

EXPERT OPINION

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Immunotherapy for recurrent ovarian cancer: a further piece of the puzzle or a striking strategy?

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Introduction: Treatment of ovarian cancer has been long standardized with the inclusion of surgery and chemotherapy based on platinum and taxanes, this strategy reaching high remission rates. However, when this treatment fails, further options are available with little benefit. Since ovarian cancer has specific immunologic features, actually immunotherapy is under evaluation to overcome treatment failure in patients experiencing recurrence.

Areas covered: Immunogenicity of ovarian cancer and its relationship with clinical outcomes is briefly reviewed. The kinds of immunotherapeutic strategies are summarized. The clinical trials investigating immunotherapy in recurrent ovarian cancer patients are reported.

Expert opinion: The results of these clinical trials about immunotherapy are interesting, but little clinical benefit has been achieved until now. For this reason, we could conclude that immunotherapy is quite different from other treatment options and it could change the global approach for recurrent ovarian cancer treatment. However, to date only fragmentary findings are available to define the real role of immunotherapy in this setting.

Keywords: antibody, cancer vaccines, cell transfer, immune system, immunotherapy, ovarian cancer, recurrence, treatment strategies

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1. Introduction

Ovarian cancer is the fifth most common cause of cancer-related death in women, with an estimated incidence in the USA in 2013 of almost 22,000 cases and a mortality of 14,000 for the current year. According to EUCAN data, the estimated number of new diagnosis is near to 44,200 with a standardized rate of 12.6/100,000 and a mortality of almost 30,000 per year [1,2]. Ninety percent of ovarian cancers are epithelial and arise from the epithelium on the ovarian surface or Mullerian derivatives including the distal Fallopian tube. WHO classification identifies six principal histotypes: serous, mucinous, endometrioid, clear cell, transitional cell and squamous. Each type can be classified into three prognostic categories: benign, malignant and intermediate, the latter known as tumors of borderline malignancy or low malignant potential and atypical proliferative tumors. Ovarian carcinomas are further sub-classified for their architectural features into three grades, according to the percentage (< 5%, 5 – 50% and > 50%) of solid growth on glandular and papillary component [2].

Treatment of epithelial ovarian cancer is based on the combination of cytoreductive surgery and combination chemotherapy using taxane and platinum. However, in our opinion there is a large expectation for improved prognosis in ovarian carcinoma

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Article highlights.

- The decision-making for treatment of advanced ovarian cancer is guided by platinum sensitivity. To date new therapeutic options have provided further chances of survival.
- Ovarian cancer can be associated with immunologic changes, regarding T regulatory lymphocytes, immunosuppressive cytokines and $\gamma\delta$ T cells, which have an impact on clinical outcomes.
- The immunologic strategies, which have been developed for cancer treatment, include anti-Id therapy, cancer vaccines, tumor cell vaccines, adoptive cell transfer and immunomodulation strategies.
- Some of these treatment strategies have been applied to recurrent ovarian cancer. However, little clinical benefits were found.
- Immunotherapy in recurrent ovarian cancer has a valid biologic rationale and interesting perspective. Further studies are needed to clarify the role of these strategies in this setting.

This box summarizes key points contained in the article.

as a consequence of the use of new biological agents. Adjuvant chemotherapy for early stage ovarian cancer is still controversial but some studies have shown its benefit under confined conditions [3]. According to the results of two studies from the International Collaborative Ovarian Neoplasm group and the EORTC, patients with IA or IB FIGO stage, non-clear-cell histology, well-differentiated (G1) tumors, and an 'optimal' surgery (performed according to international guidelines, with pelvic and retroperitoneal assessment), appear not to benefit from chemotherapy [4,5].

The standard treatment for patients with advanced ovarian cancer is maximal surgical cytoreduction (total abdominal hysterectomy, bilateral salpingo-oophorectomy, pelvic and para-aortic lymphadenectomy and omentectomy) followed by systemic platinum-based chemotherapy and, actually, is reasonable to expect a 5-year survival for 10 – 30% of women diagnosed with ovarian cancer at Stage III or IV [6,7]. Despite the activity of first-line chemotherapy, which gives response rates up to 80% in first-line treatment, the majority of patients die because of their recurrent disease. Therefore, a large proportion of patients are candidates for second-line treatment. Platinum sensitivity, which is defined by a response to first-line platinum-based therapy, has been found to predict the response to subsequent retreatment with a platinum-containing regimen frequently used for salvage therapy. In general, patients who progress or have stable disease during first-line treatment or who relapse within 1 month are considered to be 'platinum-refractory'. Patients who respond to primary treatment and relapse within 6 months are considered 'platinum-resistant', and patients who relapse > 6 months after completion of initial therapy are characterized as 'platinum-sensitive' [8]. The optimal treatment for patients with partially platinum-sensitive

