Immunotherapy in ovarian cancer: hype or hope?

Interview with B.J. Monk[1] and E. Pujade Lauraine[2] *by* C. Marchetti[3]

In favor of immunotherapy:
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Against immunotherapy:
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Introduction

Ovarian cancer (OC) is the most important cause of gynecologic cancer-related mortality, with the majority of women presenting with advanced disease. Although surgery and chemotherapy can improve survival rates, alternative strategies need to be integrated to improve outcomes. Advances in understanding about the role of the immune system in the pathogenesis of cancer have contributed to the rapidly-evolving field of immunotherapy, which might facilitate a sustained immune system response against recurring cancer cells. However, to date there are only limited data on which the role of immunotherapy in OC can be based. In this interview, two experts will discuss the main data, as well as the next challenges and limitations in the development of immunotherapy for the treatment of OC.

1. Can we consider ovarian cancer as an immunogenic disease?

Pro immunotherapy

Immunogenicity is the ability of antigens to elicit an immune response. The first cancer vaccine in humans was attributed to William Coley in 1893 [1]. He observed that some patients with cancer benefitted from bacterial infection, resulting in tumor shrinkage. This was the first step for the field of immunotherapy in cancer-related disease. Since then there are been many more steps forward and, in the last two decades, advances in the understanding of OC immunogenicity have further opened the door to immunotherapeutic approaches.

Immunotherapy as a potential approach for the treatment of OC is based on the following evidence:

- ovarian cancers express tumor-associated antigens, including HER2/neu [2], MUC1 [3], OA3 [4], membrane folate receptor [5], NY-ESO-1 [6], and many others, which can serve as targets for humoral and cellular immune responses;
- the presence of tumor infiltrating lymphocytes (TILs) correlates strongly with survival [7];
- ovarian cancers express peptide/major histocompatibility complex (MHC) complexes, which can be recognized by CD8+ T lymphocytes;
- the dynamic interaction between host immunity and cancer indicates that the balance between the two forces can be tipped to favor the host immunity, with the ever increasing arsenals of immunology.

Taken together, it has been hypothesized that immunotherapy could be an innovative and effective supportive therapy for OC.

Against immunotherapy

There is much evidence in favor of this assumption, such as documentation of spontaneous antitumor immune response and the association between this and longer survival, evidence of tumor immune evasion mechanisms and a link with short survival, and pilot data supporting the efficacy of immune therapy [8]. Indeed, OC can no longer be considered as an immunologically inert class of tumors. Nevertheless, the mechanisms of immune surveillance and immune-escape in cancer patients seem to be quite complex and have not been fully explained. In fact, immune suppressive signals are often dominant, and may prevent effective clearance of tumor cells by the immune system. A conspicuous group of immune suppressive factors and cells halt the generation and clonal expansion of antitumor immunity. Furthermore, genetic changes in tumor cells allow them to be ignored by an immune response. This may

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explain why, until recently, that only important changes in immunological features have been observed and there is a lack of relevant clinical results.

2. How should we interpret data coming from clinical trials on antibody-based immunotherapies for ovarian cancer?

Pro immunotherapy

Antibodies are fascinating anti-cancer agents given their high specificity for antigens, stability, and ability to be mass-produced by bioengineering technology. They can potentially induce tumor cell apoptosis via a number of mechanisms, including complement-dependent cytotoxicity, mediated when the Fc portion of immunoglobulins activates the complement system; antibody-dependent cellular cytotoxicity; limiting tumor growth by binding to growth receptors, preventing interactions with endogenous ligands and hence inhibiting downstream signaling events; and by inducing tumor cell death through modulation of anti-tumor immunity via blockade of immune checkpoint inhibitors. Antibody-based cancer immunotherapy has now become standard practice in the treatment of lymphoma and other cancers.

Many attempts at immunotherapy have been undertaken in OC. After controversial results with the use of anti-idiotypic antibodies [9, 10], interesting data have been obtained with antibodies able to inhibit immune checkpoints and activate effector T cells. In the near future we will see the effects of combining different monoclonal antibodies and this may truly be the right way forward.

