

The Relationship Between Programmed Death-Ligand 1 and Cancer Stem Cells in Ovarian Cancer: Implications for Immunotherapy

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Abstract

Objective: To review the relationship between programmed death-ligand 1 (PD-L1) expression and cancer stem cells (CSCs) in ovarian cancer and to explore their implications for tumor progression, immune evasion, therapeutic resistance, and immunotherapy strategies. **Materials and Methods:** A narrative review was conducted using major electronic databases, including PubMed, Scopus, Web of Science, and Google Scholar, focusing on studies investigating PD-L1 expression, CSC biology, tumor microenvironment interactions, and key signaling pathways in ovarian cancer. **Results:** Emerging evidence indicates that CSCs play a pivotal role in tumor progression, recurrence, and resistance to conventional therapies in ovarian cancer. PD-L1 has been suggested to contribute not only to immune suppression but also to the maintenance of stem-like properties through activation of multiple signaling pathways, including Notch signaling and epithelial–mesenchymal transition-related mechanisms. Components of the tumor microenvironment, particularly myeloid-derived suppressor cells, further enhance CSC phenotypes and PD-L1 expression via cytokine-mediated signaling. Elevated PD-L1 expression in CSC populations may partly explain the limited efficacy of immune checkpoint inhibitors in ovarian cancer, although further clinical validation is still required. **Conclusion:** The interplay between PD-L1 and cancer stem cells represents a key mechanism underlying immune evasion, tumor progression, and therapeutic resistance in ovarian cancer. Current evidence suggests that CSC-associated PD-L1 expression is closely linked to stemness maintenance and immunosuppressive tumor microenvironment dynamics. Although most findings are derived from preclinical studies, targeting the PD-L1–CSC axis, particularly in combination with CSC-directed or microenvironment-modulating strategies, may enhance the effectiveness of immunotherapy and improve clinical outcomes.

Keywords: Ovarian cancer, PD-L1, cancer stem cells, immunotherapy, tumor microenvironment, drug resistance

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Introduction

Ovarian cancer remains a major global health problem and is one of the leading causes of cancer-related mortality among women worldwide. In 2020, approximately 313,959 new cases and 207,252 deaths were reported globally, with an age-standardized mortality rate of 4.2 per 100,000 women, highlighting its status as one of the most lethal gynecologic malignancies [1]. More than half of global cases and deaths occur in Asia, contributing to

approximately 54.9% of incidence and 52.9% of mortality, while in the United States, ovarian cancer ranks fifth in both incidence and mortality among women and has the highest mortality rate among gynecologic cancers [2, 3]. Despite advances in surgical techniques and maintenance therapies, the overall 5-year survival rate remains below 50% in most countries [4]. In Indonesia, survival outcomes vary significantly depending on tumor

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type, differentiation, and FIGO stage, with particularly poor prognosis observed in aggressive subtypes such as serous carcinoma [5]. The limited effectiveness of current therapies, including chemotherapy and radiotherapy, is partly attributed to the presence of cancer stem cells (CSCs), a subpopulation capable of surviving treatment and driving disease recurrence [6].

Emerging evidence suggests that CSCs play a critical role in tumor initiation, metastasis, and therapeutic resistance in ovarian cancer. CSCs possess self-renewal and differentiation capabilities, enabling them to sustain tumor growth and contribute to recurrence. In parallel, immune evasion mechanisms have been increasingly recognized as key contributors to tumor persistence, particularly those mediated by programmed death-ligand 1 (PD-L1), which suppresses antitumor immune responses and facilitates tumor immune escape [7–9]. Recent studies indicate a potential interplay between CSCs and PD-L1 expression, suggesting that CSCs may exploit immune checkpoint pathways to enhance their survival and resistance to therapy. This interaction is further influenced by the tumor microenvironment, including immunosuppressive cell populations such as myeloid-derived suppressor cells (MDSCs), which contribute to CSC maintenance and immune evasion [10–12]. Therefore, understanding the relationship between PD-L1 expression and CSCs is crucial for elucidating mechanisms of immunotherapy resistance and identifying novel therapeutic strategies in ovarian cancer. This review aims to synthesize current molecular and clinical evidence regarding the PD-L1–CSC axis and its implications for tumor progression, immune evasion, and treatment outcomes.

Materials and Methods

A narrative review of current molecular and clinical evidence was conducted, focusing on studies investigating PD-L1 expression, CSC biology, tumor microenvironment interactions, and key signaling pathways involved in ovarian cancer progression and treatment resistance.

Search Strategy and Selection Criteria

This narrative review was conducted to synthesize current evidence regarding the relationship between programmed death-ligand 1 (PD-L1) expression and cancer stem cells (CSCs) in ovarian cancer. A comprehensive literature search was performed using electronic databases including PubMed, Scopus, Web of Science, and Google Scholar. The search strategy combined relevant keywords and Medical Subject Headings (MeSH) terms, including: “ovarian cancer”, “PD-L1”, “programmed death-ligand 1”, “cancer stem cells”, “CSC”, “tumor microenvironment”, and “immunotherapy”. Boolean operators (AND, OR) were applied to refine the search strategy. Studies published between 2010 and 2025 were considered to ensure inclusion of the most relevant and up-to-date evidence.

Additional articles were identified through manual screening of the reference lists of selected studies. The inclusion criteria comprised original research articles, reviews, and clinical studies that discussed

PD-L1 expression, cancer stem cell (CSC) biology, or their interaction in ovarian cancer, and were published in English. Studies were excluded if they were not related to ovarian cancer, lacked relevance to PD-L1 or CSCs, or were conference abstracts, editorials, or non-peer-reviewed reports. A total of approximately 120 studies were screened, of which 67 articles were included based on their relevance to the topic. Due to the narrative nature of this review, study selection was based on relevance and scientific contribution rather than a formal systematic review methodology. This approach allows for a comprehensive and integrative understanding of the topic while acknowledging the potential for selection bias inherent in narrative reviews.

Ovarian Cancer

Several major risk factors contribute to the development of ovarian cancer, including advancing age, a family history of breast or ovarian cancer, and hereditary genetic alterations such as BRCA1 and BRCA2 mutations and defects in mismatch repair genes [10]. Reproductive and hormonal factors also play an important role, where nulliparity, early menarche, late menopause, and the use of hormone replacement therapy are associated with increased risk, whereas oral contraceptive use, multiple pregnancies, and breastfeeding confer protective effects by reducing lifetime ovulatory cycles [11]. Recent evidence further highlights the importance of genetic risk assessment and screening in high-risk groups as a strategy for prevention and for guiding personalized treatment approaches [12].

Ovarian cancer is a heterogeneous disease with several histological and molecular subtypes. Approximately 90% of cases are epithelial ovarian cancers (EOC), with high-grade serous carcinoma being the most common and aggressive, followed by endometrioid, clear cell, and mucinous carcinoma [13]. Non epithelial ovarian cancers, including germ cell tumors and sex cord–stromal tumors, are much less common but clinically significant due to their unique biology and therapeutic responses. Molecular profiling has further refined classification, identifying subgroups with distinct genomic alterations and immune microenvironment signatures that influence prognosis and treatment response [14].

