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Review

Cellular immunotherapy in ovarian cancer: Targeting the stem of recurrence

Christina Wefers [a,b], Laurens J. Lambert [b,1], Ruurd Torensma [b], Stanleyson V. Hato [b,*]

[a] Department of Obstetrics and Gynecology, Radboud University Medical Center, Nijmegen, The Netherlands
[b] Department of Tumor Immunology, Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands

HIGHLIGHTS

- Review immune system in ovarian cancer and state of cellular immunotherapy
- Present cancer stem cells as targets for clinical application of immunotherapy
- Postulate the use of adjuvant DC vaccination to complement current treatment

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ABSTRACT

Ovarian cancer is a devastating disease with a high relapse rate. Due to a mostly asymptomatic early stage and lack of early diagnostic tools, the disease is usually diagnosed in a late stage. Surgery and chemotherapy with taxanes and platinum compounds are very effective in reducing tumor burden. However, relapses occur frequently and there is a lack of credible second-line options. Therefore, new treatment modalities are eagerly awaited. The presence and influx of immune cells in the ovarian cancer tumor microenvironment are correlated with survival. High numbers of infiltrating T cells correlate with improved progression free and overall survival, while the presence of regulatory T cells and expression of T cell inhibitory molecules is correlated with a poor prognosis. These data indicate that immunotherapy, especially cell-based immunotherapy could be a promising novel addition to the treatment of ovarian cancer. Here, we review the available data on the immune contexture surrounding ovarian cancer and discuss novel strategies and targets for immunotherapy in ovarian cancer. In the end the addition of immunotherapy to existing therapeutic options could lead to a great improvement in the outcome of ovarian cancer, especially when targeting cancer stem cells.

Contents

* Corresponding author at: Department of Tumor Immunology, Institute for Molecular Life Sciences, Radboud University Medical Centre, 6500 HB Nijmegen, The Netherlands.
E-mail address: Stanleyson.Hato@radboudumc.nl (S.V. Hato).
[1] Current address: Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142, USA.

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Introduction

Epithelial ovarian cancer (EOC) is the most lethal gynecological cancer. Approximately 22,000 new cases of ovarian cancer were diagnosed in 2014 in the US alone. Annually, ovarian cancer results in over 14,000 deaths [1]. Over the last 20 years only a small decrease in those figures was seen. The median age at the time of diagnosis is 63 years [2], although women with a high risk genetic predisposition typically develop ovarian cancer 10 years earlier [3]. Due to a lack of reliable screening tools in the early phase of the disease, which is usually asymptomatic, more than 75% of patients are diagnosed in an advanced stage International Federation of Gynecology and Obstetrics (FIGO) stages III–IV [4].

The current standard for first-line therapy of ovarian cancer consists of cytoreductive surgery and adjuvant chemotherapy based on platinum drugs in combination with Taxanes. Unfortunately, a large proportion of patients (20–40%) do not respond to first-line chemotherapy [2]. Furthermore, recurrence rates are 25% in early stage patients and higher than 80% in advanced stage patients [4]. The median survival time of patients with advanced stage disease is 65 months. Current second-line therapies are generally not curative, resulting in short-term progression-free survival for most patients [2]. The route of administration of adjuvant chemotherapy also has an effect on therapy outcome. Intraperitoneal chemotherapy leads to a longer progression-free and overall survival compared to intravenous chemotherapy. However, it is not widely adopted due to toxic side effects, intraperitoneal delivery problems and other complications [5]. Intensification of the treatment by addition of a third chemotherapeutic or extended duration of platinum or Taxane chemotherapy showed no further improvements in clinical outcome in phase 3 trials. The sequence of treatment modalities was also studied. Initial treatment with chemotherapy (neoadjuvant therapy) followed by surgical cytoreduction had no clinical benefit over surgical cytoreduction followed by chemotherapy [2].

Looking back at the last 30 years, no substantial decrease in death rates has been achieved. Thus, there is a desperate need for novel treatment strategies for ovarian cancer. One such strategy involves activating the patient's own immune system for therapeutic benefit in cancer, referred to as immunotherapy [6]. Immunotherapy has shown considerable clinical promise in recent years and has the potential for a life-long cure of cancer. In this review, we examine the immunological contexture in ovarian cancer, evaluate the clinical promise of immunotherapeutic approaches and discuss innovative combination modalities.

Rationale for cellular immunotherapy

Although not considered as an 'immunogenic' tumor such as melanoma, clinical evidence hints at a role for the immune system in EOC. Infiltration of $CD3^+$ immune cells in ovarian tumors correlates with improved progression free survival [7], suggesting that ovarian cancer is vulnerable to immunological attack. The presence and activation state of other immune effector cells such as Natural Killer (NK) cells and γδ-T cells also correlate with improved clinical outcomes. There is accumulating evidence suggesting that surgery and chemotherapy also modulate the immune system. Surgery can significantly reduce the numbers of suppressive regulatory T cells (Tregs) in EOC patients leading to an improvement in the ratio of CD8/Treg. Additionally, peripheral $CD8^+$ T cells in these patients produce higher levels of IFNγ after surgery [8]. Recent studies demonstrate that chemotherapeutic compounds trigger the immune system [9,10]. These chemotherapeutic compounds, including the platinum-based compounds, can induce tumor cell stress and death that leads to the induction of an anti-tumor immune response. Platinum compounds were also shown to enhance the recognition and killing of tumor cells by immune cells, as well as, enhancing dendritic cell (DC) function [11–13]. Additionally, patients that have $CD3^+$ T cells present in the tumor have improved responses to chemotherapy and are more frequently optimally debulked [7].

