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Are dendritic cells the most appropriate therapeutic vaccine for patients with ovarian cancer?

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Are dendritic cells the most appropriate therapeutic vaccine for patients with ovarian cancer?

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Abstract

New treatments are urgently needed in patients with ovarian cancer (OC), as diagnosis is delayed in many instances, resulting in 85% recurrence of the disease following surgery and standard chemotherapy. OC is considered to be an immunological type of cancer, despite its limited response to current immunotherapy options, including vaccination. Thus, additional interventions may improve their efficacy. Dendritic cells (DCs) are the most widely used cellular vaccination therapy in patients with OC due to their critical role in the initiation and development of immune response. There are viable options for DC-vaccination with a favorable toxicity profile, but specific alternatives should consider the limited therapeutic effectiveness of DC-vaccination in OC treatment. In this respect, B-cells and macrophages provide additional possibilities that may be explored for immunotherapy. Here we consider the current state-of-the-art of immunotherapy strategies for OC treatment and evaluate their potential for future improvements.

Introduction

The most lethal form of gynecological malignancies is OC, with more than 300,000 cases and 190,000 deaths predicted worldwide by 2020[1], with a 5-year survival rate of 45% for all types of OC. In addition, most patients have recurring and chemotherapy-resistant disease after initial responses[2], although significant progress has been made in surgical and systemic therapy. For instance, the recent advent of poly-ADP-ribose-polymerase inhibitors has provided new chances to dramatically influence OC outcomes[3], although some populations still need different approaches. Platinum-resistant OC patients are those with disease recurrence within 6 months of completion of first-line platinum-based chemotherapy, although this is now applied more broadly to include patients who progress within 6 months after multiple chemotherapy lines. The average life expectancy of these platinum-resistant OC patients is not greater than 1 year, and the effective management of such patients remains an important medical requirement. Therefore, new and more potent strategies are urgently needed to improve clinical outcomes.

Early observations in OC patients demonstrated a correlation between T-cell tumor infiltration, enhanced clinical benefit and improved survival[4]. This evidence was also later confirmed by a meta-analysis of specimens of more than 1,800 patients with OC[5], indicating that the immune system had a major role to play in patients' outcomes and has supported numerous immunotherapy treatments[1,2]. The recent use of antibodies that disrupt immune checkpoints has presented a key breakthrough in cancer therapy[6]. Immune checkpoints comprise different control mechanisms that promote self-tolerance, prevent autoimmunity and avoid tissue damage following the activation of the immune response by pathogens (reviewed elsewhere[7]). It is therefore surprising that the clinical benefit of both immune checkpoint inhibition and

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adoptive T-cell transfer have been rather disappointing in OC treatment[1], given their success in other types of cancer, such as melanoma and non-small cell lung cancer[6]. These modest results have been usually linked to the strongly immunosuppressive tumor microenvironment, a characteristic of OC tumor tissue (figure 1). OC cells coordinate a network of surface exposed or secreted molecular signals[1,8,9], which, collectively, i) inhibit immune cytotoxic responses, ii) promote immune cell dysfunction, and iii) recruit immune suppressive cells (such as regulatory T-cells, or myeloid derived suppressor cells), leading to tumor growth and poor prognosis[10,11]. These mechanisms cooperate to hijack the immune system, resulting in T-cell exhaustion and DC dysfunction. While therapy using immune checkpoint inhibitors may reverse tumor-induced T-cell exhaustion, DC dysfunction may still prevent full immune function restoration, which may be counteracted by vaccination with functional antigen-presenting cells (APCs). Below, the new developments, prospects, and remaining obstacles for effective therapeutic vaccination using APCs will be briefly addressed in the context of OC. Briefly, three main approaches are currently under investigation, with regard to the more adequate antigen-presenting cells to be used in therapeutic vaccination for OC treatment: first, the use of dendritic cells, the most potent APCs in the immune system; second, the alternative use of B-cells to exploit their APC potential; and third, the potential of using macrophages for this purpose.