Against immunotherapy

Results with immunotherapy in OC are controversial. The tumor-associated antigen CA125, a well-known surface glycoprotein that is frequently expressed in OC, was thought to be the most interesting target. Two monoclonal antibodies with different mechanisms of action, abagovomab and oregovomab, have been developed. Abagovomab has one of the most captivating mechanisms of action, which is that of the anti-idiotypic antibodies. The term idiotype (Id) means the typical antigenic determinant of the antibody variable region that allows differentiation between Ig binding to different antigens. The most immediate application of this relates to the targeting of tumor antigens to which the Id is directed. On the basis of this information, anti-Id antibodies were developed to mimic tumor antigens. After promising results in early phase trials [11], abagovomab was found to be safe and had the ability to induce measurable immune responses, but without prolongation of relapse-free and overall survival (OS) [9].

Oregovomab is a monoclonal antibody that recognizes CA125 and forms circulating immune complexes that can elicit immunity against both tumor antigen and tumor. Oregovomab was not associated with a significant survival advantage when given as maintenance therapy after front-line treatment [10]. In addition, although it elicited an immune response when combined with standard chemotherapy, no tumor-antigen specific T cell immunity could be detected in the circulation [12]. Results from the trials of abagovomab and oregovomab highlight two important lessons. Firstly, they caution against becoming overly excited about novel drugs that show promise in phase I and/or II clinical trials because high expectations are often not met in phase III studies. Secondly, immune reactivity is common when patients are treated with immunomodulatory drugs and is more frequent in patients with a favorable prognosis, which accounts for the better prognostic of immune-reactive compared with non-immune reactive patients, but generating specific anti-tumor T cells instead of a non-specific immune response remains one of the most difficult, but ultimate, goals of immunotherapy.

3. Immune checkpoint inhibitors have emerged as promising therapies in several tumors and interesting data have recently been presented in recurrent ovarian cancer. Do they have the potential to be incorporated into clinical practice in this setting?

Pro immunotherapy

As mentioned previously, we have recently seen the clinical development and approval of immunomodulators, also known as immune checkpoint inhibitors. Briefly, T cell activation is triggered via the T cell receptor (TCR) by recognition of the cognate antigen complexed with the MHC. This activation is regulated by complex signals downstream of the CD28 family of immune receptors, which includes co-stimulatory (CD28 and ICOS) and inhibitory (CTLA-4, PD-1, and BTLA) receptors. 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 monoclonal antibodies that block CTLA-4 or PD-1 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. After promising results in preclinical studies, many clinical trials have demonstrated the acceptable safety and efficacy profiles of immune checkpoint inhibitors in a variety of cancers.

The first approved immune checkpoint inhibitor was ipilimumab, an anti-CTLA-4 (cytotoxic T lymphocyte anti-

gen-4) monoclonal antibody, in the setting of advanced melanoma. Several clinical trials with ipilimumab have also included patients with OC and anti-tumor effects have been noted [13, 14].

There are now many antibodies targeting CTLA-4, PD-1 or PD-L1 undergoing clinical trials in OC (nivolumab, atezolizumab, durvalumab, avelumab). Preliminary results of some of these studies have been recently presented and showed very promising results [15, 16]. Also, there is currently an ongoing a study investigating the combination of ipilimumab and nivolumab (a monoclonal antibody targeting PD-1) (NRG-GY003).

The rationale of this trial is based on the non-redundant nature of CTLA-4- and PD-1-mediated T cell inhibition, as well as the co-expression of CTLA-4 and PD-1 evident on molecular profiling of tumor-reactive infiltrating T cells (CD137+) from ovarian cancers. Furthermore, in animal studies, co-administration of anti-PD-1 antibodies and anti-CTLA-4 antibodies reversed TIL dysfunction and induced tumor regression in 50% of the mice compared with 25% of mice when either agent was given as a monotherapy [17]. This is definitely an active area of research and, considering experiences in other solid tumors, it is reasonable to hypothesize that these agents might be incorporated into OC treatment strategies at some point in the near future.

Against immunotherapy

Recent findings demonstrate that a variety of functionally non-overlapping co-inhibitory receptors can be expressed by T cells to turn off their effector function [18]. These inhibitory receptors include CTLA-4, PD-1, TIM-3, BTLA, PD-L1.