Natural killer cells

Natural killer (NK) cells are the cytotoxic cells of the innate immune system, which are involved in the killing of tumor cells. NK cells can be divided in two subsets, based on expression of surface molecule CD56 [14]. $CD56^{bright}$ $CD16^-$ NK cells produce high amounts of cytokines upon activation, but exhibit low cytotoxicity. $CD56^{dim}$ $CD16^+$ NK cells produce low amounts of cytokines, but exhibit high cytotoxicity and the ability to mediate antibody-dependent cellular cytotoxicity (ADDC) through CD16. NK cells recognize and eliminate allogeneic or stressed cells, such as infected or tumor cells [14]. EOC exploits various mechanisms to limit NK cell-mediated tumor killing. Firstly, low numbers of NK cells infiltrate primary EOC [7]. Secondly, infiltrating NK cells are enriched for the less cytotoxic $CD56^{bright}$ cells compared to autologous peripheral blood (32% versus 10%) [15]. Furthermore, EOC also suppresses NK cells through the expression of surface molecules or secretion of soluble factors like CA-125, which shields tumor cells from cytotoxicity [16,17]. Surprisingly, the presence of $CD16^+$ NK cells was significantly correlated with decreased overall survival of ovarian cancer patients [18]. In summary, although it is still unclear whether the presence of NK cells has a beneficial effect on the outcome of EOC, there are several mechanisms in the tumor microenvironment that abolish NK cell anti-tumor immunity.

$CD8^+$ T cells

$CD8^+$ T cells are the cytotoxic effector cells of the adaptive immune system responsible for killing of tumor cells. A seminal study by Zhang and colleagues showed that the presence of tumor-infiltrating lymphocytes (TILs) correlates with favorable clinical outcome [7]. The median progression-free survival of these patients was 22.4 months and median overall survival 50.3 months. In contrast, patients without TILs had a median progression-free survival of 5.8 months and a median overall survival of 18 months. Only a small percentage (4.5%) of these survived up to 5 years, whereas this percentage was significantly higher (38%) in

patients with TILs. This demonstrates that infiltrating T cells in EOC is a strong prognostic factor for favorable clinical outcome. Later the $CD3^+$ T cell population was further refined to $CD3^+$ $CD8^+$ cytotoxic T cells [19]. Since then, other studies and meta-analyses have confirmed that the presence of TILs is a predictor of favorable clinical outcome in EOC [20,21]. This was further corroborated by an immunohistological study of post-surgical ovarian carcinoma biopsies (stages I–IV), which demonstrated that intraepithelial $CD8^+$ T lymphocyte count is a positive prognostic factor [22].

$CD4^+$ Th17 cells

Recently, a number of studies looked at the role of Th17 cells in ovarian cancer. Th17 cells are a subset of $CD4^+$ T cells that secrete a special set of cytokines, including IL-17A, IL-17F, and IL-23. The pro-inflammatory function of Th17 cells in autoimmune diseases has been well established, but their role in cancer is less clear [23]. Th17 cells can be found in both, EOC tumors and malignant ascites [24–26]. Furthermore, Th17 cells are enriched in the tumor compared to tumor-draining lymph nodes, cancer patient peripheral blood and healthy donor peripheral blood [24–26]. IFN-γ and IL-17 produced by Th17 cells attract $CD8^+$ T cells to the tumor microenvironment in a dose-dependent manner. Thus, Th17 cells might have beneficial effects on anti-tumor responses. Indeed, IL-17 production was shown to be a significant predictor for overall ovarian cancer survival, 78 vs. 27 months overall survival for patients with high vs. low levels of IL-17 [25].

Ovarian cancer generates an immunosuppressive microenvironment

As highlighted above, the immune system is able to target EOC tumors and influence overall survival. However, EOC cells can exploit several mechanisms to evade immunological elimination. These include the expression of T cell inhibitory receptors on tumor cells and immune cells and the recruitment of immunosuppressive cells such as Tregs and tumor associated macrophages. This immunosuppressive environment is further augmented in ovarian cancer. Below we will discuss some of the most important suppressive mechanisms in place.

