC mixed with keyhole limpet hemocyanin (KLH) in Patients with no evidence of disease after standard treatment for HER2/Neu Expressing Malignancies (including ovarian cancer)
NCT00027534	I	To study the effectiveness of autologous DCs infected with recombinant fowlpox-CEA-TRICOM vaccine in patients with advanced or metastatic malignancies expressing CEA (including ovarian cancer)
NCT00101257	I	To study the side effects and best dose of Autologous CD4+ Antigen-Specific T cells following in vitro stimulation with NY-ESO-1 pulsed DCs in patients with stage III or stage IV EOC or PPC
NCT01456065	I	To determine the safety of active immunotherapy with fully mature, Telomerase Reverse Transcriptase-mRNA and Survivin peptide double loaded DCs (Procore®) in patient with advanced ovarian cancer, enrolled within 12 weeks after completing primary therapy
NCT00603460	I	To determine the feasibility and safety of administering vaccine-primed, ex vivo CD3/CD28-costimulated autologous peripheral blood T cells in combination with DCVax-L vaccination, following lymphodepletion with high dose cyclophosphamide/fludarabine.
	II	To assess the distribution of progression-free survival at 6 mo for patients treated with maintenance DCVax-L vaccination plus oral metronomic cyclophosphamide as well as patients treated with ex vivo CD3/CD28-costimulated vaccine-primed peripheral blood autologous T cells after lymphodepletion with high dose cyclophosphamide / fludarabine, followed by DCVax-L boost vaccination and metronomic oral cyclophosphamide.
NCT01132014	0	To determine the feasibility, safety and immunogenicity of OC-DC, an autologous vaccine comprised of autologous dendritic cells (DC), administered intranodally alone or in combination with either intravenous Daclizumab or with a combination of Daclizumab and intravenous Bevacizumab.
NCT00703105	II	To determine if immunoregulatory T-cell inhibition by Ontak followed by the administration of an autologous tumor lysate-loaded dendritic cell vaccine enhances the immune response in patients with relapsed/refractory ovarian cancer
NCT00683241	I	To determine the feasibility and safety of administering DCVax-L intradermally combined with intravenous bevacizumab and oral metronomic cyclophosphamide in patients with recurrent ovarian or primary peritoneal cancer
Combination/Other		
NCT00194714	I/II	To determine the safety and efficacy of monthly HER2/Neu Specific Cytotoxic T Cell vaccination in patients with HER2 over-expressing Stage IV breast and ovarian cancer who are on maintenance trastuzumab alone after being treated with chemotherapy and trastuzumab or trastuzumab alone
NCT00004021	II	Evaluate the efficacy of immunotherapy with irradiated autologous tumor cell vaccine and sargramostim (GM-CSF) followed by monoclonal antibody OKT3- activated T lymphocytes and interleukin-2 in combination with standard therapy in terms of response rate in patients with stage III or IV ovarian cancer
NCT00496860	I	To evaluate the safety, determine the maximum-tolerated dose (MTD) and characterize the pharmacokinetic profile of ALT-801(a biologic compound composed of IL-2 genetically fused to a human soluble T-cell receptor directed against p53) in previously treated patients with progressive metastatic malignancies, including ovarian cancer
NCT01384253	I	To determine the toxicity profile of intraperitoneal ²¹² Pb-TCMC-Trastuzumab infusion, its dose-limiting toxicities, and its anti-tumor effects in patients with HER-2 positive intraperitoneal cancers
NCT00019084	II	To study the effectiveness of tumor specific mutated p53 or Ras peptides alone or in combination with cellular immunotherapy with peptide activated lymphocytes (PALs) along with subcutaneous IL-2 in treating patients who have advanced cancer (including ovarian cancer)

30. Diefenbach CS, Gnjatich S, Sabbatini P, Aghajanian C, Hensley ML, Spriggs DR, et al. Safety and immunogenicity study of NY-ESO-1b peptide and montanide ISA-51 vaccination of patients with epithelial ovarian cancer in high-risk first remission. *Clin Cancer Res* 2008; 14:2740-8; PMID:18451240; <http://dx.doi.org/10.1158/1078-0432.CCR-07-4619>.