Dendritic cells: the cornerstone of antigen presentation

Dendritic cells are capable of engulfing, processing, and presenting foreign and pathogen-associated antigens on their cell surface, with the aim of mounting an antigen-specific immune response[12]. DC-vaccines were therefore the cells of choice to be implemented as a customized cell-based therapy for cancer treatment in adjuvant settings[13]. While generally safe and with very low toxicity, DC-vaccines have shown only modest clinical benefits to date, rarely reaching ~15% of objective clinical response in most indications[14].

Choosing an antigen source is one of the crucial aspects of DC-vaccines; this is usually one or more carefully selected tumor-associated antigen(s) used in the form of synthetic proteins, peptides, RNA, DNA, or rather, a whole tumor lysate. Using a whole tumor lysate has many advantages, including targeting a complete antigen range, which strongly prevents the occurrence of antigen-loss tumor variants, and lack of limitation to specific patient haplotypes. Significantly, two recent studies have improved the preparation of whole lysates from OC-tumors for DC-vaccination. Fucikova and colleagues used high hydrostatic pressure to prepare tumor lysates, which induced immunogenic cell death in human cancer cells (including ovarian), enhanced DC uptake, cytokine release profile, and activation of T-cells[15]. The same group showed in a subsequent study that the antigens are stable in hydrostatic pressure lysates (at least when applied to a certain threshold) [16]. The hydrostatic pressure lysate preparations are currently the basis for several ongoing OC (NCT03905902), non-small cell lung cancer (NCT02470468), and prostate cancer (NCT03514836) Phase I clinical trials.

Alternatively, our group recently demonstrated that oxidation of ovarian tumor cells with hypochlorous acid, subsequently lysed and used to pulse monocyte-derived DCs, significantly increased the uptake of antigens from tumor lysate and their IL-12 production, inducing potent downstream T-cell immune responses[17]. This procedure has been taken up in a clinical Phase I trial in OC patients showing that the induction of T-cell antitumor immunity was associated with improved survival in a follow-up analysis[18]. In a preclinical mouse model, our group also demonstrated an increased immunogenicity of mouse OC cells when treated with squaric acid, promoting a more immunogenic cytokine secretion pattern, and efficiently prolonging animal survival when used in combination with IFNα-differentiated DCs[19,20]. Antigen modulation is thus a promising approach to increase the immunogenicity of DC-vaccines and should be pursued in conjunction with other developments, such as improving the DC's migration capacity, or selecting a more potent DC sub-population, or optimizing routes of administration [21].

One aspect that affects DC-vaccination's efficacy in OC is the tumor microenvironment. It is known that the ovarian tumor microenvironment is particularly immunosuppressive, which can at least partly explain the modest responses to immunotherapy, such as immuno-checkpoint inhibition therapy or adoptive T-cell transfer, otherwise much more successful in the treatment of other types of cancer (e.g. melanoma)[2,22]. Cytotoxic T Lymphocyte-Associated protein-4

(CTLA-4) and Programmed cell Death-1 (PD-1) are two of several molecules implicated in immune checkpoints (reviewed elsewhere[7]). The first-in-class antibody blocking an immune checkpoint was anti-CTLA-4, initially tested successfully in melanoma[23], although antibodies blocking PD-1 or its ligand (PD-L1) result in lower toxicity and higher response rates than anti-CTLA-4 monotherapy[24]. Recent studies have identified many mechanisms that lead to this local immunosuppression in OC tumors and generally suggest an important involvement of DCs[22]. High levels of PD-L1 expressed in DCs infiltrating ovarian tumors is a newly identified feature associated with dampened T-cell activation via the PD1/PD-L1 signaling axis[25]. However, recent work by Karyampudi and colleagues has also revealed a novel immunosuppressive function for the PD-1 expression in DCs infiltrating ovarian tumors, induced by the tumor microenvironment. In this study, the authors demonstrated that PD-1 is upregulated on DCs infiltrating the OC tumor microenvironment, resulting in suppression of NF-κB signaling and ultimately downregulating DC functions such as expression of co-stimulatory molecules, antigen presentation and cytokine release[26]. In a subsequent mouse model study, the authors also demonstrated that DC function was fully restored only upon dual blockade for both PD-1 and interleukin 10 (IL-10), leading to improved survival and activation of the immune system. However, both monotherapies (blocking either PD-1 or IL-10) failed, indicating the presence of countervailing mechanisms[27].