recurrent ovarian cancer is not clearly defined. Patients with platinum refractory and resistant are quite suitable for novel investigational approaches and studies of drug resistance. Single-agent therapy is considered the standard treatment in these patients [9]. Low response rates are recorded in these patients with the use of topotecan, docetaxel, oral etoposide, pegylated liposomal doxorubicin (PLD), gemcitabine, ifosfamide and hexamethylmelamine. However, PLD has favorable pharmacokinetic properties such as a lower plasma concentration peak, lower clearance, smaller distribution volume, longer half-life and higher AUC, resulting in a different and more convenient toxicity and efficacy profile [10]. One of the most investigated and promising molecular targeted drugs in ovarian cancer is bevacizumab, a monoclonal antibody directed against VEGF. VEGF expression is higher in ovarian cancer tumors than in normal ovarian tissue or benign ovarian tumors, and increasing VEGF expression in either cytosolic fractions derived from ovarian cancer tumors or serum VEGF levels in preoperative serum is considered to be associated with advanced stage and worse survival [11]. Recently the anti-VEGF monoclonal antibody bevacizumab was studied in combination with carboplatin/paclitaxel in Phase III clinical trials for the upfront setting of advanced ovarian cancer. A statistically significant increase of progression-free survival (PFS) was observed when patients received maintenance bevacizumab after upfront treatment. However, the benefit appears modest and further studies are needed to clarify the role of this drug in ovarian cancer treatment [12,13].

2. Mechanisms of immunogenicity and immunoediting in ovarian cancer and clinical effects

Human immune system is able to build a number of mechanisms to recognize and destroy foreign cells such as those of malignant tumors with the implication of both the two compartments: innate and adaptive [14,15]. First of all ovarian cancers express tumor-associated antigens, for example, HER2/neu [16], MUC1 [17], OA3 [18], membrane folate receptor [19], TAG-72 [20], mesothelin [21], NY-ESO-1 [22] and sialyl-Tn [23], which can serve as targets for humoral and cellular immune responses [24]. The mechanisms of immunosurveillance and immunoescape in cancer patients seem to be quite complex and not fully explained but they could be summarized as follows. In the immune response to cancer, several actors play different roles through three phases: elimination, equilibrium and escape [25]. The ensemble of these events is called immunoediting [26]. T cells are the principal actors of the first two phases (elimination and equilibrium) in which the immune system recognizes and eliminates cancer cells with the result of a complete control of cancer that despite of these mechanisms becomes clinically relevant, when its cells acquire the capacity of escaping the immune

system's control by suppressing it. The phase of elimination starts when the growth and developing of cancer's tissues induces the release of cytokines which make elements of the innate compartment (NK, $\gamma\delta$ -cells and macrophages) to interact with the surface of the tumor itself. The resulting production of IFN- γ and IL-12 (with a positive feedback) leads to the tumor killing by suppression of its proliferation and neoangiogenesis and conduces its cells to apoptosis. In addition, macrophages and NK cells activated by IFN- γ , kill tumor cells thus giving the start for the adaptive compartment. The dendritic cells (DCs) activated by cytokines or directly by NKs are located in the tumor site [27]. After the ingestion of cancer cells' components, they migrate to local lymph nodes, where they activate cancer-specific Th1 (CD4) with the result of the recruitment of cancer-specific CD8⁺ CTLs. Th1 CD4⁺ and CD8⁺ lymphocytes migrates in the cancer site and kill cancer cells. For these reasons, the presence of a T cells infiltration is considered as the 'primum movens' of the game and it has been also demonstrated that in the case of ovarian cancer, it has a prognostic significance [28]. In addition, CD3⁺ CD56⁺ cells, containing the NK-like T cytotoxic cells, play a role in this phase and their finding in the ascitic fluid in ovarian cancer patients seems to correlate with better prognosis [29]. The second stage, called equilibrium, can last many years. During this long period tumor cell clones, characterized by a high genetic instability, mutate generating new antigenic variants without a complete control by the immune system. Escaping is the third phase of the game in which cancer cells evade immune system and become immunologically autonomous and it could be explained by the presence of T lymphocytes with immune-suppressive functions (Treg) and with tumor's production of inhibiting cytokines like IL-10 and TGF- β . Lower levels of immunogenicity in the phase of escape are also probably due to a reduced sensitivity to IFN- γ , a loss of MHC in tumor's surface and a higher level of NKG2D ligands (MICB) [30,31]. The presence of a high count of Treg cells has been correlated with a reduced level of IL-2, IFN- γ and TNF- β [32]. Other mechanisms of immune-escaping in ovarian cancer were found in a study by Raspollini *et al.* in which the presence of $\gamma\delta$ -T lymphocytes infiltration in 95 advanced ovarian carcinomas, is correlated with a worse prognosis, confirming the immune-inhibiting role of these cells [33]. Clinical studies have also shown efficacy of immunotherapies already used for other malignancies, in patients with epithelial ovarian cancer that is interleukin-2 (IL-2) and CTLA-4 antibody [34,35]. These and other data available in literature confirm the strong connection between the immune system and the natural history of epithelial ovarian carcinomas: on the basis of such evidences it seems that therapeutic options for this malignancy could represent a valid alternative after conventional chemotherapy regimens.