31. Rahma OE, Ashtar E, Czysowska M, Szajnik ME, Wiecekowi E, Bernstein S, et al. A gynecologic oncology group phase II trial of two p53 peptide vaccine approaches: subcutaneous injection and intravenous pulsed dendritic cells in high recurrence risk ovarian cancer patients. *Cancer Immunol Immunother* 2012; 61:373-84; PMID:21927947; <http://dx.doi.org/10.1007/s00262-011-1100-9>.
32. Leffers N, Lambeck AJ, Gooden MJ, Hooeboom BN, Wolf R, Hamming IE, et al. Immunization with a P53 synthetic long peptide vaccine induces P53-specific immune responses in ovarian cancer patients, a phase II trial. *Int J Cancer* 2009; 125:2104-13; PMID:19621448; <http://dx.doi.org/10.1002/ijc.24597>.
33. Leffers N, Vermeij R, Hooeboom BN, Schulze UR, Wolf R, Hamming IE, et al. Long-term clinical and immunological effects of p53-SLP vaccine in patients with ovarian cancer. *Int J Cancer* 2012; 130:105-12; PMID:21328579; <http://dx.doi.org/10.1002/ijc.25980>.
34. Dohi S, Ohno S, Ohno Y, Takakura M, Kyo S, Soma G, et al. WT1 peptide vaccine stabilized intractable ovarian cancer patient for one year: a case report. *Anticancer Res* 2011; 31:2441-5; PMID:21873157.
35. Ohno S, Kyo S, Myojo S, Dohi S, Ishizaki J, Miyamoto K, et al. Wilms' tumor 1 (WT1) peptide immunotherapy for gynecological malignancy. *Anticancer Res* 2009; 29:4779-84; PMID:20032435.
36. Sandmaier BM, Oparin DV, Holmberg LA, Reddish MA, MacLean GD, Longenecker BM. Evidence of a cellular immune response against sialyl-Tn in breast and ovarian cancer patients after high-dose chemotherapy, stem cell rescue, and immunization with Theratope STn-KLH cancer vaccine. *J Immunother* 1999; 22:54-66; PMID:9924700; <http://dx.doi.org/10.1097/00002371-199901000-00008>.
37. MacLean GD, Reddish MA, Koganty RR, Longenecker BM. Antibodies against mucin-associated sialyl-Tn epitopes correlate with survival of metastatic adenocarcinoma patients undergoing active specific immunotherapy with synthetic STn vaccine. *J Immunother Emphasis Tumor Immunol* 1996; 19:59-68; PMID:8859725; <http://dx.doi.org/10.1097/00002371-199601000-00007>.
38. Leffers N, Daemen T, Boezen HM, Melief KJ, Nijman HW. Vaccine-based clinical trials in ovarian cancer. *Expert Rev Vaccines* 2011; 10:775-84; PMID:21692699; <http://dx.doi.org/10.1586/erv.11.42>.
39. Odunsi K, Jungbluth AA, Stockert E, Qian F, Gnjatich S, Tammela J, et al. NY-ESO-1 and LAGE-1 cancer-testis antigens are potential targets for immunotherapy in epithelial ovarian cancer. *Cancer Res* 2003; 63:6076-83; PMID:14522938.
40. Vermeij R, Leffers N, Hooeboom BN, Hamming IL, Wolf R, Reyniers AK, et al. Potentiation of a p53-SLP vaccine by cyclophosphamide in ovarian cancer: A single-arm phase II study. *Int J Cancer* 2011; In press; PMID:22139992.
41. Wang B, Kaumaya PT, Cohn DE. Immunization with synthetic VEGF peptides in ovarian cancer. *Gynecol Oncol* 2010; 119:564-70; PMID:20822802; <http://dx.doi.org/10.1016/j.ygyno.2010.07.037>.
42. Morse MA, Secord AA, Blackwell K, Hobeika AC, Sinnathamby G, Osada T, et al. MHC class I-presented tumor antigens identified in ovarian cancer by immunoproteomic analysis are targets for T-cell responses against breast and ovarian cancer. *Clin Cancer Res* 2011; 17:3408-19; PMID:21300761; <http://dx.doi.org/10.1158/1078-0432.CCR-10-2614>.