Similar inhibitory effects on the immunogenic phenotypes and functions of DCs infiltrating in the OC tumor microenvironment have also recently been shown to be induced by high lipid accumulation in the OC tumor microenvironment, controlled by FASN (fatty acid synthase), the key metabolic enzyme of *de novo* lipogenesis[28]. In addition, analogous functional impairments, particularly associated with the local high levels of IL-10 and PGE_2 (prostaglandin-E2) present in the OC tumor microenvironment [29], have recently been reported in monocyte-derived DCs exposed *ex vivo* to ascites or peritoneal fluid, representative of the OC tumor microenvironment milieu[30]. Of note, PGE_2 is synthetized by the key enzyme COX2 upon synergistic induction of IFNγ and TNFα [31], which are critical soluble mediators of type-1 immune effector cells, and it has been shown that PGE_2 together with vascular endothelial growth factor (VEGF) limit T-cell homing to tumors[32]. Additionally, VEGF can induce defects in DC function and maturation[33] and upregulates PD-L1 in myeloid DCs[34]. Such (and other[22]) DC dysfunctions caused by OC cells may well explain the documented evidence that OC progression is driven by a tumor-induced phenotypic change in DCs to immunosuppression, at least in late-stage disease[35].

Additionally, these immunosuppressive factors may coopt (i.e. force a tolerogenic adaptation) even in exogenous DCs *in vivo,* and therefore still constitute a major barrier for DC vaccine efficacy. This, together with a large body of evidence suggesting the poor migration ability and lymphoid homing of DCs, inadequate antigenic stimulation, and/or suboptimal cell maturation (leading to low or insufficient expression of co-stimulatory signals in T-cells[14,36,37]), lack of proliferative potential of DC, and the expensive and cumbersome process for manufacturing[38,39], have so far hampered the full clinical development of DC-based cancer vaccine therapeutic approaches. On the other hand, mounting evidence is currently emerging suggesting that B-cells potentially constitute a valid and even more potent alternative to DC-vaccination

Current evidence and prospects for a novel B-cell-based immunotherapy for cancer

The role of B-cells in immunity is generally described based on their ability to produce antibodies and their central role in humoral immunity. Nevertheless, B-cells are also active APCs and there is evidence suggesting that B-cell-vaccines could be an alternative to DC-vaccines for the treatment of cancer. One major advantage of using B-cells as cancer vaccines is that, unlike DCs, circulating B-cells are present in large numbers in peripheral blood and can be easily purified, activated and/or expanded *ex-vivo*[40,41]. This allows for a faster and cheaper vaccine production that could be standardized among several cell therapy centers. Furthermore, the capacity of *ex-vivo* expanded B-cells to induce anti-tumor immunity was demonstrated by several groups[40,42,43].

The activation step of B-cells is the key determinant of their inhibitory *vs* activating function. Thus, while lipopolysaccharide-activated B-cells induce T-cells anergy and apoptosis[44,45],

CD40L-activated B-cells strongly trigger T-cells[46]. In addition to the triggering agent used, the choice of antigens to be used for B-cells loading is of outmost importance. As for DC-vaccines, both tumor-lysate[42] and tumor neo-antigens may be used. Due to intrinsic genetic instability in tumors, truncal and clonal somatic mutations may generate neoepitopes. These mutated neo-antigens are recognized by the patient's own T-cell repertoire as non-self, which may generate high-avidity tumor-specific T-cell responses, in sharp contrast to self-antigens[47]. Although OC was initially considered unsuitable for neo-antigen-specific vaccination based on a murine ovarian tumor model[48], our group has recently identified neoepitope-specific CD8+ T cells either in blood or in tumors in 19 immunotherapy-naïve, chemotherapy-pretreated patients with recurrent advanced early OC[49]. Quite interestingly, B-cells electroporated with neo-antigen mRNA were shown *in-vitro* to promote neo-antigen specific tumor-infiltrating T-cell expansion[50].

