JPET Fast Forward. Published on February 8, 2019 as DOI: 10.1124/jpet.118.254979
This article has not been copyedited and formatted. The final version may differ from this version.

TITLE PAGE

Nanotechnology and immunotherapy in ovarian cancer: tracing new landscapes

Bruna Corradetti, Simone Pisano, Robert Steven Conlan, Mauro Ferrari

Affiliations

BC: Department of Nanomedicine, Houston Methodist Research Institute, 6670 Bertner Ave., Houston, TX 77030, USA; Swansea University Medical School, Singleton Park, Swansea, UK.

SP: Department of Nanomedicine, Houston Methodist Research Institute, 6670 Bertner Ave., Houston, TX 77030, USA; Swansea University Medical School, Singleton Park, Swansea, UK.

RSC: Swansea University Medical School, Singleton Park, Swansea, UK; Department of Nanomedicine, Houston Methodist Research Institute, 6670 Bertner Ave., Houston, TX 77030, USA.

MF: Department of Nanomedicine, Houston Methodist Research Institute, 6670 Bertner Ave., Houston, TX 77030, USA; Department of Medicine, Weill Cornell Medical College, New York, NY 10065, USA

RUNNING TITLE PAGE

Running title: Surveying nanotechnology and immunotherapy in ovarian cancer

Corresponding author: Bruna Corradetti, PhD

Department of Nanomedicine, Houston Methodist Research Institute,

6670 Berner Ave, 77030, Houston TX, USA

Telephone: +1 3462381001

Email: bcorradetti2@houstonmethodist.org

Text pages: 25

Number of Figures: 2

Number of Tables: 2

Abstract: 222 words

Introduction words: 684

Discussion words: n/a

Text words: 5347 words

List of non-standard abbreviations:

APC: antigen presenting cells

CAR-T: chimeric antigen receptor engineered T cells

CTA: cancer testis antigen

CTLA-4: cytotoxic T-lymphocyte antigen 4

DC: dendritic cells

DMAEMA: 2-(dimethylamino)ethyl methacrylate

EOC: epithelial ovarian cancer

Ep-CAM: epithelial cell adhesion molecule

FA: folic acid

FGF-b: fibroblastic growth factor beta

ICAM: intercellular cell adhesion molecule

IFN-g: interferon-gamma

IL: interleukin

LPN: lipid-polymer nanoparticles

MAGE: melanoma antigen-encoding gene

MHC: major histocompatibility complex

MIF: macrophage inhibitory factor

Mn: mannose ligand

NKT: natural killer T cells

NP: nanoparticles

OC: ovarian cancer

PARP: poly(adenosine diphosphate [ADP]-ribose) polymerases

PD-1: programmed death-ligand 1

PEG: polyethylene glycol

PEI: polyethylenimine

PLGA: poly(lactic-co-glycolic acid)

PyMT: polyoma middle T

TAM: tumor-associated macrophages

TIL: tumor infiltrating lymphocytes

TLR: toll-like receptor

Treg: T regulatory cells

UPR: unfolded protein response

VCAM: vascular cell adhesion molecule

VEGF: vascular endothelial growth factor

WTA: whole tumor antigens

Section: Drug Discovery and Translational Medicine

Keywords: Cancer immunotherapy, nanotechnology, ovarian cancer, checkpoint inhibitors, exosomes, RNA therapeutics

JPET Fast Forward. Published on February 8, 2019 as DOI: 10.1124/jpet.118.254979
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

Ovarian cancer (OC) is the seventh most common cancer in women worldwide. Standard therapeutic treatments involve debulking surgery combined with platinum-based chemotherapies. Of the patients with advanced stage cancer that initially respond to current treatments 50%-75% relapse. Immunotherapy-based approaches aimed at boosting anti-tumor immunity have recently emerged as promising tools to challenge tumor progression. Treatments with inhibitors of immune checkpoint molecules have shown impressive results in other types of tumors. However, only 15% of checkpoint inhibitors evaluated have proven successful in OC due to the immunosuppressive environment of the tumor and the transport barriers. This limits the efficacy of the existing immunotherapies. Nanotechnology-based delivery systems hold the potential to overcome such limitations. Various nanoformulations including polymeric, liposomes, and lipid–polymer hybrid nanoparticles have already been proposed to improve the biodistribution and targeting-capabilities of drugs against tumor-associated immune cells, including dendritic cells and macrophages. In this review, we examine the impact of immuno-therapeutic approaches that are currently under consideration for the treatment of OC. In this review we also provide a comprehensive analysis of the existing nanoparticle-based synthetic strategies, their limitations and advantages over standard treatments. Furthermore, we discuss how the strength of the combination of nanotechnology with immunotherapy may help to, overcome the current therapeutic limitations associated with their individual application and unravel a new paradigm in the treatment of this malignancy.