The possibility of overcoming this immunosuppressive phenomena and stimulating a tumor response is obviously intriguing. Clinical data with anti-PD-L1/anti-PD-1 agents in several tumors are promising, as are recently reported data in OC, with a 10-20% objective response rate in heavily pre-treated OC patients including some patients with very prolonged disease control [13-16]. Although these results are still very preliminary, it is becoming clear that individual anti-PD-L1/anti-PD-1 agents will need to be combined to optimize their action. Several combinations including checkpoint inhibitors will be tested in future studies. Anti-PD-L1/anti-P1 will be tested with chemotherapy, either with standard carboplatin-paclitaxel combination or with pegylated liposomal doxorubicin, the immunomodulatory properties of which are well known. However, this type of immunotherapy depends on a healthy immune system and conventional cancer treatments, including chemotherapy and radiation therapy, often have immunosuppressive effects and could therefore decrease the efficacy of Anti-PD-L1/anti-P1 immunotherapy. Anti-PD-L1/anti-P1 molecules will also be evaluated in combination with bevacizumab because vascular endothelial growth factor (VEGF) is inversely correlated with T cell epithelial infiltration in OC. Finally, the question of combining checkpoint inhibitors with different mechanism of action remains an unresolved topic in OC.

A strategy to selectively manipulate the tumor microenvironment rather than systemic promotion of T cell immunity is desirable. This is particularly important because many tumor cells express ligands for the co-inhibitory receptors, whereas there is minimal expression of these molecules in normal tissues [19]. Also, the potential to combine multiple T cell checkpoint blockade strategies to maximize anti-cancer immunity needs to be further investigated, in line with some published results [17]. Blockade of CTLA-4 with antibody, however, led to significant tumor regression in patients with advanced melanoma but severe autoimmune toxicity was evident in 15% of patients [13]. Therefore, the next step will be to integrate checkpoint inhibitors into the therapeutic armamentarium for OC, although optimization of their use in clinical practice may be a bit further away.

4. What is the possible role of adoptive cell therapy in ovarian cancer therapy? Are chimeric TCReng T cell-based strategies really promising?

Pro immunotherapy

Adoptive cellular therapy (ACT) has received much attention as a realistic technique for cancer treatment. Cancer immunotherapy is dependent on the presence of TILs, which correlate with survival in OC. Chimeric antigen receptors (CARs) are artificial cell receptors that allow T cells to target a tumor-associated antigen (TAA); CARs bypass the downregulation of MHC-I and antigen presentation (a common immune evasion mechanism of tumor cells), and provide engineered T cells without MHC restriction and with potent costimulatory signals. Furthermore, antibody-antigen affinity is several times stronger than natural TCR-mediated recognition. A large number of CARs targeting diverse tumors have been developed [20]; however, clinical pilot studies are only just beginning. The first study of adoptive transfer of CAR T cells in OC demonstrated the safety of this approach but activity was 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 [21].

Persistence can be improved by adding costimulatory signaling capabilities to the intracytoplasmic domain of CARs; second and third generation CARs now include additional co-stimulatory signaling domains, such as those from CD28, CD27 and 41-BB, all designed to enhance immune activation and T cell persistence. A phase I clinical trial is investigating the safety, feasibility and preliminary activity of the adoptive transfer of autologous T cells transduced with CAR recognizing the folate receptor alpha (FRa) and carrying the CD3z domain along with the 41-BB co-stimulatory signaling domain to address the issue of persistence of FRa-specific CAR-T cells [22]. Eligible patients have FRa-positive epithelial stage II-IV OC that had relapsed after two or more chemotherapy regimens; all are receiving untransduced autologous peripheral blood lymphocytes intravenously to contain the exponential expansion of CAR-T cells [22, 23].

Against immunotherapy

Immune cells, including T cells, natural killer (NK) cells, dendritic 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. After early data in melanoma tumors established the feasibility of adoptive T cell transfer, attention has focused on optimizing the anti-tumor efficacy of this therapy. Given that the availability of 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 TCRs or CARs. CARs are recombinant receptors that combine the specificity of an antigen-specific antibody with the activating functions of a T cell. This is clearly a very interesting process that, intuitively, may help to obtain a more specific immune response. Nonetheless, the process by which these CARs are generated is technically difficult, labor-intensive and time-consuming. Furthermore, even if this treatment is thought to be safe, the clinical benefits have yet to be clearly defined [21]. It is reasonable to hypothesize that the existence of a number of different immunosuppressive pathways can limit the full potential of CAR T cell therapies. The interaction of inhibitory molecules on activated T cells and their ligands on tumor cells compromises T cell function. This includes the increased expression of inhibitory immune receptors such as T cell membrane protein-3 (TIM-3), CTLA-4, and/or PD-1 on T cells following T cell activation, which can limit the duration and strength of the adaptive immune response [24]. The cost-effectiveness of manufacturing a drug for each patient will remain a challenge for these therapies, even if their efficacy is proven.