In vivo studies have also shown tumor protective effects of B-cell adoptive transfer upon *ex vivo* stimulation with tumor antigen(s)[46,51,52]. This is of particular interest knowing that the ability or B-cells to stimulate T-cells does not decrease in the presence of IL-10, TGF-β and VEGF, the primary mediators of immunosuppression present in the tumor microenvironment[53]. This contrasts with what has been demonstrated with DCs[54], and underlines the potential of using B-cells instead of DCs as cancer vaccines. Interestingly, while there are very few studies of B-cells in OC, a fresh review that listed recent work with other cancer models has established that B-cells are an attractive target for immunotherapy[55]. In the case of OC, there may be a similar scenario, but many questions remain to the optimal use of B-cells.

Macrophages as an alternative source

The role of macrophages in homeostasis is significant, both as prodigious phagocytic cells required during tissue remodeling to clear apoptotic cells and prevention of chronic inflammation, but also in the sensing of damaged or infected cells, and in subsequent initiation of the immune response[56]. Present in almost all tissues, macrophages have a strong environmental-influenced plasticity. Their plasticity range from pro-inflammatory to pro-tolerogenic, respectively classified as M1 and M2 macrophages[57].

Macrophages may be produced in large amounts after the differentiation of blood-extracted monocytes[58] or isolated directly from body cavity lavage[59,60]. In 1974, Fidler and colleagues showed for the first time the capacity of macrophages to serve as cell source for cancer vaccine immunotherapy[61]. Their study reported a decrease in pulmonary metastases in mice following administration of *ex vivo*-stimulated peritoneal macrophages[61], which supported the notion that macrophages play a key role in regulating tumor growth and metastasis spread. After that, several papers were published based on murine cancer models, and the therapeutic approach was eventually translated into human clinical studies[62]. While security and tolerability were demonstrated, the lack of clinical efficacy shifted the focus to the use of DCs as cell source for vaccine cancer therapy[63]. These early clinical trials, however, were performed using macrophage-based vaccines before better understanding of macrophage phenotypes and plasticity was derived[62,64].

Tumor cells in OC induce polarization of macrophages to an M2-like phenotype known as tumor-associated macrophages, which have been shown to promote tumor growth, increase metastasis spread and prevent T-cells from coming into direct contact with the tumor[65-67]. Consistently, the increased frequency of CD163+ macrophages (which identifies them as M2-like macrophages) is a poor prognostic factor[68]. A high M1/M2 ratio, on the other hand, has been associated with increased survival in OC[69], as well as an elevated IFNγ signaling in ascites-associated macrophages[70]. Interventions inducing macrophage M1 polarization were shown to cooperate with T-cells in tumor regulation[71,72]. Based on these findings, several molecules, such as CD40 agonists or anti-CD47 antibodies, are currently being developed in the clinic aiming to reeducate M2 macrophages toward anti-tumoral M1 phenotypes[73,74]. Such drugs could theoretically be combined with macrophage-based therapy to prevent the shift of *ex vivo* educated macrophages into tumor-associated phenotypes after their reintroduction into the tumor microenvironment. Indeed, preserving an anti-tumor phenotype *in situ* following the administration of *ex vivo* educated macrophages may be crucial to sustaining the effect of this therapeutic intervention on tumor control.

In addition, clinical trials of macrophage-based vaccines have centered on the development of cytotoxic cells to destroy tumors directly[62], without the use of the potent macrophage ability to trigger tumor-specific T-cell reactions[75,76]. Although mononuclear blood cells have been used for the generation of macrophages in these clinical trials[62], peritoneal macrophages may be an alternative source of cells for OC. A large number of peritoneal macrophages can be isolated from ascites and act as effective tumor-APCs to induce T-cell responses after *ex vivo* stimulation[59]. Indeed, OC peritoneal macrophages are preloaded with tumor antigens based on their phagocytic character, and are readily sensitive to *ex vivo* stimulation to act as effective APCs and promote tumor-reactive T-cell responses (unpublished data)[59].

Conclusions

Recent evidence suggests that OC cells orchestrate a signaling network that can reprogram DCs infiltrating in the OC tumor microenvironment to local immunosuppressive functions, resulting in immune tolerance and tumor progression. These processes must be carefully considered and counteracted not only in the context of DC-vaccination but also in the design of future OC immunotherapy regimes. For these reasons, new and more effective immunotherapy strategies, especially in adjuvant environments and against recurrent or chemo-resistant types, are urgently needed in the treatment of OC despite promising evidence using DC vaccines.