The current standard treatment for ovarian cancer generally involves cytoreductive surgery followed by platinum-based chemotherapy, typically carboplatin and paclitaxel [15]. Although many patients initially respond well, relapse and resistance remain critical challenges. Targeted therapies have reshaped management, particularly Poly (ADP-ribose) Polymerase inhibitor (PARPi), which have demonstrated significant benefit in patients with BRCA mutations and homologous recombination deficiency (HRD) [16]. Recent clinical trials and meta-analyses have expanded their use as maintenance therapy across broader patient populations, although long-term toxicities require careful management [17]. Anti-angiogenic agents such as bevacizumab have been shown to improve progression-free survival when used in combination with chemotherapy and as maintenance

therapy in patients with advanced or recurrent ovarian cancer. Meanwhile, immunotherapy with PD-1/PD-L1 inhibitors has shown variable results compared to other cancers, prompting ongoing research into combination strategies with chemotherapy, anti-angiogenic agents, and PARPi to enhance efficacy [15].

Cancer Stem Cells in Ovarian Cancer

One of the crucial factors in ovarian cancer malignancy is the presence of cancer stem cells (CSCs), a subpopulation of cancer cells capable of self-renewal and differentiation into cancer cells that are resistant to therapy. Cancer stem cells contribute to tumor initiation, progression, metastasis, recurrence, and resistance to various therapies [18]. These cells can survive cytotoxic chemotherapy and stimulate tumor regrowth after initial treatment, making them a potential target for the development of new therapies for EOC [19].

Cancer stem cells can be identified through the detection of specific surface markers. Although CSC phenotypes in ovarian cancer vary, several markers have been identified, such as CD44, CD133, CD117, and aldehyde dehydrogenase 1 (ALDH1) [20] (Figure 1). The ALDH1 enzyme plays a crucial role in cellular detoxification, retinoid biosynthesis, and the regulation of stem cell proliferation and differentiation. Populations of ovarian cancer cells with high ALDH1 activity exhibit typical CSC characteristics, including regenerative capacity and resistance to environmental stress [21]. Increased ALDH1 expression within the CSC population of ovarian cancer has been associated with enhanced tumor aggressiveness, angiogenesis, chemoresistance, and poor clinical prognosis. Therefore, ALDH1 is considered a potential prognostic biomarker as well as a therapeutic target for ovarian cancer CSCs. Nevertheless, its specificity as a CSC marker is limited, as ALDH1

expression is not exclusively restricted to CSCs and may also be observed in normal stem/progenitor cells and certain non-stem cancer cell populations. Moreover, in vitro studies suggest that inhibition of ALDH1 activity may enhance cancer cell sensitivity to chemotherapy [22].

CD133, also known as prominin-1, is a transmembrane protein that has been widely studied as a CSC marker in various cancers, including ovarian cancer. In ovarian cancer, the expression of CD133 positive CSCs was associated with high regenerative potential, therapy resistance, recurrence and tumor progression [23, 24]. Tao et al [24]. showed that CD133 expression strongly correlates with more aggressive tumor characteristics, such as advanced disease stage and lower differentiation grade. The study by Masadah et al [23]. also demonstrated that CD133 expression was significantly increased in metastatic HGSC ovarian cancer. This suggests that CD133 may play a role in the progression and malignancy of ovarian cancer and may serve as a potential therapeutic target; however, further studies are required to confirm its role.

CD44 is a cell surface glycoprotein involved in cell adhesion, migration, and epithelial-mesenchymal transition (EMT), and has been widely studied as a CSC marker. CD44 promotes tumor growth and metastatic spread, and contributes to therapy resistance through the inhibition of apoptotic pathways. High CD44 expression correlates with lower overall survival and progression-free survival (PFS) in patients with ovarian cancer, whereas low CD44 expression is associated with better prognosis [25]. These data indicate that CD44 could be used to identify patients at high risk for treatment failure and recurrence, making it a potential target for CSC-specific therapies.

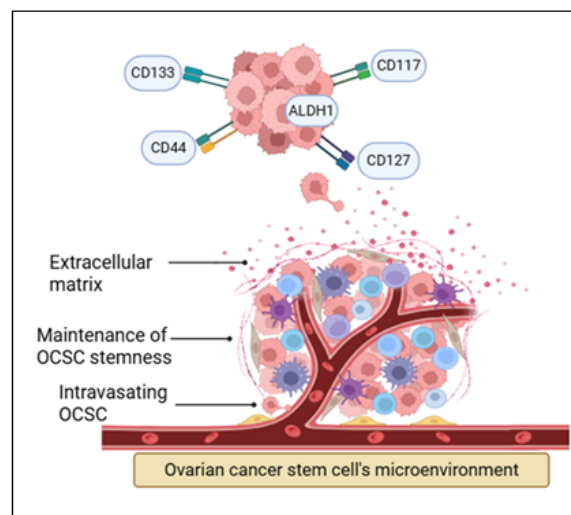


Figure 1. Potential Markers of OCSCs (Created with Biorender). Ovarian cancer stem cells (OCSCs) are characterized by the expression of various surface markers, such as CD133, CD44, CD117, and CD127, and the enzymatic activity of aldehyde dehydrogenase (ALDH1). These markers play a role in identifying and maintaining the stemness of OCSCs. The tumor microenvironment, including the extracellular matrix, contributes to the maintenance of stemness, invasion and intravasation of OCSCs into the blood vessels. Ovarian cancer stem cells (OCSCs), prominin-1 (CD133), cluster of differentiation 44 (CD44); c-KIT (CD117); interleukin 7 receptor alpha (CD127/IL-7R α), aldehyde dehydrogenase 1 (ALDH1), extracellular matrix (ECM).

Functional and Regulatory Dynamics of PD-L1 in Cancer Stem Cells

Although the interaction between PD-L1 on tumor cells and PD-1 on T cells has been well characterized, the intrinsic role of PD-L1 in CSCs and the mechanisms regulating its expression remain incompletely understood [26]. Notch3 has been identified as being highly overexpressed in breast cancer cells characterized by elevated PD-L1 expression and stem-like properties. Interestingly, the inhibitory effect of Notch pathway blockade on PD-L1 overexpression in CSCs was completely abolished when mTOR was knocked down, suggesting that PD-L1 upregulation in CSCs is at least partially mediated through the Notch signaling cascade via the Notch3/mTOR axis [27].