Expression of inhibitory receptors on tumor cells

The presence of TILs is invariantly correlated with improved survival and interestingly, $CD8^+$ T cell count was inversely correlated with the level of expression of programmed cell death ligand 1 (PD-L1 or B7-H1) in ovarian carcinoma tissues. PD-L1 is expressed on a wide range of peripheral tissues and suppresses T cell responses [27]. PD-L2 is expressed on macrophages and dendritic cells [28]. Both ligands induce T cell anergy or apoptosis through binding to PD-1 on the T cells. In patients with high expression of PD-L1, overall 5-year survival rate was lower than in patients with low PD-L1 [22]. PD-L2 expression was not significantly correlated with overall patient survival. Thus, intratumoral T cell infiltration and PD-L1 expression are two independent, opposing prognostic factors for clinical outcome. PD-L1 likely limits $CD8^+$ T cell invasion in the tumorigenic tissues. Currently, the blockade of PD-L1 by monoclonal antibodies is being explored in a clinical trial (NCT01772004).

Regulatory T cells

Classical Tregs are defined by the expression of CD4, CD25 and FoxP3. Under steady state conditions, Tregs limit autoimmune responses through modulation of DC and $CD8^+$ T cell activation [29]. Recently, a novel, $FoxP3^-$ regulatory T cell was described that expresses high levels of PD-L1. This subset was induced by IFN-β, a cytokine secreted in huge amounts by plasmacytoid DCs. It remains to be seen if those Tregs are also found in EOC, but plasmacytoid DCs are abundantly present. Classical Tregs are recruited to and infiltrate solid ovarian tumors and metastatic lesions in ascites [30,31]. A study by Woo and colleagues found that 30% of $CD4^+$ T cells co-expressed CD25 in ovarian tumor samples [30], whereas Tregs represented 23 ± 11% (mean ± s.e.m.) of the tumor-infiltrating $CD4^+$ T cell population [31]. $CD4^+$ $CD25^+$ T cells expressing FoxP3 were able to suppress the proliferation of $CD3^+$ $CD25^-$ T cells in vitro, indicating that the Tregs were fully functional and mature. Furthermore, the presence of Tregs was negatively correlated with overall survival of ovarian cancer patients [31]. Follow-up studies reported conflicting results for Treg influx and overall survival [19,32,33]. In two studies, positive ratios (>1) of $CD8^+$/Treg correlated with better overall survival [19,32], whereas no correlation was found in a third study [33]. The basis for the difference in clinical outcome between these studies is unclear but the discrepancy might be explained by recent data indicating that FoxP3 is also expressed by a number of epithelial tumor cells [34] and by in vitro activated T cells [35].

In the section above, positive correlation between a higher frequency of Th17 cells and improved survival was highlighted. However, the frequency of these cells in EOC tumors decreases with cancer progression, with stage I tumors having the highest and advanced stages having the lowest infiltration of Th17 cells. In contrast, Tregs increase with disease progression and are inversely correlated to Th17 cell number [25,26]. This observation might be explained by the presence of high quantities of TGF-β. Whereas low TGF-β concentrations promote expansion of Th17 cells, high concentrations actually induce differentiation to $FoxP3^+$ Treg cells. Additionally, Tregs directly suppress production of IL-17 [25].

Dendritic cells

Despite their potential to elicit strong anti-tumor immunity, the beneficial effects of DC antigen-presentation are rarely engaged in EOC. Rather, the ovarian tumor microenvironment suppresses DC maturation and function. $CD11c^+$ myeloid DCs (mDCs) isolated from ovarian tumor draining lymph nodes express higher levels of PD-L1 compared to non-tumor lymph nodes and blood monocytes. These $PD\text{-}L1^{high}$ mDCs were impaired in their ability to stimulate T cell responses. Blocking PD-L1 restored T cell proliferation and reduced tumor growth in a murine EOC model [36]. In support of these findings, human ovarian cancer $CD11b^+$ $CD11c^+$ DCs were found to co-express PD-L1 and PD-1 and suppressed T cell proliferation through the PD-L1/PD-1 pathway in a contact-dependent manner [37]. Plasmacytoid DCs (pDCs) were also reported to contribute to immunosuppression in the tumor microenvironment and promote tumor growth, either through promotion of neoangiogenesis or by promoting Treg expansion [38,39]. Conversely, the presence of pDCs in the tumor correlates with poor progression free survival [40].

Tumor-associated macrophages

Macrophages are the phagocytic cells of innate immunity and, similar to DCs, they can also induce T cell activation although they are a lot weaker than DCs in that respect. Macrophages can be broadly divided in two phenotypes: classically activated (M1-polarized) and alternatively activated (M2-polarized) macrophages [41]. M1 macrophages are involved in Th1 immune responses, while M2 macrophages are involved in Th2 responses, which are associated with tumor progression. Tumor-associated macrophages (TAMs) closely resemble M2 macrophages, and are thought to be gradually polarized towards immunosuppressive macrophages during tumor progression [42,43]. TAMs are abundantly present in the ovarian tumor and malignant ascites [43–45]. The number of TAMs significantly correlates with malignancy in serous and mucinous ovarian carcinomas [45]. In the tumor microenvironment TAMs recruit Tregs through the secretion of CCL22 [31,43]. In turn, Tregs also potentiate the suppressive activity of TAMs by the production of IL-10, which induces high expression of the surface molecule

B7-H4 on TAMs [46]. Like its family members, PD-L1 and PD-L2, B7-H4 also negatively regulates T cell responses. The expression level of B7-H4 in ovarian cancer is quite heterogeneous and high expression levels correlate with decreased overall survival of patients [47].