There is currently ample collective evidence suggesting that B-cells are a powerful and promising vehicle for the development of novel anti-cancer vaccines to be used alone or in combination with other immunotherapy modalities; in particular, for those tumor types characterized by a high immunosuppressive tumor microenvironment (such as OC) for which canonical immunotherapy has so far been inefficient.

Moreover, while early attempts to use macrophages for cell therapy have not shown therapeutic efficacy, the knowledge gained over the last few decades may rekindle interest in such therapeutic intervention. Consideration should therefore be given to the versatility of macrophages, and the use of alternate cell sources for tumor macrophages may be crucial to the successful development of new immunotherapy approaches.

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Declaration of interests

None.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- ● of special interest
- ●● of outstanding interest

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Cassetta et al, 2018
● This review summarizes the current knowledge on macrophages polarization and therapeutic strategies followed nowadays to reprogram pro-tumoral activities of TAM

Goossens et al, 2019
●● This study presents a novel mechanism by which ovarian tumor cells can promote macrophage re-wiring and shift toward a tumor-promoting phenotype

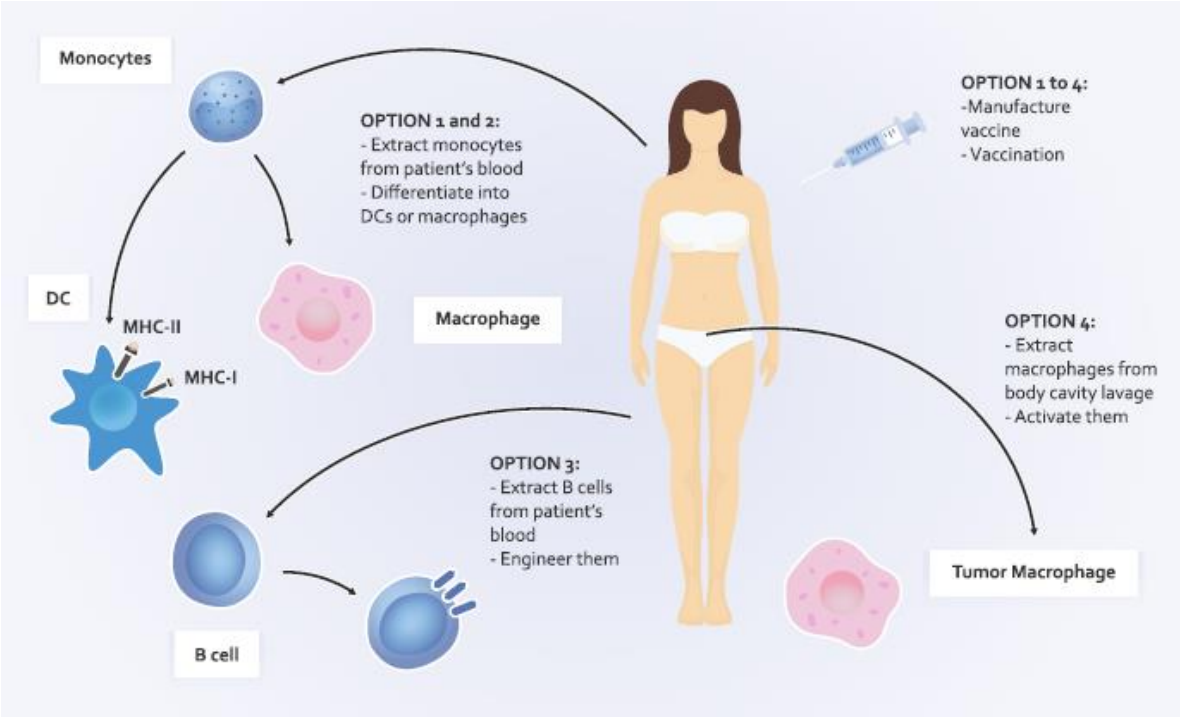
Jiang et al, 2018
●● This study showed that high levels of the metabolic enzyme FASN in the TME are linked with immunological dynsfuctions of tumor infiltrated dendritic cells (TIDCs), highilighting also the importance of metabolic networks and signals controlled by tumor cells in the ovarian TME.