1. Introduction

Ovarian cancer (OC) ranks as the seventh leading cause of death in women worldwide. According to the American Cancer Society 14,070 deaths and approximately 22,240 new cases are predicted for 2018 in the United States (Siegel, 2018). Of the patients with advanced stage cancer that initially respond to current treatments 50%-75% relapse. The asymptomatic nature of early stage ovarian cancer is the main reason for its late diagnosis, which normally occurs at a metastatic stage, drastically reducing the chances of a successful outcome of the treatment (Das, Bast, & Jr, 2008; Rauh-Hain et al., 2011). Despite the continuous improvement in screening methods, OC-associated mortality rates remain high due to the absence of routine early detection approaches. The lack of specificity of the available tests and the limitations associated to the application of imaging techniques further complicate the diagnostic process (Russell et al., 2017; Sarojini et al., 2012; Terry et al., 2016). OC comprises five histological subtypes: low-high grade serous, mucinous, clear cells, and endometrioid cancer. Serous OC represents the most common carcinoma and accounts for more than 50% of all cases. It is associated with specific genetic mutations (i.e. *BRCA1, BRCA2, MMR, TP53, BARD1, CHEK2, RAD51, and PALB2*) spanning from single nucleotides polymorphisms to high frequency of somatic gene copies or epigenetic features, indicative of defects in homologous recombination repair and gene methylations (Ducie et al., 2017; Kaldawy et al., 2016). These subtypes metastatize to the same area, within the peritoneal cavity.

Currently, the treatment of OC includes debulking surgeries, which are meant to excise tumor masses, coupled with extensive chemotherapy, radiotherapy or a combination of the three depending on the stage and type of the cancer. Recommended first line

JPET Fast Forward. Published on February 8, 2019 as DOI: 10.1124/jpet.118.254979
This article has not been copyedited and formatted. The final version may differ from this version.

treatments for OC are platinum-based and taxols drugs (www.nccn.org/guidelines). In some cases, after genetic screening, patients may be eligible for monoclonal antibodies therapies such as Bevacizumab, which blocks tumoral angiogenesis by inhibiting the vascular endothelial growth factor (VEGF) signaling. Other approaches include using Olaparib, Rucaparib, and Niraparib, known as inhibitors of the poly(adenosine diphosphate [ADP]-ribose) polymerases (PARPs) and involved in DNA repair. The use of the latter treatments has been specifically recommended for patients with BRCA genes' mutations (Coward et al., 2015). **Table 1** explains the current therapies available for OC including standard and targeted chemotherapies. The state-of-the-art nanotherapies currently being use or tested in clinical trials are also mentioned.

The 5-year survival rate for women with advanced stage OC is approximately 40% (Timmermans et al., 2018; Torre et al., 2018) but increases if the ovarian tumor has more infiltrating T cells (L. Zhang et al., 2003). The lack of a curative therapeutic regime, the frequency of relapse, and the mortality levels underlie the effort needed to refine the current treatment options and improve patient outcomes. The diversity of physiopathology (Nezhat et al., 2015) between OC types and the heterogeneity of cells infiltrating the peritoneum calls for the identification of effective approaches to maintain the bioactivity of the payload, precisely aim the target, and preferentially accumulate the drug at the site of interest while reducing cytotoxicity.

Nanomedicines are frequently employed as engineered drug delivery systems that support the prolonged circulation of drugs, maintain their bioactivity, reduce their side effects, and selectively target diseased cells (Blanco et al., 2015). Targeted nanomedicines include liposomal nanocarriers (siRNA-EphA2, OSI-211, Myocet) (Eitan

et al., 2014; Seiden et al., 2004; H. Shen et al., 2013), polymeric nanoparticles (abraxane, CRLX101) (Pham et al., 2015; Srinivasan et al., 2014), and antibody-drug conjugates (Howard, Garcia-parra, et al., 2016). Nanotechnology-based strategies for diagnostic tools have been also developed to detect biomarkers and genetic mutations (Engelberth et al., 2014), as well as to combine nano-enabled therapeutic and diagnostic capabilities, giving rise to “nano-theranostics” (Yaari et al., 2016).