Immunotherapy

As outlined above, there is ample evidence for natural pre-existing, anti-tumor immune responses in ovarian cancer, which are manipulated and suppressed by the tumor microenvironment. The goal of cancer immunotherapy is to initiate an anti-tumor immune response or to re-activate and boost a pre-existing one. Effective rejection of the tumor by the host immune system depends on sufficient numbers of effector cells that infiltrate the tumor and exhibit cytotoxicity towards tumor cells. Cellular immunotherapies have the potential to generate large numbers of effector cells that are directed towards tumor cells. Besides raising large numbers of effector cells, their functional time window has to be expanded. This can be achieved by inhibiting suppressive factors like the inhibitory receptors or suppressive cells.

Tumor-associated antigens as target for cellular immunotherapy

T cell activation involves the presentation of tumor-derived antigens on major-histocompatibility complex (MHC) molecules by DCs. The balance between co-stimulatory and co-inhibitory molecules on the surface of antigen presenting cells (APCs) determines an activating or tolerogenic T cell response. During malignant transformation, tumor cells often start to express tumor-associated antigens (TAA). These are antigens, which are exclusively or preferentially expressed on tumor cells and can elicit a specific host immune response. A number of bona fide ovarian TAAs have been identified, such as HER-2/neu, MAGE-A1, folate binding protein (FBP), p53, NY-ESO-1, EpCAM and CA-125. These antigens have been explored as single agent or combination vaccines in phase I/II trials. The peptide vaccines were generally well tolerated, toxicity was limited to local grade 1 and 2 inflammatory reactions. Although the vaccines led to induction of specific T cells in a significant number of patients, clinical efficacy was lacking [48–52]. However, it should be noted that most of these were very small phase I/II trials, set up to test toxicity and immunological responses. Some of these trials could benefit from larger, placebo-controlled phase III trials. One such promising example is an NY-ESO-1 vaccine containing recombinant vaccinia and fowlpox vectors to further boost an immune response. In the phase II trial involving 22 recurrent ovarian cancer patients, 3 were seropositive for NY-ESO-1 at the start of the trial, and 8 other patients seroconverted during vaccination. Median overall survival was 48 months, which is much longer than the 15 months for patients that lacked immune activation [53].

Cellular immunotherapies

Adoptive cell transfer (ACT) therapy

Early studies established the feasibility of ex vivo expansion of TILs using recombinant IL-2, and persistence and survival of the TILs after re-infusion in patients [54,55]. Inclusion of autologous antigen-pulsed DCs to the ex vivo expansion phase was shown to promote the expansion of tumor-specific effector $CD8^+$ T cells [56,57]. A pilot trial involving 13 recurrent ovarian cancer patients (stages II–IV) tested ACT of TILs after optimal debulking surgery and cisplatin chemotherapy [58]. The control group was not treated with TILs after primary surgery and chemotherapy. Progression-free survival was significantly higher in the TIL-treated group compared to the control group. Even though patients were not randomly assigned to both arms, this trial nonetheless illustrates the potency of ACT TIL therapies for ovarian cancer.

However, ACT can have considerable drawbacks. Some ovarian tumors do not yield sufficient tumor-specific lymphocytes for ex vivo expansion. Additionally, cellular immunotherapy has to overcome tolerance to self-antigens. A strategy to overcome these drawbacks is to genetically engineer T cells to express either tumor-specific T cell receptors (TCRs) or chimeric antigen receptors (CARs) (Fig. 1) [59]. The first approach uses high-affinity TCRs isolated from patients, which are then retrovirally transfected into non-specific T cells. These T cells expressing high affinity tumor-specific TCRs can then be infused in patients. For the second approach, variable regions of antibodies are fused to TCR intracellular activating domains to create a receptor that is capable of activating T cells upon antigen recognition. These CARs can be transfected into T cells and infused in autologous patients.

A number of CAR strategies have been tested in vitro and in vivo settings, although much work still needs to be done in clinical studies (see Supplementary Table 1 for an overview of all clinical trials discussed in this review). T cells from 5 ovarian cancer patients were transduced with *Mov-γ*, a CAR for FBP and stably retained in vitro FBP-specificity after large expansion [60]. Presently, ACT of CAR-modified T cells has been tested in a single phase I trial. CARs were designed by fusion of an anti-folate receptor (FR) antibody to the signaling domain of FcRγ. One cohort of 8 recurrent ovarian cancer patients was treated with 3 cycles of high dose IL-2 administration and allogeneic FR-modified T cell re-infusion. The other cohort of 6 recurrent patients was treated with dual-specific FR-modified T cells that responded to both FR antigen and allogeneic antigens, followed by immunization with allogeneic peripheral blood mononuclear cells (PBMCs) to stimulate T cell proliferation in the patient. Five patients in the first cohort showed grade 3 and 4 toxicities, which were likely caused by high doses of IL-2, the remainder of the patients showed only grade 1 and 2 toxicities [61]. The number of transferred T cells rapidly declined and T cell inhibitory factors accumulated in 3 patients, further limiting an effective immune response. No tumor regression was observed in any patient during the trial indicating that the procedure has to be further optimized. A new phase I trial, based on a refined FRα CAR was initiated to test efficacy and maximum dosage in ovarian cancer patients (stages II–IV) [62]. The trial has not been completed.