Lamichhane et al, 2017

●● This study showed that only dual IL-10 and PD-1 blockade efficiently controlled tumor growth in an ovarian tumor mouse model, compared to both monotherapies. This study further demonstrated the existence of tumor adaptive mechanisms for immunoediting, supporting the use of carefully selected and synergistic combination therapies.

Tanyi et al, 2018
●● This study showed the induction of both *de novo* and boosted pre-existing anti-tumor T cell responses in OC patients upon DC vaccination with autologous tumor cells treated with hypochlorous acid. Immune responses also well correlated with improved overall survival.

Graphical abstract



Potential use of APCs for vaccination in ovarian cancer therapy: different options are shown, numbered 1 to 4, according to the cell type chosen. Once the selected source cells have been isolated, differentiated (if required) and activated, vaccine is manufactured in a GMP facility, and vaccination proceeds according to the defined clinical protocol, as approved by the corresponding regulatory institutions.

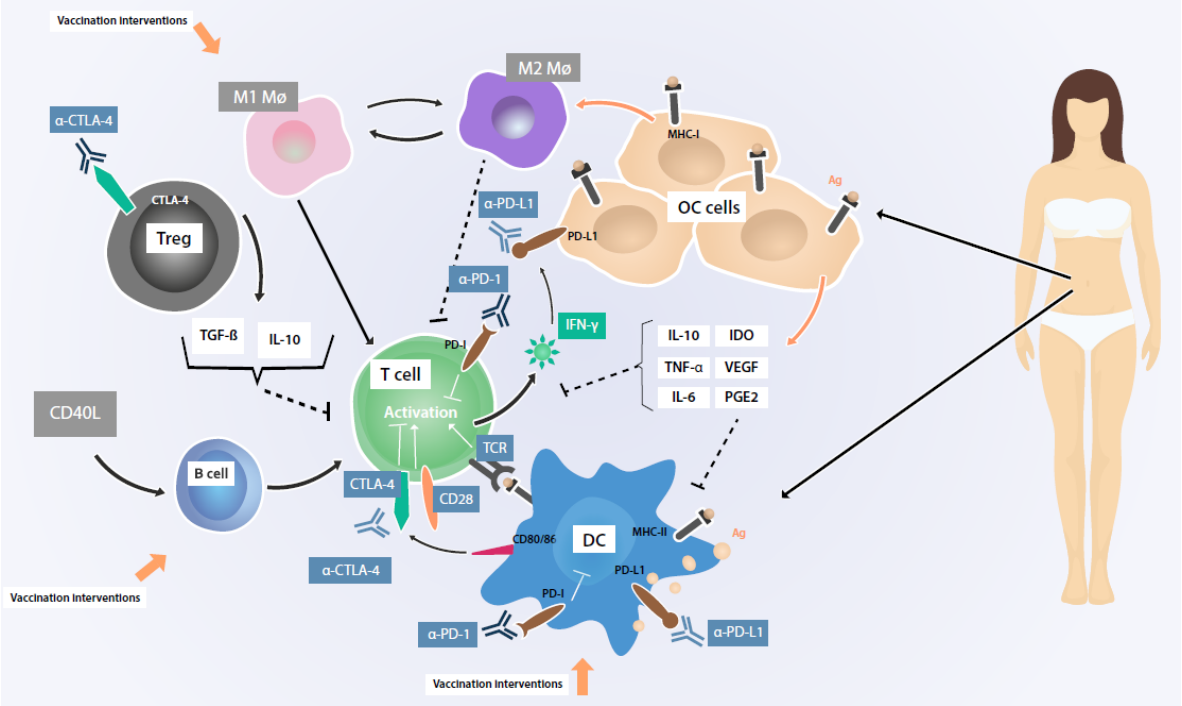


Figure 1 Summary of different mechanisms of immunosuppression acting in the OC tumor microenvironment. Dendritic cells (DCs) present tumor antigen peptides through the major histocompatibility-complex (MHC) molecule to T-cells, which recognize different MHC-peptides through their T-cell-receptor (TCR). In the absence of DC's costimulatory signals and adequate cytokines, the interaction of the peptide-MHC complex and the T-cell receptor is insufficient to contribute to T-cell activation and may induce T-cell tolerance. Mature DCs express the costimulatory ligands CD80 and CD86, which bind to CD28 in T cells and induce cytokine production, such as interleukin 2 (IL-2), which is essential for T-cell activation and proliferation, or interferon-γ (IFN-γ). However, CD80/86 on DCs