43. Chianese-Bullock KA, Irvin WP Jr., Petroni GR, Murphy C, Smolkin M, Olson WC, et al. A multi-peptide vaccine is safe and elicits T-cell responses in participants with advanced stage ovarian cancer. *J Immunother* 2008; 31:420-30; PMID:18391753; <http://dx.doi.org/10.1097/CJI.0b013e31816dad10>.
44. Sabbatini PJ, Kudryashov V, Ragupathi G, Danishefsky SJ, Livingston PO, Bornmann W, et al. Immunization of ovarian cancer patients with a synthetic Lewis(y)-protein conjugate vaccine: a phase I trial. *Int J Cancer* 2000; 87:79-85; PMID:10861456; [http://dx.doi.org/10.1002/1097-0215\(20000701\)87:1<79::AID-IJC12>3.0.CO;2-L](http://dx.doi.org/10.1002/1097-0215(20000701)87:1<79::AID-IJC12>3.0.CO;2-L).
45. Odunsi K, Lele S, et al. Diversified prime and boost vaccination using recombinant vaccinia and fowlpox expressing NY-ESO-1 efficiently induces antibody, CD4+ and CD8+ anti tumor immune responses in patients with ovarian cancer. 38th Society of Gynecologic Oncologists Annual Meeting on Women's Cancer. San Diego, CA, USA, 2007.
46. Gulley JL, Arlen PM, Tsang KY, Yokokawa J, Palena C, Poole DJ, et al. Pilot study of vaccination with recombinant CEA-MUC-1-TRICOM poxviral-based vaccines in patients with metastatic carcinoma. *Clin Cancer Res* 2008; 14:3060-9; PMID:18483372; <http://dx.doi.org/10.1158/1078-0432.CCR-08-0126>.
47. Chiang CL, Kandalaf LE, Coukos G. Adjuvants for enhancing the immunogenicity of whole tumor cell vaccines. *Int Rev Immunol* 2011; 30:150-82; PMID:21557641; <http://dx.doi.org/10.3109/08830185.2011.572210>.
48. Buckanovich RJ, Facciabene A, Kim S, Benencia F, Sasaroli D, Balint K, et al. Endothelin B receptor mediates the endothelial barrier to T cell homing to tumors and disables immune therapy. *Nat Med* 2008; 14:28-36; PMID:18157142; <http://dx.doi.org/10.1038/nm1699>.
49. Ehlen TG, Hoskins PJ, Miller D, Whiteside TL, Nicodemus CF, Schultes BC, et al. A pilot phase 2 study of oregovomab murine monoclonal antibody to CA125 as an immunotherapeutic agent for recurrent ovarian cancer. *Int J Gynecol Cancer* 2005; 15:1023-34; PMID:16343178; <http://dx.doi.org/10.1111/j.1525-1438.2005.00483.x>.
50. Gordon AN, Schultes BC, Gallion H, Edwards R, Whiteside TL, Cermak JM, et al. CA125- and tumor-specific T-cell responses correlate with prolonged survival in oregovomab-treated recurrent ovarian cancer patients. *Gynecol Oncol* 2004; 94:340-51; PMID:15297171; <http://dx.doi.org/10.1016/j.ygyno.2004.04.024>.
51. Braly P, Nicodemus CF, Chu C, Collins Y, Edwards R, Gordon A, et al. The Immune adjuvant properties of front-line carboplatin-paclitaxel: a randomized phase 2 study of alternative schedules of intravenous oregovomab chemioimmunotherapy in advanced ovarian cancer. *J Immunother* 2009; 32:54-65; PMID:19307994; <http://dx.doi.org/10.1097/CJI.0b013e31818b3dad>.
52. Berek J, Taylor P, McGuire W, Smith LM, Schultes B, Nicodemus CF. Oregovomab maintenance monotherapy does not improve outcomes in advanced ovarian cancer. *J Clin Oncol* 2009; 27:418-25; PMID:19075271; <http://dx.doi.org/10.1200/JCO.2008.17.8400>.