Many studies have demonstrated spontaneous antitumor immune responses in patients with ovarian cancers, through

the identification of tumor-reactive T cells and antibodies in peripheral blood [36,37] and tumor-reactive T cells in tumors or ascites [38-45], suggesting that ovarian cancers are intrinsically immunogenic.

It has been observed that in ovarian cancer, the presence of antitumor immune response by intratumoral infiltrating T lymphocytes was associated with significantly improved survival in patients with a complete clinical response after debulking and platinum-based therapy. This advantage has been demonstrated in 2003 by Zhang *et al.* who analyzed 186 frozen specimens from advanced-stage ovarian carcinomas to assess the distribution of tumor-infiltrating T cells (tumor-infiltrating lymphocytes, TILs) with immunohistochemical assays and correlated these data with survival and PFS. In 58% of the specimens CD3⁺ tumor-infiltrating T cells were detected, and the 5 years OS rate was significantly superior for patients whose tumors had lymphocytic infiltration (38 vs 4.5%, $p < 0.001$). In addition, a substantial advantage in 4 years - PFS rate has been found for patients with intratumoral T cells infiltrations (31 vs 8.7%, $p < 0.001$). They also observed an association between presence of TILs and the percentage of complete surgical debulking, thus suggesting that a good host immune response can confine tumor's expansion and infiltration [46]. Several studies have shown a prevalence of intraepithelial T cells in tumors with increased proliferation, indicating that improved outcome is not due to indolent tumors [47].

The intratumor presence of effector T cells, helper and cytotoxic, is associated with improved survival in patients with higher numbers of intraepithelial CD8⁺ T cells compared with patients without intraepithelial CD8 cytotoxic T cells (median survival 55 vs 26 months) [48,49]. In contrast to CD8 cytotoxic T cells, the role of CD4 helper T cell infiltration is less clear, in fact similar outcomes have been observed among patients with or without CD4⁺ T cell staining of tumors [48,50]. Moreover, high levels of IL-17 are associated with greatly improved outcome suggesting that a subset of CD4 Th cells, called Th17 and producing IL-17, may have a direct role in eradicating tumors [51]. The presence of blood NK cell activity in ovarian cancer patients before surgery is considered a predictive factor of improved PFS, whereas an increase of NK cells in peritoneal and pleural fluids of metastatic ovarian cancer suggests poorer prognosis [52,53]. Although evidence of antibody responses to ovarian cancer has been shown studies evaluating whether B cell infiltration is associated with improved survival show unclear results [37,50,53-55].

According to the all above reported observations, ovarian cancers should no longer be considered immunologically inert tumors. Pilot clinical data indicate that patients with ovarian cancer can in fact respond to the same immunotherapy approaches as patients with other immunogenic tumors, such as melanoma [56]. In the tumor milieu, however, 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, tumor cells' genetic changes allow them to be ignored by the immune response. Immune suppression is mediated by factors released from the tumor or by lymphoid or myeloid-derived suppressor cells (MDSCs) or regulatory cells that infiltrate the tumors. Among these, CD4 Tregs have an important role in the immune evasion of ovarian cancers.

Tregs are a heterogeneous T-cell subpopulation whose primary function is immune regulation by blocking both the function and the proliferation of activated T cells through immune-suppressive soluble mediators such as TGF- β and IL-10 (induced or adaptive Tregs) or by means of cytokine- or contact-dependent mechanisms (natural Treg cells) [57,58]. Tumors can recruit or induce Treg tumor infiltration as shown by numerous studies that demonstrate intratumoral localization of Tregs in several human cancers; besides, several tumor types, including ovarian, can increase the numbers of Tregs in the peripheral blood of cancer patients [59-61].

Curiel *et al.* evaluated the role of regulatory CD4⁺CD25⁺FOXP3⁺ T cells (Treg) in 104 patients with ovarian cancer. They demonstrated that the exaggerated suppression of tumor-associated antigen-reactive lymphocytes mediated by Treg cells may cause the loss of host antineoplastic immunity and they also showed that increased tumor Treg cells' amount is associated with a reduced patients' survival [62]. Moreover, Wolf *et al.* showed improved survival in patients with low levels of intratumoral Foxp3 Tregs versus patients with high levels (77 vs 30 months) [63].

DCs, involved in T-cell activation, are not activated by tumors; human ovarian cancers contain either DC or their precursors, which could promote both angiogenesis and vasculogenesis during tumor growth [64-67]. In particular, the presence of DC expressing B7-H1 is associated with poor overall survival (OS) in ovarian cancer, probably by directly inhibiting T cell proliferation and by promoting the induction of FoxP3⁺ Tregs as well [68-71]. Thus, the DC population could represent a therapeutic target in ovarian cancer; in this regard, recent murine modeling studies demonstrate improved anti-tumor immunity following specific depletion of DCs [72].