Dendritic cell-based vaccines

DCs are highly efficient activators of the adaptive immune response, and play a key role in activating naïve T cells by presenting antigens on MHC I molecules [27]. Two main subsets of DCs in circulation can be distinguished by the expression of cell surface markers: $CD11c^+$ mDCs and $BDCA2^+$ pDCs. The mDCs can be further subdivided into $BDCA1^+$ ($CD1c^+$), $BDCA3^+$, and $CD16^+$ mDCs [63]. DC vaccination involves the isolation of autologous DCs followed by ex vivo loading with TAAs, induction of DC maturation and re-infusion into the patients to initiate a tumor-specific $CD8^+$ T cell response (Fig. 1) [27]. An advantage of this strategy is that it may boost the immunogenicity of otherwise weak TAAs and will induce long-lasting immunologic memory.

Several methods of generating TAA-presenting DCs have been explored as immunotherapy for ovarian cancer. Evidently, the choice of the TAA is one of the most important parameters. Nonetheless, a response against a TAA, even one present on the majority of tumor cells, will probably not lead to complete eradication of the tumor. The tumor cells outnumber the generated CD8 cells by far. Therefore, immunotherapy can only be successful when the bulk of the tumor has been removed by other means. Surgery and/or chemotherapy could be used to this end and then followed up by immunotherapy. Such combination treatments are presently being explored in trials where the bulk of the tumor is removed and then tumor lysate-loaded DCs are infused. The tumor lysate contains TAAs present in the tumor but only the most immunodominant will end up in MHC class I. The disadvantage of using tumor lysate is that the TAA remains unknown.

A number of clinical studies on DC vaccination in ovarian cancer have been performed. Firstly, in a phase I trial, involving 3 advanced