53. Pfisterer J, du Bois A, Sehouli J, Loibl S, Reinartz S, Reuss A, et al. The anti-idiotypic antibody abagovomab in patients with recurrent ovarian cancer. A phase I trial of the AGO-OVAR. *Ann Oncol* 2006; 17:1568-77; PMID:17005631; <http://dx.doi.org/10.1093/annonc/mdl357>.
54. Reinartz S, Köhler S, Schlebusch H, Krista K, Giffels P, Renke K, et al. Vaccination of patients with advanced ovarian carcinoma with the anti-idiotypic ACA125: immunological response and survival (phase Ib/II). *Clin Cancer Res* 2004; 10:1580-7; PMID:15014007; <http://dx.doi.org/10.1158/1078-0432.CCR-03-0056>.
55. Sabbatini P, Dupont J, Aghajanian C, Derosa F, Poyner E, Anderson S, et al. Phase I study of abagovomab in patients with epithelial ovarian, fallopian tube, or primary peritoneal cancer. *Clin Cancer Res* 2006; 12:5503-10; PMID:17000686; <http://dx.doi.org/10.1158/1078-0432.CCR-05-2670>.
56. Nicholson S, Bomphray CC, Thomas H, McIndoe A, Barton D, Gore M, et al. A phase I trial of idiotype vaccination with HMGF1 in ovarian cancer. *Cancer Immunol Immunother* 2004; 53:809-16; PMID:15127236; <http://dx.doi.org/10.1007/s00262-004-0522-z>.
57. Hird V, Maraveyas A, Snook D, Dhokia B, Soutter WP, Meares C, et al. Adjuvant therapy of ovarian cancer with radioactive monoclonal antibody. *Br J Cancer* 1993; 68:403-6; PMID:8347497; <http://dx.doi.org/10.1038/bjc.1993.349>.
58. Oei AL, Verheijen RH, Seiden MV, Benigno BB, Lopes A, Soper JT, et al. Decreased intraperitoneal disease recurrence in epithelial ovarian cancer patients receiving intraperitoneal consolidation treatment with yttrium-90-labeled murine HMGF1 without improvement in overall survival. *Int J Cancer* 2007; 120:2710-4; PMID:17354223; <http://dx.doi.org/10.1002/ijc.22663>.
59. Bellone S, Siegel ER, Cocco E, Cargnelutti M, Silasi DA, Azodi M, et al. Overexpression of epithelial cell adhesion molecule in primary, metastatic, and recurrent/chemotherapy-resistant epithelial ovarian cancer: implications for epithelial cell adhesion molecule-specific immunotherapy. *Int J Gynecol Cancer* 2009; 19:860-6; PMID:19574774; <http://dx.doi.org/10.1111/JGC.0b013e3181a8331f>.
60. Heiss MM, Murawa P, Koralewski P, Kutarska E, Kolesnik OO, Ivanchenko VV, et al. The trifunctional antibody catumaxomab for the treatment of malignant ascites due to epithelial cancer: Results of a prospective randomized phase II/III trial. *Int J Cancer* 2010; 127:2209-21; PMID:20473913; <http://dx.doi.org/10.1002/ijc.25423>.
61. Baumann K, Pfisterer J, Wimberger P, Burchardi N, Kurzeder C, du Bois A, et al. Intraperitoneal treatment with the trifunctional bispecific antibody Catumaxomab in patients with platinum-resistant epithelial ovarian cancer: a phase IIa study of the AGO Study Group. *Gynecol Oncol* 2011; 123:27-32; PMID:21733566; <http://dx.doi.org/10.1016/j.ygyno.2011.06.004>.
62. Makhija S, Amel LC, Glenn D, Ueland FR, Gold MA, Dizon DS, et al. Clinical activity of gemcitabine plus pertuzumab in platinum-resistant ovarian cancer, fallopian tube cancer, or primary peritoneal cancer. *J Clin Oncol* 2010; 28:1215-23; PMID:19901115; <http://dx.doi.org/10.1200/JCO.2009.22.3354>.