The intrinsic expression of PD-L1 in CSCs is also regulated through the PI3K/AKT/mTOR pathway, as evidenced by studies demonstrating that mTOR inhibition with rapamycin reduces cancer cell stemness in a manner similar to PD-L1 knockdown [28]. Furthermore, activation of transcription factors HIF-1 α and HIF-2 α induces elevated PD-L1 expression, which is associated with enhanced self-renewal capacity and tumorigenic potential of CSCs [29]. PD-L1 has been reported to interact with Frizzled 6, potentially leading to activation of β -catenin and establishment of a positive feedback loop that promotes CSC maintenance and expansion [30]. The epithelial-mesenchymal transition process plays a crucial role in enhancing tumor invasiveness and metastatic ability. Previous studies have demonstrated that PD-L1 facilitates EMT and stemness features in cancer cells, while EMT itself promotes PD-L1 enrichment in CSCs through the EMT/ β -catenin/STT3/PD-L1 signaling axis [31, 32]. These signaling pathways are not mutually exclusive but rather form an interconnected regulatory network that collectively contributes to PD-L1 expression and CSC maintenance.

Furthermore, PD-L1 expression on the CSC surface can also be controlled through post-translational modifications such as ubiquitination and N-glycosylation [33, 34]. In various CSC models, PD-L1 has been suggested to play a key role in CSC-mediated immune evasion. In breast cancer and other cancer types, the Wnt signaling pathway regulates the co-expression of PD-L1 with CD44 and ALDH1A1, while simultaneously increasing the expression of CD200 and CD276, all of which contribute to immune escape [35]. Moreover, S100A14 a member of the S100 calcium-binding protein family, has been reported to inhibit STAT3-mediated PD-L1 expression, thereby negatively regulating stemness and immune evasion in colorectal cancer [36]. Collectively, these findings highlight the complex network of molecular mechanisms regulating CSC intrinsic PD-L1 expression, involving signaling and post-translational pathways. Understanding these regulatory processes provides crucial insight for developing combination therapies that simultaneously target CSC antigens and signaling pathways while overcoming immunosuppression, thereby enhancing the efficacy of CSC focused immunotherapy. Taken together, these findings underscore the complexity

of PD-L1 regulation in CSCs and highlight the need for further studies to clarify their relevance in ovarian cancer.

PD-L1 Expression in Ovarian Cancer Stem Cells

To avoid redundancy, the molecular mechanisms regulating PD-L1 expression in CSCs are discussed in an integrated manner in the previous section. Recent advances in immunotherapy, particularly the use of immune checkpoint inhibitors targeting the PD-1/PD-L1 axis, have revolutionized cancer treatment across various tumor types. However, in ovarian cancer clinical outcomes remain modest compared to other malignancies, suggesting the presence of intrinsic immune evasion mechanisms that limit therapeutic efficacy. Therefore, elucidating the molecular basis of immune escape in ovarian cancer is essential to improve responsiveness to immunotherapy. One of the key immune-regulatory pathways involved is the PD-1/PD-L1 signaling axis, which plays a central role in suppressing antitumor immune responses mediated by tumor cells [37, 38].

Ovarian cancer demonstrates a remarkable ability to evade immune surveillance. A key mechanism involves the expression of PD-L1, which interacts with the PD-1 receptor on T lymphocytes, leading to suppression of antitumor immune responses. Several studies suggest that CSCs may exhibit higher levels of PD-L1 expression compared to non-stem cancer cells, although findings may vary depending on tumor context and experimental models. Nevertheless, the precise molecular mechanisms driving PD-L1 accumulation in CSCs remain poorly understood [39, 40].

Recent studies have shown that PD-L1 is significantly expressed in subpopulations of ovarian cancer stem cells. Alwosaibai et al [51] reported high PD-L1 expression in ovarian cancer and identified cancer stem cell populations expressing CD44 and other stem cell markers. These findings suggest that PD-L1 may be involved in maintaining stemness properties and could potentially contribute to therapy resistance and disease recurrence, although further clinical validation is required. Masadah et al. (2024) found a positive correlation between CD133 and PD-L1 expression in ovarian cancer tissues, particularly in HGSC. Increased CD133 expression was significantly correlated with elevated PD-L1 expression, suggesting that the more stem-like cancer subpopulations (CD133 based) express PD-L1, the more capable they are of evading immune recognition and elimination. Studies using ovarian cancer models, particularly those investigating the role of myeloid-derived suppressor cells (MDSCs), demonstrated that CSCs characterized by high ALDH1 activity express PD-L1 at significantly higher levels compared to non-CSCs [41]. Interestingly, PD-L1 expression not only functions as an immune evasion mechanism but has also been associated with the maintenance and expansion of CSC populations. This is evidenced by its ability to stimulate the expression of stemness-associated genes, such as Nanog, Oct4 and SOX2 which are key regulators of stem-like properties in cancer [42]. As previously described, EMT-associated β -catenin/STT3 signaling has been implicated in

regulating PD-L1 expression and stability in CSCs. STT3-mediated N-glycosylation has been implicated in stabilizing PD-L1 expression in CSCs, as discussed in the previous section. This molecular mechanism may contribute to the immune evasion ability and maintenance of stemness in ovarian CSCs [31].

In addition to intrinsic mechanisms, components of the tumor microenvironment (TME) also play a pivotal role in regulating PD-L1 expression and CSC maintenance (Figure 2). Myeloid-derived suppressor cells, a major immunosuppressive population within the TME, have been shown to support CSC phenotypes and tumor progression. Li et al [43]. reported that co culture of ovarian cancer cells with MDSCs led to increased expression of CSC markers, including CD133, ALDH1, and SOX2, indicating that MDSCs promote or maintain CSC-like properties. The role of MDSCs in promoting CSC stemness and PD-L1 expression has been described in previous sections. Phosphorylated STAT3 (pSTAT3) has been shown to induce PD-L1 expression in several cancer types; however, this mechanism has not been fully confirmed in ovarian cancer. Nevertheless, findings by Li et al [43]. suggest that MDSC-mediated pSTAT3 activation may contribute to increased PD-L1 expression and the maintenance of stemness properties in ovarian cancer stem cells (Figure 2).

PD-L1 Expression by Cancer Stem Cells and Its Implications for Immunotherapy Resistance in Ovarian Cancer

Although PD-L1 expression has recognized prognostic significance, the immunological pathways associated with its high expression in ovarian cancer remain insufficiently explored, and the molecular mechanisms involving interactions between immune and tumor cells are still unclear [44-46]. Consistent with this limited mechanistic understanding, PD-1/PD-L1 inhibitors used as single agents generally demonstrate modest objective response rates (ORR) of approximately 19–25% across mixed solid-tumor populations, with responses being highly context-dependent [47]. In line with these observations, a meta-analysis of 160 clinical trials involving 28,304 patients treated with PD-1/PD-L1 monotherapy reported an overall ORR of 20.2% [48]. Despite these encouraging outcomes in tumor regression, cancer relapse continues to occur without a well-defined explanation, indicating that recurrence may be linked to prior PD-L1 expression and potentially driven by cancer stem cells (CSCs) [49].