Even neutrophils could have a potential immune deregulating role in ovarian cancer. A study carried out by Klink and colleagues evaluated the interactions between neutrophils and ovarian cancer cells [73] and recent studies showed that elevated neutrophil-to-lymphocyte ratio [NLR] is an independent prognostic factor associated with an increase in disease recurrence in several cancers [74-78]. Cho *et al.* showed that patients with advanced ovarian cancer and high preoperative NLR had decreased OS compared to patients with low NLR [75]. In the future, the neutrophils may become targets for immune-based therapies for advanced and metastatic ovarian cancer.

3. Results of clinical trials on immunotherapy for recurrent ovarian cancer

Although the improvement in clinical outcome from surgery and chemotherapy in ovarian cancer patients, the most of the patients relapse after first-line therapy. The evidence of immunogenicity for ovarian cancer prompted innovative immunotherapy for recurrent ovarian cancer to extend the survival of these patients.

Adoptive immunotherapy is one of the most studied kinds of immunotherapy. Cancer immunotherapy is dependent on the presence of TILs with appropriate homing and effector functions. Adoptive T cell immunotherapeutic strategies utilizing naturally occurring tumor-reactive T cells are limited by the availability of such T cells for administration and the downregulation of MHC Class I molecules and antigen processing machinery by the tumor. To overcome this problem chimeric antigen receptor (CAR)-T lymphocytes were used in preclinical and clinical studies. CARs bypass a common immune evasion mechanism of tumor cells, the downregulation of MHC-I and antigen presentation, and provide to use engineered T cells without MHC restriction and with potent costimulatory signals. A Phase I clinical trial studied the safety, feasibility and preliminary activity of the adoptive transfer of autologous T cells transduced with CAR recognizing the folate receptor-alpha (FR α), and carrying the CD3 ζ domain along with the 4-1BB costimulatory signaling domain to address the issue of persistence of FR α -specific CAR-T cells [79]. Enrolled patients had FR α -positive epithelial ovarian carcinoma stages II – IV relapsed after two or more chemotherapy regimens. Folate receptor is a target antigen expressed in 90% of epithelial ovarian carcinoma and its expression is not significantly altered even after chemotherapy, for these reasons it appears to be a safe target. All enrolled subjects received untransduced autologous peripheral blood lymphocytes intravenously to contain the exponential expansion of CAR-T cells. This study is actually ongoing. Previous findings in CLL patients showed no acute toxic effects and after 6 months from the infusion CAR-T cells persisted at high level in the blood and bone marrow continued to express the CAR.

A recent study evaluated in recurrent ovarian cancer patients a combinatorial approach encompassing DC-based autologous whole tumor vaccination and anti-angiogenesis therapy, combined with subsequent adoptive transfer of autologous vaccine-primed CD3/CD28-co-stimulated lymphocytes. Tumor lysate was obtained from prior cytoreductive surgery treatment. Subsequently intravenous bevacizumab and oral metronomic cyclophosphamide were delivered, followed by bevacizumab plus vaccination with DCs pulsed with autologous tumor cell lysate supernatants, lymphodepletion and transfer of autologous vaccine-primed T cells. Six patients receiving this vaccine showed a good tolerability. In four patients antitumor immune response was demonstrated,

and they experienced clinical benefits. Lymphodepletion and adoptive T cell transfer was applied in three out of these four patients. It resulted in a durable reduction of circulating regulatory T cells and increased CD8⁺ lymphocyte counts [80].

A Phase II study tested a maintenance immunotherapy based on IL-2 and 13-cis-retinoic acid (RA) that improved the tumor associated immunodeficiency and decreased vascular endothelial growth factor (VEGF), which is a cytokine that negatively influences the function of the immune system and it is associated with poor OS. IL-2 has pleiotropic activities on cell-mediated and humoral immunity, improving T cell proliferation, increasing the generation of cytotoxic T lymphocytes (CTL) and inducing both the activation of T and B cells and the activity of natural killer cells (NK). Retinoids increase IL-2 receptors and peripheral blood lymphoid cells expressing surface markers for T-helper cells, and cooperate with IL-2 to increase the production of IFN- γ . After 6 months of immunotherapy, the lymphocyte count, the number of NK cells and the CD4⁺/CD8⁺ ratio increased in patients with a partial response and stable disease. Patients with disease progression did not show any change in lymphocyte count. NK cells and the CD4⁺/CD8⁺ values continued to be high after 1 and 5 years only in patients who were progression-free. The therapy substantially decreased VEGF levels, also after 5 years. The progression was accompanied by a substantial decrease of lymphocyte and NK count and by increase of VEGF. After a median follow-up of 46.9 months (minimum, 34 months for living patients), 29 patients were alive and 21 patients were progression-free. The median PFS was 23.2 months, and the median OS was 52.8 months [81].