63. Bookman MA, Darcy KM, Clarke-Pearson D, Boothby RA, Horowitz IR. Evaluation of monoclonal humanized anti-HER2 antibody, trastuzumab, in patients with recurrent or refractory ovarian or primary peritoneal carcinoma with overexpression of HER2: a phase II trial of the Gynecologic Oncology Group. *J Clin Oncol* 2003; 21:283-90; PMID:12525520; <http://dx.doi.org/10.1200/JCO.2003.10.104>.
64. Chung MK, Han SS, Roh JK. Synergistic embryotoxicity of combination pyrimethamine and folic acid in rats. *Reprod Toxicol* 1993; 7:463-8; PMID:8274822; [http://dx.doi.org/10.1016/0890-6238\(93\)90091-K](http://dx.doi.org/10.1016/0890-6238(93)90091-K).
65. Molthoff CF, Prinssen HM, Kenemans P, van Hof AC, den Hollander W, Verheijen RH. Escalating protein doses of chimeric monoclonal antibody MOv18 immunoglobulin G in ovarian carcinoma patients: a phase I study. *Cancer* 1997; 80(Suppl):2712-20; PMID:9406729; [http://dx.doi.org/10.1002/\(SICI\)1097-0142\(19971215\)80:12<2712::AID-CNCR50>3.0.CO;2-B](http://dx.doi.org/10.1002/(SICI)1097-0142(19971215)80:12<2712::AID-CNCR50>3.0.CO;2-B).

66. Konner JA, Bell-McGuinn KM, Sabbatini P, Hensley ML, Tew WP, Pandit-Taskar N, et al. Farletuzumab, a humanized monoclonal antibody against folate receptor alpha, in epithelial ovarian cancer: a phase I study. *Clin Cancer Res* 2010; 16:5288-95; PMID:20855460; <http://dx.doi.org/10.1158/1078-0432.CCR-10-0700>.
67. Perren TJ, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, Kristensen G, et al.; ICON7 Investigators. A phase 3 trial of bevacizumab in ovarian cancer. *N Engl J Med* 2011; 365:2484-96; PMID:22204725; <http://dx.doi.org/10.1056/NEJMoa1103799>.
68. Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, et al.; Gynecologic Oncology Group. Incorporation of bevacizumab in the primary treatment of ovarian cancer. *N Engl J Med* 2011; 365:2473-83; PMID:22204724; <http://dx.doi.org/10.1056/NEJMoa1104390>.
69. Burger RA, Sill MW, Monk BJ, Greer BE, Sorosky JL. Phase II trial of bevacizumab in persistent or recurrent epithelial ovarian cancer or primary peritoneal cancer: a Gynecologic Oncology Group Study. *J Clin Oncol* 2007; 25:5165-71; PMID:18024863; <http://dx.doi.org/10.1200/JCO.2007.11.5345>.
70. Cannistra SA, Matulonis UA, Penson RT, Hambleton J, Dupont J, Mackey H, et al. Phase II study of bevacizumab in patients with platinum-resistant ovarian cancer or peritoneal serous cancer. *J Clin Oncol* 2007; 25:5180-6; PMID:18024865; <http://dx.doi.org/10.1200/JCO.2007.12.0782>.
71. Leffers N, Daemen T, Helfrich W, Boezen HM, Cohlen BJ, Melief K, et al. Antigen-specific active immunotherapy for ovarian cancer. *Cochrane Database Syst Rev*:CD007287
72. Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. *Nat Rev Cancer* 2008; 8:299-308; PMID:18354418; <http://dx.doi.org/10.1038/nrc2355>.
73. Dudley ME, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, et al. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol* 2005; 23:2346-57; PMID:15800326; <http://dx.doi.org/10.1200/JCO.2005.00.240>.
74. Aoki Y, Takakuwa K, Kodama S, Tanaka K, Takahashi M, Tokunaga A, et al. Use of adoptive transfer of tumor-infiltrating lymphocytes alone or in combination with cisplatin-containing chemotherapy in patients with epithelial ovarian cancer. *Cancer Res* 1991; 51:1934-9; PMID:2004379.
75. Lum LG, LeFever AV, Treisman JS, Garlie NK, Hanson JP Jr. Immune modulation in cancer patients after adoptive transfer of anti-CD3/anti-CD28-costimulated T cells-phase I clinical trial. *J Immunother* 2001; 24:408-19; <http://dx.doi.org/10.1097/00002371-200109000-00003>.