5. What are the critical hurdles and upcoming challenges in ovarian cancer immunotherapy?

Pro immunotherapy

Immune-based therapies have now demonstrated efficacy in a range of clinical studies and types of cancer. We have seen that the selective manipulation of T cell checkpoint inhibitors or the adoptive transfer of tumor-reactive T cells promises some efficacy in the treatment of OC. However, the more realistic and achievable application of immunotherapy in the short-to-medium term is probably as an adjunctive therapy rather than as a front-line monotherapy. Chemo-immunotherapy is appealing not only because chemotherapies directly induce apoptosis of tumor cells and result in the release of antigen to drive immune responses, but they often also disrupt essential immune regulatory mechanisms that limit the development of immunity, an increasingly appreciated attribute [25]. An ideal combination of chemo-immunotherapy would be where one or both agents have minimal overlapping toxicities and work via independent mechanisms, but have additive or synergistic antitumor effects. Several trials are currently ongoing to address this question, and some of the preliminary data are promising [26]. Selection of appropriate patients for clinical trial participation will be quite important because evidence to date indicates that many patients with OC display a spontaneous antitumor immune response. These patients may be best suited for vaccine therapy or TIL-based therapy because 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 because the tumor microenvironment is already conducive to T cell homing and engraftment. Additional biomarkers are needed to optimize selection of patients who may benefit from immune therapy. In conclusion, more than ever before, the field of cancer immunology is permeated with a sense of optimism. The key question today is not whether immune-based therapies will transform cancer therapy, but how will these approaches transform cancer medicine in the future.

Against immunotherapy

Innovative immunotherapeutic strategies offer the promise of enhancing host anti-tumor responses, which may improve clinical outcomes in women with OC. While many

preliminary phase I/II studies have demonstrated induction of anti-tumor responses, current data on clinical outcomes in the OC setting are controversial. To date, interesting results have been achieved by immunomodulation strategies including anti-CTLA-4, anti-PD-1 and anti-PD-L1 monoclonal antibodies, and the combination of immunotherapy with chemotherapy is another area of interest. Nonetheless, there are a number of questions to be answered and some limitations need to be considered. Primarily, although there is no lack of OC antigens due to genomic instability and accumulation of mutated genes at this point, the generation of immune responses against these antigens is likely to be unproductive in the late stage of disease due to multiple immune tolerance mechanisms.

Thus, multiple immunological 'brakes' need to be considered to increase the productive immune response. As a result, combined immunotherapeutic modalities need to be seriously considered. However, the application of multiple therapies (a combination of immunotherapies or immunotherapy plus chemotherapy) simultaneously requires careful consideration of important factors such as the potential for overlapping toxicities and an elevated risk of severe organ damage due to immune system dis-inhibition. There is also a need to optimize the timing of treatment administration, and surgery and chemotherapy are extremely immunosuppressive making them very difficult to combine with immunotherapy. Therefore, what is the best time for immunotherapy to be administered? Should immunotherapy be given first, then followed by surgery and chemotherapy? Does corticosteroid premedication before chemotherapy administration prevent checkpoint inhibitor monoclonal antibodies from being active? There should also be a focus on finding biomarkers for early diagnosis or prognosis and individualization of treatment. For example, what is the role of tumor PD-L1 expression? The presence of TIL? Or the presence of immunosuppressive immune cells such as T regulatory cells, immature dendritic cells or M2 macrophages in the tumor microenvironment? Finally, conventional response criteria (RECIST or WHO) may not reflect the patterns of response to immunotherapies and therefore the correlation between clinical and immunological response needs to be better defined. The next trials in this field should be designed to include careful selection of candidates as well as an appropriate reporting of standardized treatment responses and adverse events. This might facilitate clarification of the role of immunotherapy in the treatment of OC.

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