In another Phase II study, IL-2 is used to promote NK cell expansion. It evaluated the tumor response and *in vivo* expansion of allogeneic NK cells in recurrent ovarian and breast cancer. Twenty patients (14 ovarian, 6 breast) were first treated with fludarabine and cyclophosphamide or radiotherapy to increase a lymphodepletion. NK cells were taken from a haplo-identical related donor and incubated with IL-2 before infusion. In the weeks after, a subcutaneous IL-2 infusion was given to promote the NK cell expansion. They obtained a mean NK cell dose and donor DNA was detected 7 days after NK cell infusion, especially in patients that underwent to depletive radiotherapy (85 vs 69%). After 14 days, regulatory T cells ($p = 0.03$) and serum IL-15 levels ($p \leq 0.001$) were increased. Studies to increase *in vivo* NK cell persistence and expansion are still needed [82].

A Phase I/II study considered therapeutic effects of adoptively transferred IL-10- and IFN- γ -producing CD4 effector cells in patients with recurrent ovarian cancer. Autologous CD4 effector cells were stimulated *ex vivo* by MUC1-peptide and IL-2 and then intraperitoneally reinfused. One out of four enrolled patients with recurrent disease was disease-free after 16 months. In patients with long survival high levels of systemic CD3(+)/CD4(+)/CD25(+) and CD3(+)/CD4(+)/CD25(-) T cells were found. Such cell populations among these patients contained variable levels of 'Inducible' Tr1

[CD4(+)/CD25(-)/FoxP3(-)/IL-10(+)] and 'Natural' [CD4(+)/CD25(+)/CD45RO(+)/FoxP3(+)] regulatory T cell numbers and ratios that were associated with prolonged and/or disease-free survival. Restimulated T cells have a higher survival and apoptosis-inducing activities because of the increase of IFN- γ production and select TNF family ligands. The results of the study suggest that the immunotherapy administered could stimulate differential systemic regulatory T cell subpopulations that contribute to long-term tumor immunity and enhanced memory/effector CD4-mediated therapeutic potentials [83].

Since one of the most common ovarian cancer localizations is the peritoneal cavity, different studies exploited intraperitoneal (IP) delivery for immunotherapy. A pilot study enrolled seven patients with recurrent ovarian cancer confined to the peritoneal cavity to test the toxicity and feasibility of IP infusion of tumor-specific CTL. After leukapheresis precursor lymphocytes were collected and stimulated *in vitro* with MUC1. IP infusion was well tolerated and no toxic events were reported. Survival ranged 2 – 6 months with no evidence of disease. Survival did not correlate with the number of infusions. Not statistically significant CA125 reduction was observed just after the first month of immunotherapy, but it increased then. The immune activated T cells are induced only after the first cycle, but there was not any increasing of it after the successive cycles. Multiple cycles of immunotherapy seems to be not necessary [84].

Malignant ascites is frequently observed in association with peritoneal localization. In the 90% of ascites fluid the ovarian tumor cells overexpress the epithelial cell adhesion molecule (EpCAM), which is not expressed in the other peritoneal cells. For this reason, it could be a good target for IP immunotherapy. Catumaxomab (anti-EpCAM \times anti-CD3) is a trifunctional monoclonal antibody with two different specificities binding simultaneously to EpCAM on tumor cells and the CD3-antigen on T cells. Moreover, this monoclonal antibody selectively binds to human Fc γ I and III-receptors on accessory cells, but not to the inhibitory Fc γ II receptor expressed on B cells. These binding specificities of catumaxomab induce a simultaneous activation of different immune cell types at the tumor site, resulting in an antitumor activity. A multicenter Phase I/II study investigated tolerability and efficacy of IP catumaxomab infusion in recurrent ovarian cancer patients. Twenty-three patients, with symptomatic malignant ascites refractory to previous treatments, were enrolled after the confirmation of EpCAM overexpression on tumor cells. Safety profile appeared acceptable. The immunoreactions against tumor cells induced cytokine release, which was responsible of adverse event. The most frequent side effects were: fever (83%), nausea (61%) and vomiting (57%). Catumaxomab induced effective tumor cell destruction, as demonstrated by a reduction of EpCAM positive tumor cells in ascites fluid, with decreased ascites accumulation [85]. More recently a randomized Phase II/III trial showed similar data. The subjects were randomized (2:1) to paracentesis plus IP