76. Kershaw MH, Westwood JA, Parker LL, Wang G, Eshhar Z, Mavroukakis SA, et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clin Cancer Res* 2006; 12:6106-15; PMID:17062687; <http://dx.doi.org/10.1158/1078-0432.CCR-06-1183>.
77. Geller MA, Cooley S, Judson PL, Ghebre R, Carson LE, Argenta PA, et al. A phase II study of allogeneic natural killer cell therapy to treat patients with recurrent ovarian and breast cancer. *Cytotherapy* 2011; 13:98-107; PMID:20849361; <http://dx.doi.org/10.3109/14653249.2010.515582>.
78. Steinman RM, Banchereau J. Taking dendritic cells into medicine. *Nature* 2007; 449:419-26; PMID:17898760; <http://dx.doi.org/10.1038/nature06175>.
79. Cannon MJ, O'Brien TJ. Cellular immunotherapy for ovarian cancer. *Expert Opin Biol Ther* 2009; 9:677-88; PMID:19456205; <http://dx.doi.org/10.1517/14712590902932897>.
80. Hernando JJ, Park TW, Kübler K, Offergeld R, Schlebusch H, Bauknecht T. Vaccination with autologous tumour antigen-pulsed dendritic cells in advanced gynaecological malignancies: clinical and immunological evaluation of a phase I trial. *Cancer Immunol Immunother* 2002; 51:45-52; PMID:11845259; <http://dx.doi.org/10.1007/s00262-001-0255-1>.
81. Chu CS, Boyer J, Schullery DS, Gimotty PA, Gamerman V, Bender J, et al. Phase I/II randomized trial of dendritic cell vaccination with or without cyclophosphamide for consolidation therapy of advanced ovarian cancer in first or second remission. *Cancer Immunol Immunother* 2012; 61:629-41; PMID:22021066; <http://dx.doi.org/10.1007/s00262-011-1081-8>.
82. Brossart P, Wirths S, Stuhler G, Reichardt VL, Kanz L, Brugger W. Induction of cytotoxic T-lymphocyte responses in vivo after vaccinations with peptide-pulsed dendritic cells. *Blood* 2000; 96:3102-8; PMID:11049990.
83. Hodi FS, Butler M, Oble DA, Seiden MV, Haluska FG, Kruse A, et al. Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. *Proc Natl Acad Sci U S A* 2008; 105:3005-10; PMID:18287062; <http://dx.doi.org/10.1073/pnas.0712237105>.
84. Berd D, Mastrangelo MJ. Effect of low dose cyclophosphamide on the immune system of cancer patients: reduction of T-suppressor function without depletion of the CD8+ subset. *Cancer Res* 1987; 47:3317-21; PMID:2953413.
85. North RJ. Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor T cells. *J Exp Med* 1982; 155:1063-74; PMID:6460831; <http://dx.doi.org/10.1084/jem.155.4.1063>.
86. Ercolini AM, Ladle BH, Manning EA, Pfannenstiel LW, Armstrong TD, Machiels JP, et al. Recruitment of latent pools of high-avidity CD8(+) T cells to the anti-tumor immune response. *J Exp Med* 2005; 201:1591-602; PMID:15883172; <http://dx.doi.org/10.1084/jem.20042167>.
87. Hodi FS, O'Day SJ, McDermott DE, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010; 363:711-23; PMID:20525992; <http://dx.doi.org/10.1056/NEJMoa1003466>.
88. Hodi FS, Mihm MC, Soiffer RJ, Haluska FG, Butler M, Seiden MV, et al. Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. *Proc Natl Acad Sci U S A* 2003; 100:4712-7; PMID:12682289; <http://dx.doi.org/10.1073/pnas.0830997100>.
89. Alagkiozidis I, Facciabene A, Carpenito C, Benencia F, Jonak Z, Adams S, et al. Increased immunogenicity of surviving tumor cells enables cooperation between liposomal doxorubicin and IL-18. *J Transl Med* 2009; 7:104; PMID:20003308; <http://dx.doi.org/10.1186/1479-5876-7-104>.