One potential explanation for the limited durability of responses and the occurrence of relapse is the involvement of cancer stem cells, which are increasingly recognized as a therapy-resistant tumor subpopulation capable of immune evasion. Cancer stem cell-associated PD-L1 expression has been implicated in immune evasion and therapeutic

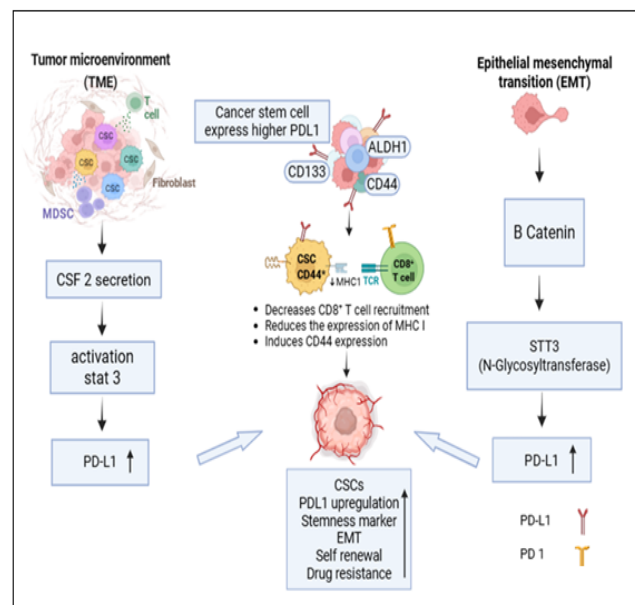


Figure 2. Tumor Microenvironment-mediated Regulation of PD-L1 Expression and Cancer Stem Cell Maintenance in Ovarian Cancer (Created with Biorender). This schematic illustrates the proposed mechanisms by which components of the tumor microenvironment (TME) contribute to the regulation of programmed death-ligand 1 (PD-L1) expression and the maintenance of cancer stem cell (CSC) properties in ovarian cancer. Myeloid-derived suppressor cells (MDSCs), a key immunosuppressive population within the TME, support CSC phenotypes through the secretion of colony-stimulating factor 2 (CSF2), leading to activation of the STAT3 signaling pathway. Phosphorylated STAT3 (pSTAT3) may induce PD-L1 upregulation and enhance stemness-associated features, including self-renewal, epithelial–mesenchymal transition (EMT), and drug resistance. Ovarian cancer stem cells exhibit elevated expression of PD-L1 and stem cell markers such as CD133, CD44, and ALDH1, which may contribute to immune evasion by reducing CD8⁺ T-cell recruitment and antigen presentation. In parallel, EMT-associated signaling involving β -catenin and STT3-mediated N-glycosylation may further promote PD-L1 stabilization and CSC maintenance. Collectively, these interactions highlight the crosstalk between the TME, immune regulation, and CSC biology in ovarian cancer progression. Programmed death-ligand 1 (PD-L1), programmed death-1 (PD-1), cancer stem cells (CSCs), epithelial mesenchymal transition (EMT), myeloid-derived suppressor cells (MDSCs), oligosaccharyltransferase complex subunit (STT3), colony-stimulating factor 2 (CSF2), signal transducer and activator of transcription 3 (STAT3).

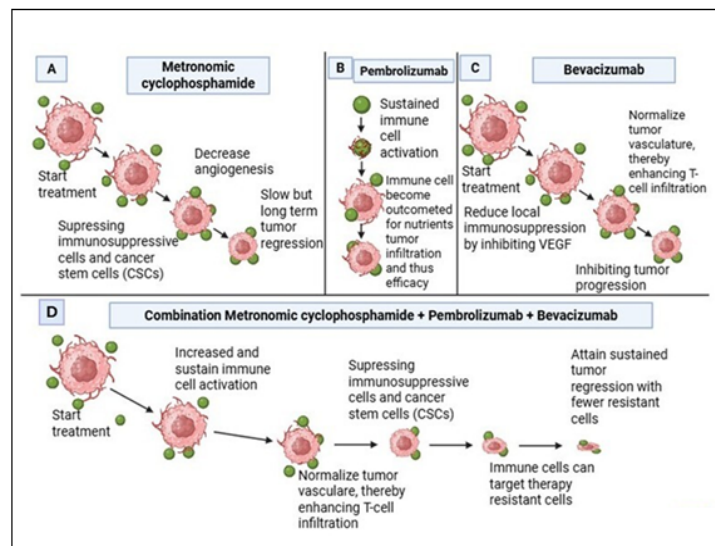


Figure 3. Synergistic Antitumor Mechanisms of Metronomic Cyclophosphamide, Pembrolizumab, and Bevacizumab in Recurrent Ovarian Cancer (Created with Biorender). Panel A illustrates that metronomic cyclophosphamide suppresses immunosuppressive cells and cancer stem cells (CSCs) while reducing angiogenesis, leading to gradual but sustained tumor regression. Panel B shows that pembrolizumab restores cytotoxic T-cell activity through PD-1/PD-L1 blockade, although immune cell infiltration may be limited when used alone. Panel C displays that bevacizumab inhibits VEGF-driven immunosuppression, normalizes tumor vasculature, and enhances T-cell infiltration. Panel D demonstrates the synergistic effect of combining all three agents, resulting in enhanced immune activation, improved vascular normalization, suppression of CSCs and immunosuppressive cells, and more durable tumor regression with fewer resistant cells. Vascular endothelial growth factor (VEGF), programmed death-1 (PD-1), programmed death-ligand 1 (PD-L1), cancer stem cells (CSCs), regulatory T cells (Tregs), tumor microenvironment (TME)

resistance [50]. PD-L1 is also frequently associated with stemness markers such as CD44, indicating a strong link between stemness status and the immune evasive potential of CSCs [51]. Consequently, PD-L1 expression on CSC surfaces is believed to play an important role in promoting chemotherapy resistance and contributing to tumor recurrence [52].

PD-L1 expression on CSCs may contribute to the establishment of an immunosuppressive tumor microenvironment. The direct interaction between PD-L1 on the surface of CSCs and PD-1 on the surface of T cells, which may lead to T cell exhaustion, anergy or apoptosis. This mechanism leads to immune evasion, allowing CSCs to escape immune surveillance even during ongoing therapy [53, 54]. CSCs actively interact with various types of cells to induce their phenotype switching and function alternation, e.g., modulating the cytokine, chemokine secretion profiles. These immunosuppressive cells include regulatory dendritic cells (DCregs), MDSCs, tumor-associated macrophages (TAMs) and regulatory T cells (Tregs). Recent studies have suggested that resistance to anti-PD-L1 therapy is in large part conferred by immunosuppressive cells. Of note, CSCs and these immunosuppressive cells express PD-1 and/or PD-L1 at various levels [53, 55].