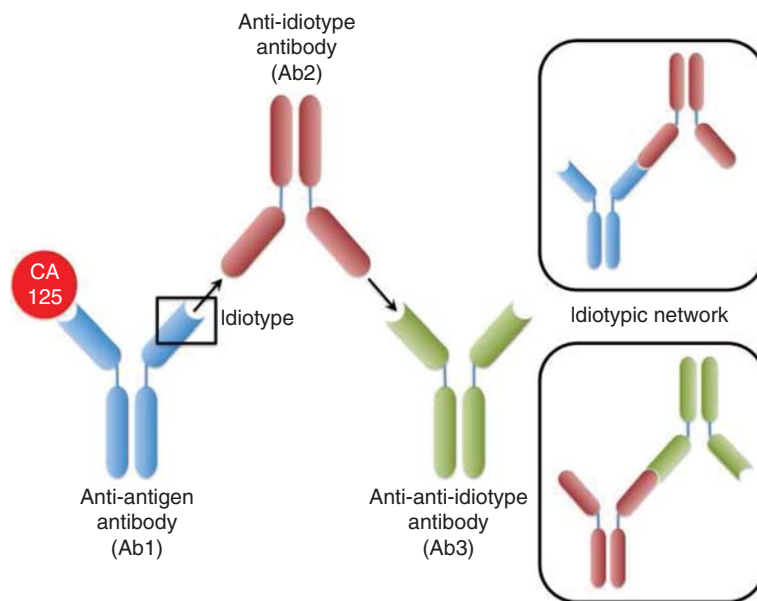


Figure 1. The immune network hypothesis. The immunization with an antigen (i.e., CA125) generates an anti-antigen antibody called Ab1, which induces the formation of an anti-idiotype antibody (Ab2). Its antigenic determinant mimics the first antigen. So it could be used to generate a third antibody Ab3 also called Ab1' which recognizes the original antigen identified by Ab1.

catumaxomab or paracentesis alone. The patients in the paracentesis plus catumaxomab group experienced a statistically significant prolongation of puncture-free-survival and of the time to next paracentesis. Moreover, it was observed a reduction of both symptoms and ascites, and also the level of EpCAM positive cells in the ascites fluid was reduced. OS, a secondary endpoint, was prolonged in about 50% of the patients treated with catumaxomab [86].

Over the last decade anti-idiotypic monoclonal antibodies were studied as anti-cancer immunotherapy. The term idiotype (Id) means the typical antigenic determinant of the antibody variable part region that allows distinguishing Ig binding to different antigens. Its most immediate application regards the targeting of tumor antigens, to which the Id is directed. On these bases anti-Id antibodies were developed to mimic tumor antigens. This strategy provided a new opportunity for the treatment of advanced solid tumor including ovarian cancer.

According to the 'immune network hypothesis' epitopes, the part of the antigens binding antibodies, are transformed in Id-determinants expressed on antibodies [87]. So an antibody takes at the same time the role of an antigen. In particular, the immunization with an antigen generates an antibody called Ab1. The latter generates Ab2 antibodies, which will have an antigenic determinant that mimics the first antigen and, therefore, may be used as its surrogates. Thus, immunization with Ab2 can generate a third antibody Ab3 also called Ab1' because it recognizes the original antigen identified by Ab1 (Figure 1) [88].

The tumor-associated antigen CA125, frequently expressed in ovarian cancer, is an interesting target for two monoclonal antibodies, Abagovomab and Oregovomab. Abagovomab is a murine monoclonal anti-idiotypic antibody directed against the Ca125 antigen. The first Phase I/II multicenter trial addressed both safety and immunogenicity of abagovomab, enrolled 119 women affected by ovarian cancer, carcinoma of the fallopian tube, peritoneal carcinoma, CA125 positive malignant tumor and relapsed after debulking surgery and platinum-based chemotherapy. The treatment induced in 81 out of 119 patients an anti-anti-idiotypic immune response (Ab3), but the development of Ca125 specific antibodies and antibody-dependent cell-mediated cytotoxicity of Ca125-positive tumor cells were also reported. The treatment was well tolerated with no serious adverse events. The OS was 19.4 months, but the group of patients which produced a specific immune response (Ab3) showed a significantly improvement of OS (23.4 months; $p < 0.0001$) [89]. The MIMOSA trial, a randomized, double-blind, placebo-controlled, multicenter trial, was designed to test Abagovomab as maintenance therapy in epithelial ovarian cancer patients, primary peritoneal cancer or fallopian tube cancer, with a first clinical remission. Although the anti-idiotypic antibody vaccine ACA125 resulted safe and induced an immune response, no statistical difference were observed in RFS and OS between the abagovomab-treated and placebo. These recent data are in contrast to the results of the previous Phase II study [90,91].

In addition, oregovomab was studied as maintenance mono-immunotherapy after front-line therapy in a Phase III

Table 1. All studies, including translational researches and Phase I – III trials, investigating immunological effects and clinical outcomes of immunotherapy in recurrent ovarian cancer are summarized.