also bind to the co-inhibitory receptor Cytotoxic T-cell Lymphocyte-4 (CTLA-4), which attenuates activation of T-cells. CTLA-4 can be blocked using specific antibodies to release T-cell activation. The Programmed cell Death-1 and its ligand (PD-1/PDL-1) is also an important pathway in T-cell regulation. The PD-1 receptor is on the surface of the activated T cells, allowing inhibition of T-cell activation, whereas PD-L1 and PD-L2 are commonly on the surface of DCs and macrophages (MØ). The interaction between PD-1 and PD-L1 ensures that the immune system is regulated in a timely manner to reduce chronic autoimmune inflammation. PD-L1 molecules present on DCs and tumor cells inhibit cytotoxic T cells, and such disabled T cells remain inhibited in the microenvironment of the tumor. Additionally, it has been recently found that PD-1 is upregulated on DCs infiltrating the OC tumor microenvironment, resulting in suppression of NF-κB signaling and ultimately downregulating DC functions. PD-1 and PD-L1 monoclonal antibody therapies are commonly used to release these breaks. In addition, several immunosuppressive paracrine, molecular pathways (TGF-β, IL-10, IL-4, VEGF, PGE2) and metabolic pathways (Indoleamine 2,3-dioxygenase [IDO]) are upregulated in the OC tumor microenvironment. Therapeutic vaccination intervention can be applied at different levels to promote T-cell activation against tumor cells. Ag, antigen; IL-4,6,10, interleukin-4,6,10; OC, ovarian cancer; PGE2, prostaglandin E2; TGF-β, tumor growth factor-β; TNF-α, tumor necrosis factor-α; Treg, T-regulatory cell; VEGF, vascular endothelial growth factor.

Are dendritic cells the most appropriate therapeutic vaccine for patients with ovarian cancer?

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Declaration of interests

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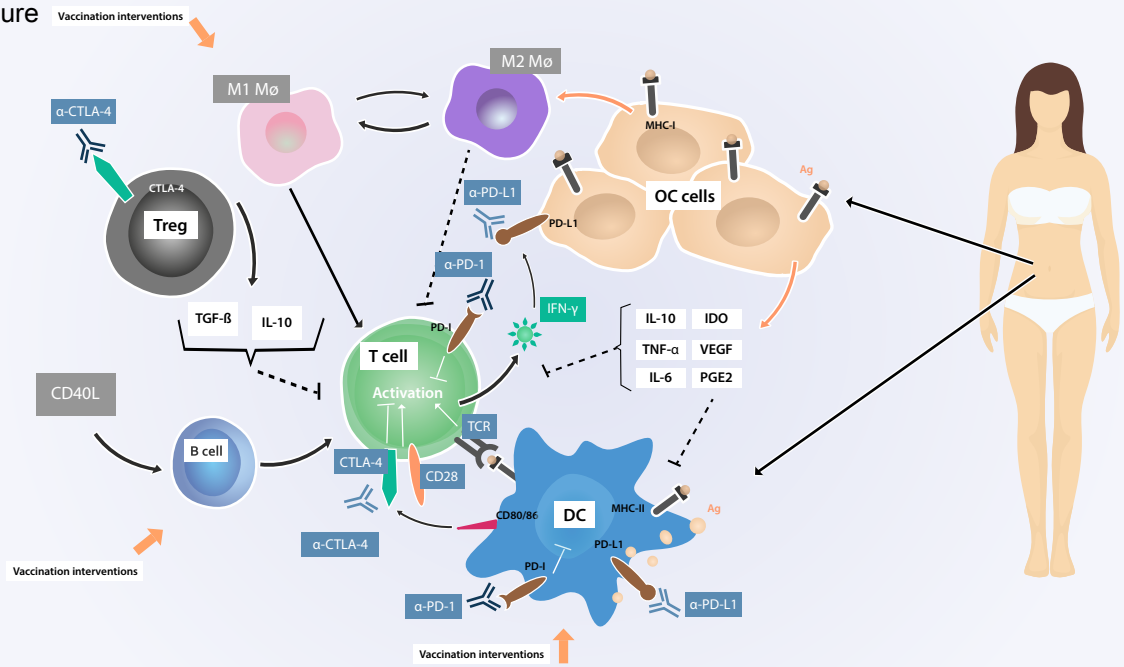
Figure