Moreover, MDSCs have been reported to promote PD-L1 expression in CSCs, potentially contributing to immunosuppression and therapeutic resistance [41]. This process often leads to the suppression of the antitumor activity of infiltrating T cells via the extrinsic PD-1/PD-L1 signaling axis. These findings may provide a strong rationale for concurrently targeting both CSCs

and the PD-1/PD-L1 pathway in cancer immunotherapy. Therefore, understanding the complex interactions between CSCs and immunosuppressive cells, particularly the regulatory role of PD-L1 in these interactions is crucial for developing more effective therapeutic strategies [53, 56]. Taken together, these findings support the association between PD-L1 expression and CSC phenotypes in ovarian cancer, although further clinical validation is still required.

Therapeutic Synergy Between VEGF Inhibition, Immune Checkpoint Blockade, and CSC Targeting in Overcoming Resistance in Ovarian Cancer

Although optimal cytoreduction and platinum taxane chemotherapy have been shown to improve survival, and PARPi such as olaparib, niraparib, and rucaparib provide significant benefits for patients with BRCA mutations or homologous recombination deficiency (HRD), resistance both intrinsic and acquired frequently occurs, making long-term responses rarely achievable [57, 58]. Ovarian cancer is known to be an immunogenic disease, where increased T-cell infiltration within tumor tissues is significantly correlated with prolonged patient survival [59]. Approximately half of ovarian cancer patients exhibit T-cell infiltration in the tumor microenvironment (TME) however, these cytotoxic T cells are often suppressed by various immunosuppressive mechanisms [60]. High PD-L1 expression on CSCs is associated with stemness phenotypes and pro-survival signaling pathways (PI3K/AKT/mTOR), which functionally suppress T and NK cell effectors and reduce sensitivity to immune checkpoint inhibitors (ICIs) [61].

Vascular endothelial growth factor (VEGF) is highly expressed in most ovarian cancer patients and serves as a key driver of tumor neovascularization and local immunosuppression [62]. Elevated VEGF levels can inhibit T-cell infiltration into the tumor microenvironment (TME) and upregulate Fas ligand expression on abnormal tumor endothelial cells, leading to T-cell apoptosis [63]. Moreover, VEGF promotes the expansion of immunosuppressive cell subsets, including regulatory T cells and MDSCs, thereby creating a profoundly immunosuppressive TME [62]. Beyond VEGF-mediated immunosuppression, additional therapeutic strategies that modulate the tumor microenvironment and target cancer stem cells may further enhance the efficacy of immune checkpoint blockade. In this context, daily metronomic chemotherapy with oral cyclophosphamide represents a low-toxicity and cost-effective approach to enhance antitumor immune responses. Metronomic administration of cyclophosphamide has been shown to reduce the tumorigenic potential of cancer stem cells and selectively deplete regulatory T cells, thereby shifting the immune balance toward increased cytotoxic activity [63]. By attenuating CSC-driven tumor growth while alleviating local immunosuppression, metronomic cyclophosphamide may function as a complementary strategy to both anti-angiogenic and immune checkpoint therapies in overcoming treatment resistance and reducing tumor recurrence [64, 65].

The phase II clinical trial conducted by Zsiros et al. [66] demonstrated that the combination therapy (Figure 3) provided significant clinical benefits in patients with recurrent ovarian cancer, including those resistant to platinum-based chemotherapy. This combination regimen achieved an objective response rate (ORR) of 47.5%, with clinical benefit observed in 95% of patients and durable responses lasting more than 12 months in 25% of cases. Based on the recent study by Steck et al [67], the combination of pembrolizumab, bevacizumab, and metronomic cyclophosphamide has been shown to be safe and well tolerated, with adverse effects generally mild to moderate and rarely leading to treatment discontinuation. Clinically, this regimen provides a moderate therapeutic benefit, achieving an objective response rate (ORR) of 21% and a clinical benefit rate (CBR) of 42%, even in patients with heavily pretreated, highly resistant disease. These findings reinforce the results of the phase II trial by Zsiros et al. [66]. The synergistic effects of these three agents arise from their complementary mechanisms of action: pembrolizumab, an immune checkpoint inhibitor, reactivates the antitumor immune response by blocking the PD-1/PD-L1 interaction and restoring cytotoxic T-cell activity; bevacizumab functions as an antiangiogenic agent that normalizes tumor vasculature and reduces VEGF-mediated immunosuppression, thereby enhancing T-cell infiltration into the tumor microenvironment; meanwhile, metronomic cyclophosphamide exerts immunomodulatory effects by suppressing immunosuppressive cell populations such as regulatory T cells (Tregs) and inhibiting cancer stem cells (CSCs), both of which contribute to therapeutic resistance

and tumor recurrence [67]. These findings suggest that this combination has strong potential as a future therapeutic strategy for recurrent ovarian cancer.

In conclusion, PD-L1 expression in CSCs is suggested to play a role in immunotherapy resistance and tumor progression in ovarian cancer, however much of the current evidence is derived from preclinical studies, and further clinical validation is needed. Beyond its classical immunosuppressive function in inhibiting cytotoxic T-cell activity, PD-L1 exerts intrinsic effects that sustain CSC stemness and survival through key signaling pathways such as PI3K/AKT/mTOR, Notch3/mTOR, and β -catenin/STT3. These mechanisms enable CSCs to adapt to therapeutic stress, contributing to disease recurrence and treatment resistance.

The interaction between PD-L1, CSCs, and the immunosuppressive tumor microenvironment forms a central axis that explains the limited efficacy of immune checkpoint inhibitors (ICIs) in ovarian cancer. Therefore, therapeutic strategies targeting the PD-L1 CSC axis, combined with modulation of VEGF signaling and other immunosuppressive components of the tumor microenvironment, represent a promising approach to enhance the effectiveness of immunotherapy. Clinical evidence from the phase II combination trial of pembrolizumab, bevacizumab, and metronomic cyclophosphamide demonstrated meaningful clinical benefits, including in platinum-resistant recurrent ovarian cancer, supporting the potential of combinatorial approaches to suppress stemness, restore antitumor immunity, and reduce recurrence.

In summary, a deeper understanding of the regulatory mechanisms of PD-L1 expression in CSCs and its interaction with the tumor microenvironment will provide a strong foundation for the development of more effective and durable combinatorial immunotherapy strategies for ovarian cancer in the future.

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Conflict of Interest

The authors declare no conflict of interest.