Citation	Treatment	Phase	Immunologic effect	Clinical outcomes
Kandalaf et al. 2012 [79]	Adoptive transfer of CAR-T cells	I	CAR-T cells persisted at high level in the blood and bone marrow continued to express the CAR	N/A
Kandalaf et al. 2013 [80]	DC-based vaccination and anti-angiogenesis therapy, with subsequent adoptive transfer of autologous vaccine-primed CD3/CD28-co-stimulated lymphocytes	Translational	Durable reduction of circulating regulatory T cells and increased CD8 ⁺ lymphocyte counts	N/A
Recchia et al. 2010 [81]	Interleukin-2 (IL-2) and 13-cis-retinoic acid (RA)	II	The lymphocyte count, the number of NK cells and the CD4 ⁺ /CD8 ⁺ ratio increased	mPFS: 23.2 months and mOS: 52.8 months
Geller et al. 2011 [82]	Infusion of NK cells incubated with IL-2	II	Regulatory T cells ($p = 0.03$) and serum IL-15 levels ($p \leq 0.001$) were increased	N/A
Dobrzanski et al. 2012 [83]	Adoptively transferred IL-10- and IFN- γ -producing CD4 T cells	I/II	The immunotherapy administered could stimulate differential systemic regulatory T cells	N/A
Wright et al. 2012 [84]	IP infusion of tumor-specific CTL	Translational	The activated T cells are induced only after the first cycle, but there was not any increase after the successive cycles	mOS: 11.5 months
Burges et al. 2007 [85]	Intraperitoneal immunotherapy with catumaxomab, a trifunctional monoclonal antibody (anti-EpCAM \times anti-CD3)	I/II	The level of EpCAM positive cells in the ascites fluid was reduced	Reduction of symptoms; OS was prolonged in about 50% of pts
Reinartz et al. 2004 [89]	Abagovomab, a monoclonal anti-idiotypic antibody against Ca125	II	An anti-anti-idiotypic immune response (Ab3) was induced, with Ca125 specific antibodies and ADCC of Ca125-positive tumor cells	Improvement of OS: 23.4 months
Grisham et al. 2011 [90]	Abagovomab, a monoclonal anti-idiotypic antibody against Ca125	III	Immunologic response similar to those observed in previous trials	No statistical difference in RFS and OS
Berek et al. 2009 [92]	Oregovomab, as maintenance mono-immunotherapy after front-line therapy	III	Elicited tumor-antigen specific T cell immunity	No differences in PFS and time-to-relapse
Braly et al. 2009 [95]	Oregovomab with carboplatin-paclitaxel	II	The immune responses were stronger than maintenance monoimmunotherapy	N/A

randomized, double-blind, placebo-controlled trial [92]. Ovarian cancer patients with high serum levels of Ca125 were treated with oregovomab, but no clinically effective benefit was observed. In fact after a 5-year follow-up there were no differences in PFS and time to relapse in these two treatment arms [93]. Although this clinical results oregovomab elicited tumor-antigen specific T-cell immunity according with the data of a pilot Phase II study [94].

A stronger immune response was induced by oregovomab if it is delivered with front-line chemotherapy (carboplatin-paclitaxel) in advanced ovarian cancer patients. A Phase II

study investigated the immune adjuvant properties of front-line chemotherapy when combined with oregovomab immunotherapy [95].

Recently, a recombinant fusion protein composed of an anti-idiotypic single chain mimicking CA125 connected with tuftsin by an artificial linker was constructed. This molecule was tested in mice. It showed stronger immunogenicity triggering humoral and cellular immune responses, inducing enhanced production of anti-anti-idiotypic antibodies and T cell response. Subsequently, a protection against tumor challenges may be achieved, so that the administration of

fusion proteins composed of anti-idiotypic antibodies and tuftsin could be employed as cancer immunotherapy [96].

Table 1 summarizes all the studies reported above about the role of immunotherapy in recurrent ovarian cancer patients.

4. Expert opinion

Ovarian cancer treatment is still one of the most relevant challenges of clinical oncology. In fact ovarian cancer of epithelial origin is usually diagnosed late, when regional or distant metastases have been already arisen. To date the standard treatment for advanced ovarian cancer is represented by the combination of carboplatin and paclitaxel. When this treatment fails because of cancer progression, further treatment options are chosen according to the treatment-free interval, which is useful to define platinum sensitivity or resistance. Various chemotherapy regimens are now available to deal with platinum resistance. These options allowed reaching better disease control against supportive care.

However, new treatment strategies different from chemotherapy have been considered to improve disease control, to increase OS and to offer an effective therapeutic option to those patients who developed resistance to all the chemotherapeutic agents actually available. Among these new treatment strategies immunotherapy represents one of the most controversial. In fact it has been developed since immunologic features were identified in tumor tissue and in the blood of cancer patients. Ovarian cancer also showed to elicit immune response as supported by some evidences. These include the tumor-associated antigen expression, which could take the role of targets for both humoral and cellular immune response; TILs within the tumor tissue, which are related to prognosis; immune-suppressive cytokines and Tregs in the peripheral blood, which are associated with worse prognosis.