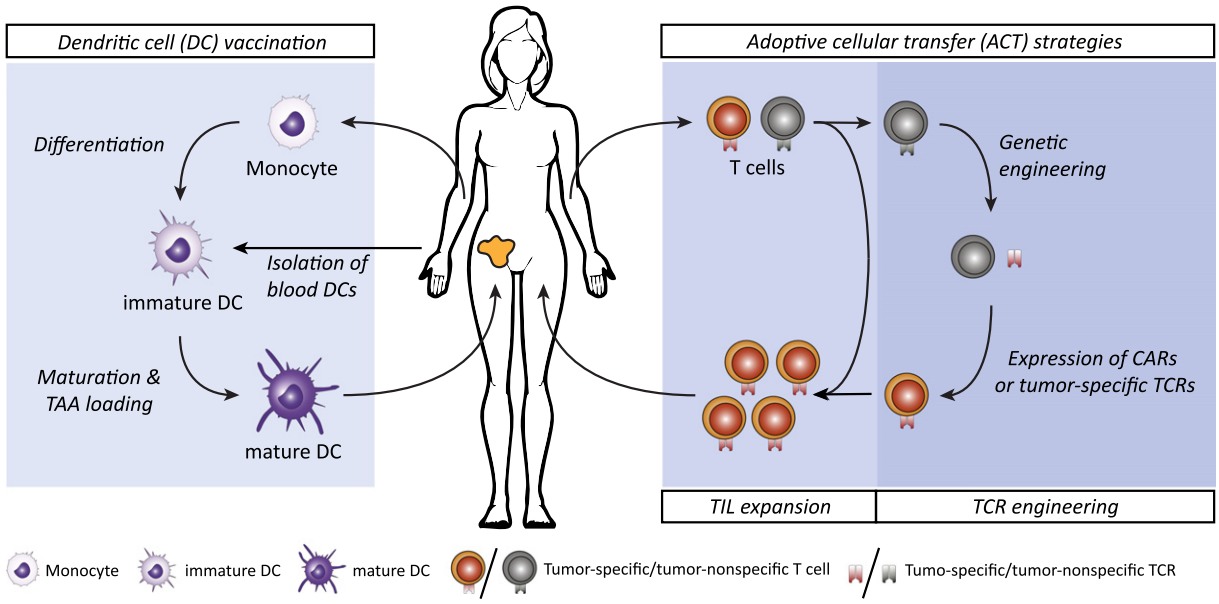

Fig. 1. *Cellular immunotherapy approaches.* There are several ways to employ the immune system in the fight against cancer. For dendritic cell (DC) vaccination, monocytes are isolated from the patient's blood and ex vivo differentiated into immature monocyte-derived DCs. Alternatively, naturally-occurring DCs can be isolated from the blood. The DCs are subsequently matured and loaded with tumor associated antigens (TAA). Both procedures generate tumor-specific DCs, which are re-infused into the patient, where they migrate to the lymph nodes. In the lymph nodes, these DCs will then activate tumor-specific T cells and thus induce a potent anti-tumor immune response. In the case of adoptive cellular transfer (ACT), tumor-infiltrating lymphocytes (TILs) are isolated from the patients. These TILs are expanded ex vivo and re-infused into the patients. If not enough tumor-specific T cells can be isolated from the patient, nonspecific T cells are modified prior to expansion and re-injection. They are either engineered to express a tumor-specific T cell receptor (TCR), isolated from tumor-specific T cells from the same patient, or an artificially constructed chimeric antigen receptor (CAR). Tumor-specific T cells migrate to the tumor were they recognize and kill the cancer cells.

stage ovarian cancer patients, DCs were pulsed with either HER-2/neu or MUC-1 peptides and re-infused in the patient [64]. After 3 cycles of DC vaccination, tumor-specific $CD8^+$ T cells were detectable in 2 patients. In a second phase I trial, involving 6 patients with ovarian cancer, DCs were pulsed with keyhole limpet hemocyanin (KLH) and whole tumor lysates before re-infusion in the patient [65]. The treatment was well tolerated, and 3 patients showed stable disease between 25 to 45 weeks during the study. T cell proliferation in response to KLH and to whole tumor lysate was observed in 6 patients and in 2 patients respectively. In a fourth phase I trial, involving 4 ovarian cancer patients, the HER-2/neu-based DC therapy Lapuleucel-T (Neuvenge, Dendreon) was evaluated for toxicity and disease progression [66]. Administration of Lapuleucel-T was well tolerated. Short-term stable disease was reported for 2 of the 4 ovarian cancer patients in response to DC vaccination (time to progression 18.3 and 15.7 weeks). In a randomized, phase I/II trial that involved 11 ovarian cancer patients (stages Ic–IV), DC therapy was explored as a maintenance therapy [67]. DCs were pulsed with HER-2/neu, hTERT, and PADRE peptides, without intravenous cyclophosphamide (arm 1) or with cyclophosphamide (arm 2) as adjuvant to deplete Tregs. All patients were also immunized with pneumococcal vaccine to additionally boost immune responses. The treatment was well tolerated; no grade 3 or 4 toxicities were reported. A single dose of intravenous cyclophosphamide (300 mg/m^2) did not reduce the number of circulating Tregs in arm 2. Immune responses to DC vaccination were modest; induction of antigen-specific T cell responses was detected in a subset of patients. The estimated 3-year progression-free and overall survival was not significant between treatment arms ($p = 0.17$ respectively $p = 1.00$). In another clinical study, involving 6 recurrent ovarian cancer patients, the combination of DC therapy with a VEGF-inhibitor, bevacizumab, was investigated [68]. DCs were isolated from patients, who subsequently received 2 cycles of bevacizumab followed by seven daily doses of cyclophosphamide. DCs were pulsed with whole tumor lysates and infused in the patient during 3 cycles. Three patients underwent a second trial, consisting of higher doses of cyclophosphamide and fludarabine plus re-infusion of CD3/CD28 stimulated T cells and ex vivo matured DCs. Both treatments were well tolerated. Treg depletion was not observed in response to cyclophosphamide treatments. Four patients responded to the initial treatment with partial responses or stable disease. Two of the three patients who enrolled in the second trial demonstrated a complete response or stable disease.

Another opportunity for DC vaccination in ovarian cancer, which is yet to be explored, is the targeting of cancer stem cells (CSCs). CSCs are cancer cells that have properties similar to normal stem cells, in particular the ability to give rise to all cell types found in a particular cancer type. These cells are generally resistant to chemotherapy and are believed to be the source of tumor relapse. As highlighted in a recent meeting [69] the exclusive property of a stem cell or cancer stem cell is self-renewal. As a consequence of this innate ability, CSCs express stem cell markers like OCT4A, SOX2 and NANOG. Maintaining the expression of these markers is the result of several derailed signaling networks. Without the maintenance of these stem cell proteins, the cell will differentiate and ultimately die. Spheroid cell cultures, which have an enriched CSC population, are far less sensitive to chemotherapy due to several mechanisms. Just as “normal” stem cells, they are mostly quiescent and thus resistant to chemotherapeutic drugs that target DNA replication. Additionally, they also express protein pumps that expel chemotherapeutic drugs [70]. These features ensure that while chemotherapy will kill the majority of the normal tumor cells, the CSCs remain and eventually lead to recurrence.