Vaccination interventions
M1 Mø
M2 Mø
α-CTLA-4
CTLA-4
Treg
MHC-I
α-PD-L1
PD-L1
OC cells
Ag
α-PD-1
PD-1
IFN-γ
TGF-ß
IL-10
T cell
Activation
IL-10
IDO
TNF-α
VEGF
IL-6
PGE2
CD40L
B cell
TCR
CTLA-4
CD28
CD80/86
DC
MHC-II
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PD-1
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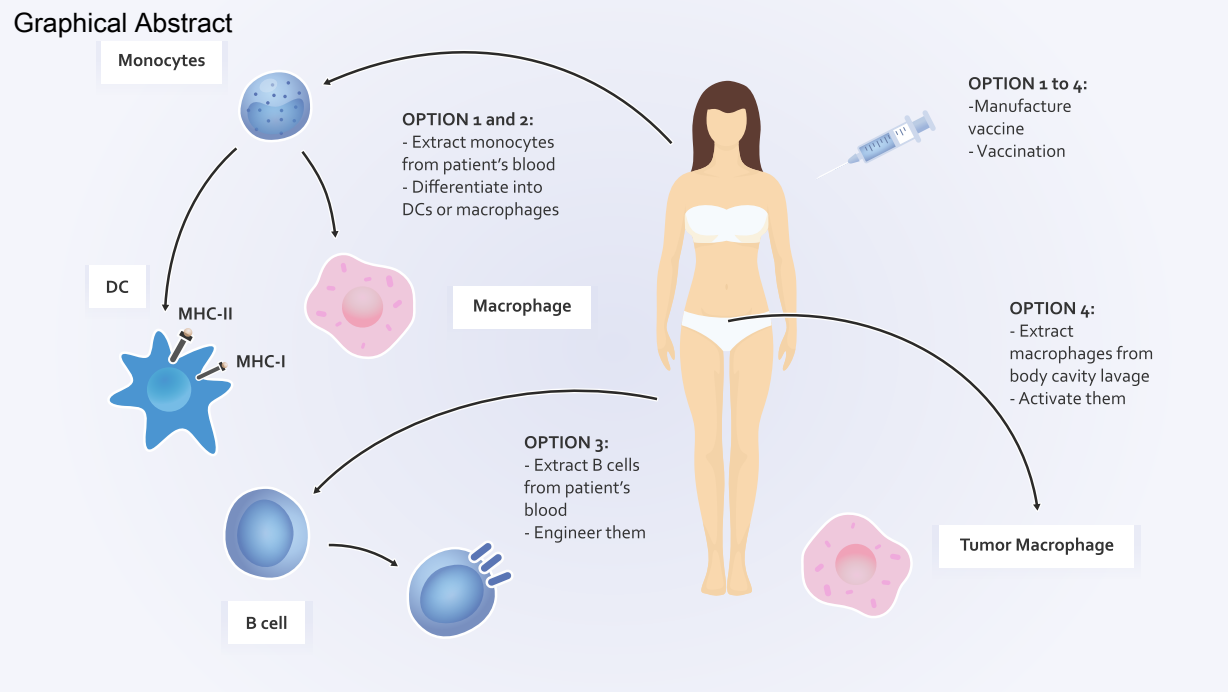
α-PD-1
α-PD-L1
Vaccination interventions
Vaccination interventions

Graphical Abstract



To: Current Opinion in Biotechnology Editors

Date: March 9, 2020

Dear Sirs,

We would like to thank the reviewers for their comments on our manuscript. We have addressed all of them, including also a figure in the text to clarify the conditions present in the tumor microenvironment specific for ovarian cancer.

Should you have any comments, please let us know.

Best regards,

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