In this review we discuss the potential of cancer immunotherapy, a recently developed field that aims at treating cancer patients by re-stimulating their immune system. Particular emphasis will be given to its applications and pitfalls in OC. We also review how a nanomedicine approach to immunotherapy may overcome the current therapeutic limitations of the treatment of OC and unravel a new paradigm in the cure of this malignancy.

2. Immunotherapy and cancer

Cancer immunotherapy aims at stimulating the immune system in order to provide cancer prevention and treatment. The first discoveries of the crucial role played by the immune regulation in cancer progression have recently led to the 2018 Nobel Prize for Medicine and Physiology to Drs. James P. Allison and Tasuku Honjo (www.nobelprize.org). Their studies unraveled fundamental mechanisms that govern immune cell (specifically T cells) responses to cancer and provided insights to overcome immune system evasion by cancer. Since then, the use of immune checkpoint blockade has been widely recognized as an effective cancer treatment. In particular, Dr. Allison and his research group have been the first to identify the Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) protein, an

immune checkpoint receptor expressed on the surface of activated T cells, believed to regulate their proliferation. When the CTLA-4 pathway is activated by co-stimulatory molecules (CD80, CD86) the result is hindrance of T-cell function, which inhibits the T-cell strong anti-cancer potential (Leach et al., 1996). Based on these observations, a specific antibody was developed to retain CTLA-4 activation and maintain T-cells in an activated status (Chambers et al., 1996). Almost simultaneously, in 1992 Honjo's group discovered Programmed Death-ligand 1 (PD-1) which also acts as a T-cell retainer, finding an alternative way to defeat the tumor-mediated immune evasion. The insights provided by such inspiring scientists have led to many FDA-approved drugs for the treatment of various cancers. These drugs span from sipuleucel-T, approved in 2010 to target the immune system for the treatment of prostate cancer (Cheever & Higano, 2011), to ipilimumab, the first monoclonal antibody against CTLA-4 for metastatic melanoma (Lipson & Drake, 2011). By 2018, eight immunotherapies have been FDA-approved for the treatment of several cancers (**Table 2)**, including durvalumab (stage 3 lung cancer), blinatumomab (acute lymphoblastic leukemia), and nivolumab (used in combination with ipilimumab for previously untreated kidney cancers) (https://www.cancer.gov/fda-approvals).

Immunotherapeutic approaches include the use of targeted antibodies and vaccines against immune checkpoint inhibitors directed towards a specific immune cell population (Ventola, 2017). For instance, due to their antigen presenting capabilities, dendritic cells (DC) have been used to develop immune vaccines (Sabado et al., 2017). Depending on the molecules employed to activate them, DC are able to re-program or launch a cell-specific cytotoxic response. Conversely, tumor-associated macrophages (TAMs) have

been shown to exert different roles in tumor microenvironment development and flourishing (Mills et al., 2016). Approaches that target this macrophage population are currently being evaluated, especially since the discovery that the blockade of TAMs potentiates the immune checkpoint inhibitors' effect (Ries et al., 2014; Y. Zhu et al., 2014). Adoptive T cell therapy to re-engineer the T-cell populations against tumor initiation is another strategy that has been widely validated (Dzhandzhugazyan et al., 2018). The Chimeric Antigen Receptor re-engineered T cells (CAR-T) system has been recently approved by the FDA for the treatment of patients with leukemia, large B-cell and non-Hodgkin lymphomas (Zheng et al., 2018). Other focuses involve the use of a different immune cell population, the Natural Killer T cells (NKT). NKT cells naturally stimulate the innate and adaptive immune system in several ways, such as the release of interferon-gamma (IFN-γ) to activate the CD8+ T cell population (Mah & Cooper, 2016). They are being investigated as potential immunotherapies both as ex-vivo expanded cell vaccines and as combinatorial therapies (Nair & Dhodapkar, 2017).