Unfortunately, eradicating CSCs is not simple. The initial paradigm was that eradicating CSCs would cure cancer as tumor cells would eventually die and not be replenished [71]. Now, it is apparent that more complex factors also play a role. Eradicated CSCs are replaced by more mature ‘stem cells’ that gain access to the vacated CSC niche and acquire self-renewal properties [72]. Thus it seems that even if CSC were susceptible to chemotherapy, this would not be enough to prevent the tumor from coming back. Immunotherapy is a prime candidate to

keep these CSCs in check. After inducing an anti-CSC response the immune system will further react with a memory response. This means that as soon as the antigen re-surfaces, due to the cells that occupy the stem cell niche acquiring stem cell features, these memory cells will become effector cells and eliminate the CSC. This is the same memory mechanism that is active against pathogenic microorganisms and forms the basis of therapeutic and prophylactic vaccination against pathogens. In ovarian cancer, OCT4A expression was found in a rare cancer cell population, akin to CSCs [73]. Moreover, T cell reactivity towards OCT4 was demonstrated, making OCKT4A an attractive and feasible target for vaccination strategies [74]. Taken together, DC therapy is feasible and well tolerated for a number of tumor antigens. Preliminary clinical results in phase I/II trials are encouraging, but larger randomized trials are needed to further improve dendritic cell immunotherapies. Finally, there is still the intriguing yet unexplored strategy of targeting CSCs with DC vaccination.

Summarizing conclusions

Ovarian cancer is an immunogenic cancer, but….

Here we reviewed the involvement of the immune system in ovarian cancer, and have demonstrated that although ovarian cancer has historically not been regarded as 'immunogenic cancer', accumulating evidence indicates that the immune system plays an important role in the outcome of intervention strategies whether it is surgery, chemotherapy, or other modalities. Multiple studies have shown that T cells infiltrate ovarian tumors and that this is associated with a favorable clinical outcome. Specifically, the presence of $CD8^+$ cytotoxic T cells delays disease progression and extends overall survival of cancer patients. In contrast, the presence of Tregs, recruited through secretion of chemokines by tumor cells, is associated with poorer clinical outcome. On the other hand, the $CD4^+$ Th17 T cell subset has beneficial effects on tumor rejection by the host immune response and is also associated with improved survival of ovarian cancer patients.

….There are many immunosuppressive mechanisms at work

Despite the presence of infiltrating lymphocytes in many patients, the tumor is thought to ultimately escape immunosurveillance by fostering an immunosuppressive environment. Natural killer cells are inactivated by contact-dependent interactions with tumor cells, resulting in downregulation of activating receptors, and by the secretion of soluble factors, which limit NK cell infiltration. Tumor-associated macrophages are polarized towards an immunosuppressive M2 phenotype and are recruited to promote neoangiogenesis, tumor invasion and inhibition of other immune cells. Furthermore, the cytotoxic activity of tumor-infiltrating $CD8^+$ T cells is thwarted through expression of co-inhibitory molecules on tumor cells that render these effector cells anergic. Finally, DCs are recruited in an immature state and are unable to efficiently present antigens to elicit adaptive immune responses. Instead, immature DCs suppress the activity of other immune cells and promote tumor growth e.g., through neoangiogenesis.

Cellular immunotherapy

The fact that the immune system plays an important role in the outcome of ovarian cancer intervention and the fact that immune evasion seems to be a requirement for disease progression, indicate that ovarian cancer might be susceptible to immunotherapy. DC vaccination against TAAs is a promising strategy to activate the immune system in ovarian cancer patients. While phase I/II trials indicate that vaccination is well tolerated and capable of inducing tumor-specific T cell responses, further testing in larger phase III trials is required to truly evaluate clinical outcome. Adoptive transfer of TILs has improved in the last decades, and novel implementation strategies for ACT are being tested in the clinic.

Future challenges

The main challenge facing immunotherapy in ovarian cancer is overcoming the immunosuppressive tumor microenvironment. There are several different immunosuppressive mechanisms (e.g., PD-L1/2 expression, anergic NK and T cells, Tregs, TAMs, and immature DCs) at work in the tumor microenvironment. These form considerable barriers that the host's immune system must overcome in order to mount an effective anti-tumor response. Fortunately, many novel strategies to break immunosuppression are currently being developed in clinical setting. Blocking of the immune checkpoint receptor PD-1, the receptor for PD-L1 and PD-L2 is showing impressive effects in an ongoing trial in melanoma patients (NCT01295827). Phase I/II trials for other cancers have also been initiated and it is only a matter of time before these will also include ovarian cancer (NCT01772004). Another immune checkpoint, CTLA-4, has also been targeted for clinical intervention. The anti-CTLA-4 monoclonal antibody, Ipilimumab, showed promising results in advanced melanoma patients, and gained FDA approval in 2011. A phase II trial in recurrent ovarian cancer patients was recently initiated, and the results are eagerly awaited (NCT01611558). Another very important issue, which is still to be addressed, is that of ovarian cancer heterogeneity.