References

- Huang J, Chan WC, Ngai CH, Lok V, Zhang L, Lucero-Prisno DE, Xu W, et al. Worldwide Burden, Risk Factors, and Temporal Trends of Ovarian Cancer: A Global Study. *Cancers*. 2022 04 29;14(9):2230. <https://doi.org/10.3390/cancers14092230>
- International Agency for Research on Cancer. Cancer site ranking. 2022. Available at: <https://gco.iarc.fr> [Accessed 5 December 2025].
- Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *CA: a cancer journal for clinicians*. 2021 01;71(1):7-33. <https://doi.org/10.3322/caac.21654>
- Nag S, Aggarwal S, Rauthan A, Warriar N. Maintenance therapy for newly diagnosed epithelial ovarian cancer- a review. *Journal of Ovarian Research*. 2022 07 28;15(1):88. <https://doi.org/10.1186/s13048-022-01020-1>
- Rustamadi P, Wiyarta E, Nuryanto KH, Anggraeni TD, Kusuma F, Purwoto G, Winarto H, et al. A Decade of Ovarian Cancer in Indonesia: Epidemiology and Survival Analysis from 2010 to 2020. *Journal of Clinical Medicine*. 2025 03 03;14(5):1692. <https://doi.org/10.3390/jcm14051692>
- Battle E, Clevers H. Cancer stem cells revisited. *Nature Medicine*. 2017 Oct 06;23(10):1124-1134. <https://doi.org/10.1038/nm.4409>
- Colak S, Medema JP. Cancer stem cells--important players in tumor therapy resistance. *The FEBS journal*. 2014 Nov;281(21):4779-4791. <https://doi.org/10.1111/febs.13023>
- Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell*. 2015 04 13;27(4):450-461. <https://doi.org/10.1016/j.ccell.2015.03.001>
- Lau EY, Ho NP, Lee TK. Cancer Stem Cells and Their Microenvironment: Biology and Therapeutic Implications. *Stem Cells International*. 2017;2017:3714190. <https://doi.org/10.1155/2017/3714190>
- Flaum N, Crosbie EJ, Edmondson RJ, Smith MJ, Evans DG. Epithelial ovarian cancer risk: A review of the current genetic landscape. *Clinical Genetics*. 2020 01;97(1):54-63. <https://doi.org/10.1111/cge.13566>
- Stewart C, Ralyea C, Lockwood S. Ovarian Cancer: An Integrated Review. *Seminars in Oncology Nursing*. 2019 04;35(2):151-156. <https://doi.org/10.1016/j.soncn.2019.02.001>
- Tavares V, Marques IS, Melo IG, Assis J, Pereira D, Medeiros R. Paradigm Shift: A Comprehensive Review of Ovarian Cancer Management in an Era of Advancements. *International Journal of Molecular Sciences*. 2024 02 03;25(3):1845. <https://doi.org/10.3390/ijms25031845>
- Kim J, Park EY, Kim O, Schilder JM, Coffey DM, Cho C, Bast RC. Cell Origins of High-Grade Serous Ovarian Cancer. *Cancers*. 2018 Nov 12;10(11):433. <https://doi.org/10.3390/cancers10110433>
- Taylor EC, Irshaid L, Mathur M. Multimodality Imaging Approach to Ovarian Neoplasms with Pathologic Correlation. *Radiographics: A Review Publication of the Radiological Society of North America, Inc*. 2021;41(1):289-315. <https://doi.org/10.1148/rg.2021200086>
- Goldlust IS, Guidice E, Lee J. PARP inhibitors in ovarian cancer. *Seminars in Oncology*. 2024;51(1-2):45-57. <https://doi.org/10.1053/j.seminoncol.2024.01.001>
- Wang L, Zhang Q, Wang X, Dong Z, Liu S, Wang Q, Zhang Z, Xing J. Therapeutic landscape of ovarian cancer: recent advances and emerging therapies. *Biomarker Research*. 2025 08 12;13(1):103. <https://doi.org/10.1186/s40364-025-00818-7>
- Ji S, Chen L, Yu Y, Chen X, Wei L, Gou L, Shi C, Zhuang S. A comprehensive comparison of PARP inhibitors as maintenance therapy in platinum-sensitive recurrent ovarian cancer: a systematic review and network meta-analysis. *Journal of Ovarian Research*. 2025 01 30;18(1):18. <https://doi.org/10.1186/s13048-025-01599-1>
- Wilczyński JR, Wilczyński M, Paradowska E. Cancer Stem Cells in Ovarian Cancer-A Source of Tumor Success and a Challenging Target for Novel Therapies. *International Journal of Molecular Sciences*. 2022 02 24;23(5):2496. <https://doi.org/10.3390/ijms23052496>
- Ma H, Tian T, Cui Z. Targeting ovarian cancer stem cells: a new way out. *Stem Cell Research & Therapy*. 2023 02 14;14(1):28. <https://doi.org/10.1186/s13287-023-03244-4>
- Frąszczak K, Barczyński B. The Role of Cancer Stem Cell Markers in Ovarian Cancer. *Cancers*. 2023 Dec 20;16(1):40. <https://doi.org/10.3390/cancers16010040>
- Clark DW, Palle K. Aldehyde dehydrogenases in cancer stem cells: potential as therapeutic targets. *Annals of Translational Medicine*. 2016 Dec;4(24):518. <https://doi.org/10.21037/atm.2016.11.82>
- Wei Y, Li Y, Chen Y, Liu P, Huang S, Zhang Y, Sun Y, et al. ALDH1: A potential therapeutic target for cancer stem cells in solid tumors. *Frontiers in Oncology*. 2022;12:1026278. <https://doi.org/10.3389/fonc.2022.1026278>
- Masadah R, Ikram D, Riadi R, Tangdiung Y, Nelwan BJ, Ghaznawie M, Rauf S, Faruk M. CD133, CD47, and PD-L1 Expression in Ovarian High-grade Serous Carcinoma and Its Association with Metastatic Disease: A Cross-sectional Study. *Asian Pacific journal of cancer prevention: APJCP*. 2024 01 01;25(1):249-255. <https://doi.org/10.31557/APJCP.2024.25.1.249>
- Tao Y, Li H, Huang R, Mo D, Zeng T, Fang M, Li M. Clinicopathological and Prognostic Significance of Cancer Stem Cell Markers in Ovarian Cancer Patients: Evidence from 52 Studies. *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology*. 2018;46(4):1716-1726. <https://doi.org/10.1159/000489586>
- Sihombing UHM, Andrijono A, Purwoto G, Gandamihardja S, Harahap AR, Rustamadi P, Kekalih A, Widyawati R, Fuady DR. CD44+/CD24- Expression as predictors of ovarian cancer chemoresistance: immunohistochemistry and flow cytometry study. *Journal of the Egyptian National Cancer Institute*. 2022 Oct 24;34(1):44. <https://doi.org/10.1186/s43046-022-00143-2>
- Yamaguchi H, Hsu J, Yang W, Hung M. Mechanisms regulating PD-L1 expression in cancers and associated opportunities for novel small-molecule therapeutics. *Nature Reviews. Clinical Oncology*. 2022 05;19(5):287-305. <https://doi.org/10.1038/s41571-022-00601-9>
- Mansour FA, Al-Mazrou A, Al-Mohanna F, Al-Alwan M, Ghebeh H. PD-L1 is overexpressed on breast cancer stem cells through notch3/mTOR axis. *Oncoimmunology*. 2020;9(1):1729299. <https://doi.org/10.1080/2162402X.2020.1729299>
- Almozyan S, Colak D, Mansour F, Alaiya A, Al-Harazi O, Qattan A, Al-Mohanna F, Al-Alwan M, Ghebeh H. PD-L1 promotes OCT4 and Nanog expression in breast cancer stem cells by sustaining PI3K/AKT pathway activation. *International Journal of Cancer*. 2017 Oct 01;141(7):1402-1412. <https://doi.org/10.1002/ijc.30834>
- Golan H, Shukrun R, Caspi R, Vax E, Pode-Shakked N, Goldberg S, Pleniceanu O, et al. In Vivo Expansion of Cancer Stemness Affords Novel Cancer Stem Cell Targets: Malignant Rhabdoid Tumor as an Example. *Stem Cell Reports*. 2018 09 11;11(3):795-810. <https://doi.org/10.1016/j.stemcr.2018.08.001>