3. Ovarian cancer: a "cold" enemy

The characterization of the topographic distribution of immune cells within the tumour in a panel of 177 human samples with different cancer types has recently led to their categorization in inflamed ("hot"), non-inflamed ("cold") and "immune excluded" patterns according to where the cells are positioned (Kather et al., 2018). Cold tumors are malignancies that display a very limited response to immunotherapies compared to other cancer types. OC is considered a "cold" tumor (Preston et al., 2011) despite the significant association between tumor immunity and ovarian patient outcomes and the strong

correlation between the presence of infiltrating lymphocytes in the primary tumor with patient survival (L. Zhang et al., 2003). The reasons behind this lack of effectiveness has yet to be clarified. A possible explanation, proposed for pancreatic cancer, suggests that the difference between hot and cold tumors reflects the way tumor infiltrating immune cells are recognized by cancer cells or engage in the tumor. If such, the properties of the microenvironment make a tumor hot or cold. Hot tumors are more sensitive to treatments that activate the T cell population, as they are considered to be the main drivers of the adaptive immune response, against tumor initiation (Haanen, 2017).

The tumor microenvironment is a complex hub where different cell types interact with each other and with the extracellular matrix and it is plausible that other cells, including antigen presenting cells (APC), play an active role in downregulating the immune system. APC, including the aforementioned DC, are highly responsive to external stimuli and the tumor surroundings can negatively affect their physiological behavior. Indeed, it has been shown that endoplasmic reticulum stress is also crucial for triggering cancer resistance mechanisms by activating the unfolded protein response, which in turn disrupts the physiological immune response (Yadav et al., 2014). Specifically, through the constitutive activation of the endoplasmic reticulum stress response factor XBP1, DC undergo an abnormal lipid accumulation that leads to their ineffective functioning (Cubillos-Ruiz et al., 2015). While low infiltration of immune cells both inside and outside the tumor is found in OC samples, the co-existence of different immune-microenvironments within the same patient partly explains the heterogeneity in the response to treatment often observed in patients with recurrent disease (Jiménez-Sánchez et al., 2017).

Currently there are no FDA-approved immunotherapies for OC, although there are several ongoing clinical trials. Of the 98 total clinical trials, 26 have been completed, 40 are actively recruiting patients, and 9 have been terminated before their planned end due to the inefficacy determined by the limitations described in the previous paragraphs (https://clinicaltrials.gov). In 2016, Gaillard et al. reported a comprehensive analysis of all clinical trials on checkpoint inhibitors, and discovered that, on average, the efficacy of these treatments is surprisingly poor on OC patients (Gaillard et al., 2016). The positive outcome was found to be around 10 to 15%. A schematic representation of the immunotherapy-based approaches used in OC and the interactions between different immune players and tumor cells is provided in **Figure 1**.

3.1 Monoclonal Antibodies in OC

In the attempt to enhance treatments for OC, a number of targets involved in tumor progression and immune suppression have been used to develop monoclonal antibodies capable of inhibiting their functions. Catumaxomab is a monoclonal antibody directed against the epithelial cell adhesion molecule (EpCAM), a glycoprotein highly expressed in OC (Tayama et al., 2017). This antibody is currently being evaluated in a phase II clinical study on patients resistant to chemotherapy (J. S. Berek et al., 2014). Following the identification of the Cancer Antigen 125 (CA125), which is the most renowned OC marker (Bast et al., 1981), its role as a suppressant of both natural killer cell activity (Patankar et al., 2005; Tyler et al., 2012) and antibody-dependent cellular toxicity (Kline et al., 2017) has been widely investigated. Several anti-CA125 monoclonal antibodies have been developed and tested, including oregovomab (J. Berek et al., 2009) and abagovomab (Sabbatini et al., 2013), although they did not prove to be effective in

improving the outcomes in advanced OC when used as a single-agent maintenance immunotherapy. Anti-CD25, daclizumab, has been clinically tested for its capacity to suppress the T regulatory (Treg) cell populations, responsible for shorter patient survival rates when infiltrated within the tumor (Barnett et al., 2010). While the trial has been completed, the results have not been released yet. The translational potential of anti-CD25-based platforms is limited by their non-specific binding as CD25 is widely expressed on T-cells populations.