Some studies evaluated immunotherapy as adjuvant treatment after surgery. In fact the real aim of vaccination is to prevent the recurrence of cancer during a quiescent phase.

However, many studies evaluated immunotherapeutic approaches as further treatment option after failure of standard chemotherapeutic regimens. In this perspective, many researchers are trying to answer the question about the most effective role of immunotherapy in the whole strategy to improve quality of life and survival of the advanced ovarian cancer patients. In other words we try to clarify whether immunotherapy could change the therapeutic landscape for these patients. We reported a wide analysis about the actual landscape of immunotherapy developed and studied for recurrent ovarian cancer. On the basis of this analysis, we could conclude that interesting treatment options have been developed. However, not relevant clinical results were observed, but important changes in immunological features have been observed as a consequence of these treatments. In particular, IL-2 treatment with RA achieved interesting high median PFS and OS and effects on immune cells are associated with prolonged progression-free time. In addition,

catumaxomab obtained prolonged OS, even though symptom and ascites reduction was the real aim of this study. For anti-Id immunotherapy an improvement in OS was observed in patients who produced Ab3 specific immune response as reported in the first Phase I/II trial with abagovomab. This finding was not confirmed in subsequent trials. In the other studies, the development of immune response related to the kind of immunologic strategy was observed, but clinical endpoints were not clearly defined. In our opinion to achieve significant benefit in common clinical endpoints, such as PFS and OS, the proper clinical setting, the combination with appropriate cytotoxic treatment and the optimal phase of disease natural history should be defined. To date the available trials for immunotherapy in ovarian cancer patients tried to test the ability to develop specific immunologic changes and concomitantly affect on common clinical endpoints were reported. Specific translational studies are needed to establish when an immune effect could be translated in improvement of PFS and OS. The effect of cytotoxic drugs on immune response development by specific strategies has not been discovered yet.

However the findings that OS could be improved in those patients experiencing immunologic activation as a consequence of immunotherapy, let us argue that patients who receive these treatments have to be selected. A possible way to explore this field could consider the prognostic classification according with the development of immune response during immunotherapy. For those patients who do not experience significant immunologic changes, immune escape mechanisms should be evaluated to identify further immunotherapy strategies. The main role for this escape could be attributed to regulatory T cells, but other mechanisms have to be considered involving co-stimulatory molecules (CD28/B7, CTLA-4/B7, PD1/PD-L1, PD1/PD-L2, ICOS/ICOS-L, CD27/CD70, CD30/CD30L, CD40L/CD40, OX40/OX40L, GITR/GITRL, TIM family molecules) [97]. In fact as we have found that antitumor immune response could fail because of immune escape, a specific treatment option has been developed to overcome these hurdles. The combination of antitumor immune modulation and the overcoming of immune escape mechanisms would represent the new proposal for redesigning the immunotherapy treatment strategy both to prevent and to control ovarian cancer recurrence. In fact the main goal of these treatments in recurrent ovarian cancer is to control the disease for as long time as possible with minimal toxic effects, which could impair quality of life. Immunologic strategies appear to hold this potential but they could be integrated with other strategies to avoid escape. To date interesting results have been achieved in other malignancies by immunomodulation strategies including the anti-CTLA-4, anti-PD1 and anti-PD-L1 moAbs. We argue that in the next years new trials could be designed with these agents in recurrent ovarian cancer. The findings of an anti-CTLA-4 antibody in some previously vaccinated metastatic melanoma and ovarian cancer patients suggest that

CTLA-4 blockade increases tumor immunity [98]. More recently a preclinical study showed that the combination of the blockade of both PD-1 and CTLA-4 with GVAX vaccination induced rejection of tumors in a model of colon and ovarian carcinoma [99]. Actually a Phase II trial is recruiting ovarian cancer patients for monotherapy with the anti-CTLA-4 antibody ipilimumab. Anyway anecdotal data from initial studies about these immunomodulating agents suggest that to achieve a relevant and prolonged clinical benefit the immune escape mechanisms have to be blocked.

5. Conclusions

Anticancer treatment in advanced ovarian malignancy reached high rates of remission. However, the onset of treatment failure is associated with poor outcomes since further therapeutic options achieve little clinical benefit. For this reason researchers are attempting a general change of treatment strategy for

these patients. Since immunologic phenomena in ovarian cancer could impact on outcomes, immune system modulation has been exploited for the widening of treatment chances. Cancer vaccines using both tumor antigens and DCs, adoptive immune cell transfer and anti-Id antibodies are the most interesting immunotherapy strategies studied for recurrent ovarian cancer. The clinical outcomes are limited, but the associated immunization let us argue great therapeutic potential. To date defining its role in the general strategy has not been possible yet. Next studies will improve these clinical results by overcoming immune escape mechanisms. The possibility of using immunotherapy in ovarian cancer is still restricted to clinical trials.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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