- org/10.1016/j.stemcr.2018.07.010
30. Katoh M, Katoh M. WNT signaling and cancer stemness. *Essays in Biochemistry*. 2022 09 16;66(4):319-331. <https://doi.org/10.1042/EBC20220016>
 31. Hsu J, Xia W, Hsu Y, Chan L, Yu W, Cha J, Chen C, et al. STT3-dependent PD-L1 accumulation on cancer stem cells promotes immune evasion. *Nature Communications*. 2018 05 15;9(1):1908. <https://doi.org/10.1038/s41467-018-04313-6>
 32. Koh YW, Han J, Haam S. Expression of PD-L1, cancer stem cell and epithelial-mesenchymal transition phenotype in non-small cell lung cancer. *Pathology*. 2021 02;53(2):239-246. <https://doi.org/10.1016/j.pathol.2020.07.009>
 33. Li C, Lim S, Xia W, Lee H, Chan L, Kuo C, Khoo K, et al. Glycosylation and stabilization of programmed death ligand-1 suppresses T-cell activity. *Nature Communications*. 2016 08 30;7:12632. <https://doi.org/10.1038/ncomms12632>
 34. Chen M, Sharma A, Lin Y, Wu Y, He Q, Gu Y, Xu ZP, et al. Insulin and epithelial growth factor (EGF) promote programmed death ligand 1(PD-L1) production and transport in colon cancer stem cells. *BMC cancer*. 2019 02 15;19(1):153. <https://doi.org/10.1186/s12885-019-5364-3>
 35. Castagnoli L, Cancila V, Cordoba-Romero SL, Faraci S, Talarico G, Belmonte B, Iorio MV, et al. WNT signaling modulates PD-L1 expression in the stem cell compartment of triple-negative breast cancer. *Oncogene*. 2019 05;38(21):4047-4060. <https://doi.org/10.1038/s41388-019-0700-2>
 36. Min H, Cho J, Sim JY, Boo H, Lee J, Lee S, Lee Y, et al. S100A14: A novel negative regulator of cancer stemness and immune evasion by inhibiting STAT3-mediated programmed death-ligand 1 expression in colorectal cancer. *Clinical and Translational Medicine*. 2022 07;12(7):e986. <https://doi.org/10.1002/ctm2.986>
 37. Hamanishi J, Mandai M, Matsumura N, Abiko K, Baba T, Konishi I. PD-1/PD-L1 blockade in cancer treatment: perspectives and issues. *International Journal of Clinical Oncology*. 2016 06;21(3):462-473. <https://doi.org/10.1007/s10147-016-0959-z>
 38. Siminiak N, Czepczyński R, Zaborowski MP, Iżycki D. Immunotherapy in Ovarian Cancer. *Archivum Immunologiae Et Therapiae Experimentalis*. 2022 08 09;70(1):19. <https://doi.org/10.1007/s00005-022-00655-8>
 39. Tang Q, Chen Y, Li X, Long S, Shi Y, Yu Y, Wu W, et al. The role of PD-1/PD-L1 and application of immune-checkpoint inhibitors in human cancers. *Frontiers in Immunology*. 2022;13:964442. <https://doi.org/10.3389/fimmu.2022.964442>
 40. Mathan SV, Singh RP. Cancer Stem Cells Connecting to Immunotherapy: Key Insights, Challenges, and Potential Treatment Opportunities. *Cancers*. 2025 06 23;17(13):2100. <https://doi.org/10.3390/cancers17132100>
 41. Komura N, Mabuchi S, Shimura K, Yokoi E, Kozasa K, Kuroda H, Takahashi R, et al. The role of myeloid-derived suppressor cells in increasing cancer stem-like cells and promoting PD-L1 expression in epithelial ovarian cancer. *Cancer immunology, immunotherapy: CII*. 2020 Dec;69(12):2477-2499. <https://doi.org/10.1007/s00262-020-02628-2>
 42. Gupta HB, Clark CA, Yuan B, Sareddy G, Pandeswara S, Padron AS, Hurez V, et al. Tumor cell-intrinsic PD-L1 promotes tumor-initiating cell generation and functions in melanoma and ovarian cancer. *Signal Transduction and Targeted Therapy*. 2016;1:16030. <https://doi.org/10.1038/sigtrans.2016.30>
 43. Li X, Wang J, Wu W, Gao H, Liu N, Zhan G, Li L, et al. Myeloid-derived suppressor cells promote epithelial ovarian cancer cell stemness by inducing the CSF2/p-STAT3 signalling pathway. *The FEBS journal*. 2020 Dec;287(23):5218-5235. <https://doi.org/10.1111/febs.15311>
 44. Zhang H, Lai R. STAT3 in Cancer-Friend or Foe?. *Cancers*. 2014 07 03;6(3):1408-1440. <https://doi.org/10.3390/cancers6031408>
 45. Marzec M, Zhang Q, Goradia A, Raghunath PN, Liu X, Paessler M, Wang HY, et al. Oncogenic kinase NPM/ALK induces through STAT3 expression of immunosuppressive protein CD274 (PD-L1, B7-H1). *Proceedings of the National Academy of Sciences of the United States of America*. 2008 Dec 30;105(52):20852-20857. <https://doi.org/10.1073/pnas.0810958105>
 46. Pawłowska A, Kwiatkowska A, Suszczyk D, Chudzik A, Tarkowski R, Barczyński B, Kotarski J, Wertel I. Clinical and Prognostic Value of Antigen-Presenting Cells with PD-L1/PD-L2 Expression in Ovarian Cancer Patients. *International Journal of Molecular Sciences*. 2021 Oct 26;22(21):11563. <https://doi.org/10.3390/ijms222111563>
 47. Carbognin L, Pilotto S, Milella M, Vaccaro V, Brunelli M, Calìo A, Cuppone F, et al. Differential Activity of Nivolumab, Pembrolizumab and MPDL3280A according to the Tumor Expression of Programmed Death-Ligand-1 (PD-L1): Sensitivity Analysis of Trials in Melanoma, Lung and Genitourinary Cancers. *PloS One*. 2015;10(6):e0130142. <https://doi.org/10.1371/journal.pone.0130142>
 48. Zhao B, Zhao H, Zhao J. Efficacy of PD-1/PD-L1 blockade monotherapy in clinical trials. *Therapeutic Advances in Medical Oncology*. 2020;12:1758835920937612. <https://doi.org/10.1177/1758835920937612>
 49. Pan Y, Yuan C, Zeng C, Sun C, Xia L, Wang G, Chen X, et al. Cancer stem cells and niches: challenges in immunotherapy resistance. *Molecular Cancer*. 2025 02 25;24(1):52. <https://doi.org/10.1186/s12943-025-02265-2>
 50. Wu Y, Chen M, Wu P, Chen C, Xu ZP, Gu W. Increased PD-L1 expression in breast and colon cancer stem cells. *Clinical and Experimental Pharmacology & Physiology*. 2017 05;44(5):602-604. <https://doi.org/10.1111/1440-1681.12732>
 51. Alwosaibai K, Aalmri S, Mashhour M, Ghandorah S, Alshangiti A, Azam F, Selwi W, et al. PD-L1 is highly expressed in ovarian cancer and associated with cancer stem cells populations expressing CD44 and other stem cell markers. *BMC cancer*. 2023 01 05;23(1):13. <https://doi.org/10.1186/s12885-022-10404-x>
 52. Peng J, Hamanishi J, Matsumura N, Abiko K, Murat K, Baba T, Yamaguchi K, et al. Chemotherapy Induces Programmed Cell Death-Ligand 1 Overexpression via the Nuclear Factor-κB to Foster an Immunosuppressive Tumor Microenvironment in Ovarian Cancer. *Cancer Research*. 2015 Dec 01;75(23):5034-5045. <https://doi.org/10.1158/0008-5472.CAN-14-3098>
 53. Zheng F, Zhang S, Chang AE, Moon JJ, Wicha MS, Wang SX, Chen J, et al. Breaking Immunosuppression to Enhance Cancer Stem Cell-Targeted Immunotherapy. *International Journal of Biological Sciences*. 2025;21(4):1819-1836. <https://doi.org/10.7150/ijbs.101025>
 54. Rouzbahani E, Majidpoor J, Najafi S, Mortezaee K. Cancer stem cells in immunoregulation and bypassing anti-checkpoint therapy. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*. 2022 Dec;156:113906. <https://doi.org/10.1016/j.biopha.2022.113906>
 55. Borlongan MC, Saha D, Wang H. Tumor Microenvironment: A Niche for Cancer Stem Cell Immunotherapy. *Stem Cell Reviews and Reports*. 2024 01;20(1):3-24. <https://doi.org/10.1007/s12015-023-10639-6>
 56. Hudson K, Cross N, Jordan-Mahy N, Leyland R. The Extrinsic and Intrinsic Roles of PD-L1 and Its Receptor