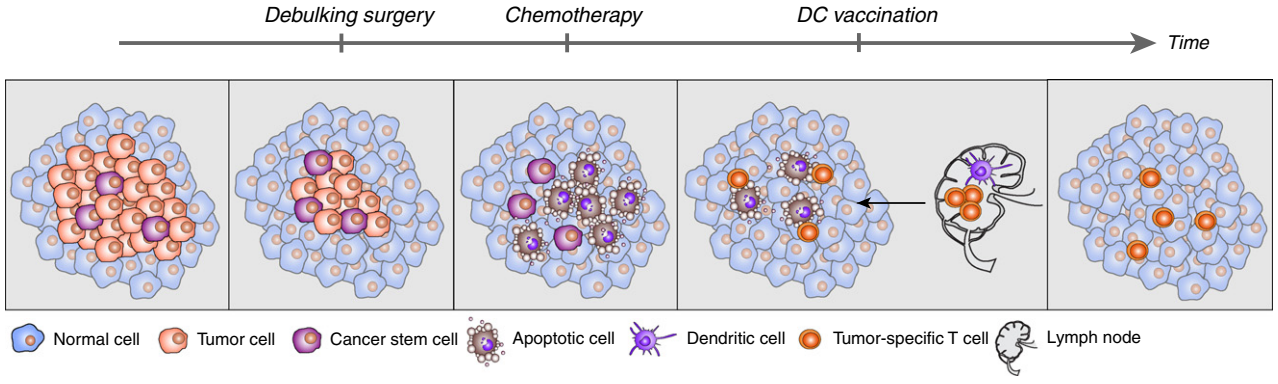

Fig. 2. *Ovarian cancer treatment strategy*. Tumors are very heterogeneous and consist of many different cell types. They are not only composed of rapidly dividing tumor cells, but also contain slowly dividing cells with stem cell-like properties, called cancer stem cells (CSC). CSCs form a rare cell population, which is self-renewing and gives rise to new cancer cells. First line therapy in ovarian cancer is debulking surgery, aiming at removal of the main tumor mass. This is followed by chemotherapy, to destroy tumor cells that were not removed during surgery. Chemotherapy targets cells with a high proliferation rate and CSCs therefore survive. These CSCs can give rise to a new tumor and have been proposed as the reason for disease relapse. DC vaccination could be used to specifically target CSCs. The tumor burden of a patient is drastically reduced after surgery and chemotherapy, rendering DC vaccination more effective. Injection of autologous DCs, pulsed with CSC-specific peptides, into lymph nodes might induce a potent immune response against CSC. CSC-specific T cells could migrate to the tumor were they kill CSCs. DC vaccination furthermore generates a long-lasting memory response, which would be activated if new cancer cells are arise, thus preventing disease recurrence.

Recent studies show that EOC encompasses a number of histologically, clinically and molecularly diverse subtypes. Gene expression profiling has identified 5 distinct subtypes, including one that has been described as 'immunoreactive', and another as 'chemo-sensitive' [75,76]. These studies clearly demonstrate that these subtypes have very different prognoses. The clinical studies described in this review did not select the patients on these molecular subtypes but it would be very interesting to see whether the patients that respond to immunotherapy have tumors belonging to the immunoreactive subtype. Molecular subtyping of the tumors of patients included in new and ongoing trials would provide very valuable data in order to address this question. In the end, this could result in novel criteria for the selection of patients for immunotherapy based on the tumor molecular subtype.

Realistically, the short-term application of immunotherapy in ovarian cancer might be adjuvant therapy rather than first-line monotherapy (Fig. 2). Most patients are diagnosed with ovarian cancer in advanced stages with considerable tumor burden and a long evolution of tumor immune evasion. Mounting a durable anti-tumor immune response might simply be a too formidable task for the host immune system in late stages of disease progression, even in those patients that have an immunoreactive tumor subtype. Cytoreductive surgery and chemotherapy offer very efficient tumor debulking, severely lowering the tumor burden. Adjuvant immunotherapy would then be a method to eradicate remaining (micro)metastases leading to a complete cure. An additional advantage of such a sequential treatment modality is that patients can also benefit from the immunological effects of the surgery and especially the chemotherapy. A number of chemotherapeutic compounds, including the platinum compounds used during ovarian cancer, are known to have immunostimulatory effects that could be exploited following therapy [9,11,12]. The induction of immunogenic cell death by these drugs can lead to DC activation and initiation of immune responses against TAAs. Furthermore, they may also disrupt immunosuppressive networks by downregulation of PD-L1 and 2 and sensitization of tumor cells to T cell killing. It will be important to address how to synergize chemotherapy with immunotherapy in order to achieve disruption of immunosuppression and subsequent activation of the immune system. In terms of preferred immunotherapeutic modality, DC vaccination might be the best option, as it synergizes with platinum chemotherapy [12] and can be targeted to cancer stem cells, which are resistant to chemotherapy and are responsible for relapse. In conclusion, although more research is needed and is ongoing, immunotherapy is a viable addition and can have a (much needed) positive contribution to existing intervention strategies to ovarian cancer.

Conflict of interest statement

The authors declare no conflict of (financial) interests.

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