- PD-1: Implications for Immunotherapy Treatment. *Frontiers in Immunology*. 2020;11:568931. <https://doi.org/10.3389/fimmu.2020.568931>
57. Kulkarni S, Seneviratne N, Tosun Ç, Madhusudan S. PARP inhibitors in ovarian cancer: Mechanisms of resistance and implications to therapy. *DNA repair*. 2025 05;149:103830. <https://doi.org/10.1016/j.dnarep.2025.103830>
 58. Noordermeer SM, Attikum H. PARP Inhibitor Resistance: A Tug-of-War in BRCA-Mutated Cells. *Trends in Cell Biology*. 2019 Oct;29(10):820-834. <https://doi.org/10.1016/j.tcb.2019.07.008>
 59. Steinhart B, Jordan KR, Bapat J, Post MD, Brubaker LW, Bitler BG, Wrobel J. The Spatial Context of Tumor-Infiltrating Immune Cells Associates with Improved Ovarian Cancer Survival. *Molecular cancer research: MCR*. 2021 Dec;19(12):1973-1979. <https://doi.org/10.1158/1541-7786.MCR-21-0411>
 60. Blanc-Durand F, Clemence Wei Xian L, Tan DSP. Targeting the immune microenvironment for ovarian cancer therapy. *Frontiers in Immunology*. 2023;14:1328651. <https://doi.org/10.3389/fimmu.2023.1328651>
 61. O'Donnell JS, Massi D, Teng MWL, Mandala M. PI3K-AKT-mTOR inhibition in cancer immunotherapy, redux. *Seminars in Cancer Biology*. 2018 02;48:91-103. <https://doi.org/10.1016/j.semcancer.2017.04.015>
 62. Fukumura D, Kloepper J, Amoozgar Z, Duda DG, Jain RK. Enhancing cancer immunotherapy using antiangiogenics: opportunities and challenges. *Nature Reviews. Clinical Oncology*. 2018 05;15(5):325-340. <https://doi.org/10.1038/nrclinonc.2018.29>
 63. André N, Carré M, Pasquier E. Metronomics: towards personalized chemotherapy?. *Nature Reviews. Clinical Oncology*. 2014 07;11(7):413-431. <https://doi.org/10.1038/nrclinonc.2014.89>
 64. Wysocki PJ, Łobacz M, Potocki P, Kwinta Ł, Michałowska-Kaczmarczyk A, Słowik A, Konopka K, Buda-Nowak A. Metronomic Chemotherapy Based on Topotecan or Topotecan and Cyclophosphamide Combination (CyTo) in Advanced, Pretreated Ovarian Cancer. *Cancers*. 2023 02 07;15(4):1067. <https://doi.org/10.3390/cancers15041067>
 65. Kareva I. A Combination of Immune Checkpoint Inhibition with Metronomic Chemotherapy as a Way of Targeting Therapy-Resistant Cancer Cells. *International Journal of Molecular Sciences*. 2017 Oct 13;18(10):2134. <https://doi.org/10.3390/ijms18102134>
 66. Zsiros E, Lynam S, Attwood KM, Wang C, Chilakapati S, Gomez EC, Liu S, et al. Efficacy and Safety of Pembrolizumab in Combination With Bevacizumab and Oral Metronomic Cyclophosphamide in the Treatment of Recurrent Ovarian Cancer: A Phase 2 Nonrandomized Clinical Trial. *JAMA oncology*. 2021 01 01;7(1):78-85. <https://doi.org/10.1001/jamaoncol.2020.5945>
 67. Steck AR, Mahar B, Martin JY, McElrath TJ, Timmins PF, Barlin JN. Efficacy of pembrolizumab in combination with bevacizumab and oral metronomic cyclophosphamide in the treatment of platinum resistant recurrent ovarian cancer: a retrospective observational study. *Gynecologic Oncology Reports*. 2025 08;60:101793. <https://doi.org/10.1016/j.gore.2025.101793>



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