3.2 Dendritic cell vaccines in OC

Dendritic cells have a pivotal role in launching the immune response due to their capacity of activating CD4+ or CD8+ T-cells (Sallusto & Lanzavecchia, 2002). Their role in the tumor microenvironment is the subject of an active contemporary research (Pfirschke et al., 2017). As plastic APC, DC are currently harnessed for their potential to boost the immune system against tumor initiation and progression. Scarlett et al. applied an inducible p53-dependent model of aggressive ovarian carcinoma to demonstrate that DC display differential immunostimulatory capacity during tumor initiation and escape (Scarlett et al., 2012). These changes correspond to significantly lower levels of MHC-II and CD40 on their surface. DC are tunable cells, capable of inducing either an immune surveillance effect or to release malignant growth, by activating or suppressing anti-tumor T-cells activity, respectively. DC-based vaccines have also been conceived in the context of OC, by ex vivo pulsing DC with tumor-derived components, as single tumor-associated peptides or peptide combinations (Liao & Disis, 2013). Cancer testis antigens (CTA) that are typically expressed in multiple types of tumors have also gained interest for their potential applicability in immunotherapy (Gjerstorff et al., 2015; Seifi-Alan et al., 2018).

NY-ESO-1, a member of the CTA family, has been used to produce either DC-based vaccines (NCT number NCT02387125) or adoptive T-cell therapies (NCT number NCT01567891). Similar immunotherapeutic approaches are being developed using melanoma antigens (i.e MAGE-A1, MAGE-A4, MAGE-A3, and MAGE-A10) that represent another subgroup of the CTA category (Daudi et al., 2014). Zitvogel et al. are among the first researchers to use tumor antigen-pulsed DC to treat mice with fibrosarcoma (Zitvogel et al., 1996). They also demonstrated that patient-derived DC pulsed with a cocktail of tumor antigens (whole tumor antigen; WTA) can trigger a tumor growth suppression through the activation of CD4+ and CD8+ T-cell when reintroduced into the patient. In their study, the activation of T-cells correlates with a better prognosis in patients with recurrent ovarian, fallopian tube, or primary peritoneal cancer (Kandalaft et al., 2013; Tanyi et al., 2018). Recently, a pilot study employing autologous WTA-pulsed DC-based vaccine demonstrated to be safe and effective in combination with cyclophosphamide and bevacizumab (Tanyi et al., 2018). By priming DC with patient-derived WTA, Tanyi et al. were able to overcome two of the limitations associated with the use of immunotherapy for the treatment of OC, namely the lack of an efficient antigen-specific active treatment and the inability of tumor-specific T cells to home to tumors.

3.3 Adoptive cell therapy in OC

Adoptive cell therapies show potential for the treatment of OC. For example, it is shown that tumor-infiltrating lymphocytes (TILs) derived from OC biopsies-derived cells can be expanded ex vivo and be re-activated to produce anti-tumor cytokines (Owens et al., 2018). Similarly, the abundance of TILs in patients' ascitic fluid has prompted their evaluation as re-injectable immunotherapies after their demonstrated cytotoxic effect on

tumor cells (Abe et al., 2018). CAR-T-based therapy produced by combining programmable antigen receptor specificity with T-cell activation also holds an attractive opportunity for the treatment of OC (Dzhandzhugazyan et al., 2018). The lack of a demonstrable efficacy of this approach is mainly due to the poor T-cell trafficking and the immunosuppressive microenvironment (Jindal et al., 2018; Mirzaei et al., 2017; B.-L. Zhang et al., 2016). Despite the potential pitfalls of this approach, clinical trials evaluating its efficacy on OC are currently active and specifically target mesothelin (NCT 02580747, 01583686), MUC16 (NCT 02498912), HER2 (NCT 01935843), NY-ESO-1 (NCT 02366546) among all (X. Zhu et al., 2017).

4. Nanomedicines and Immunotherapy in Ovarian Cancer

Synthetic and natural nanotechnologies are currently being investigated to deliver immunotherapies, as they have the potential to improve patient treatment outcomes and reduce the mortality rates (H. Shen et al., 2017). This includes the use of nanoparticles for the delivery of immunostimulatory and immunosuppressive molecules in combination with chemo- or radiotherapy or as adjuvants to other immunotherapies (Sapiezynski et al., 2016). Nanoparticles have also been designed to produce vaccines to stimulate T cell response against tumor growth (Fan & Moon, 2015), allowing for the co-delivery of antigen and adjuvants (A. Dunkle et al., 2013), contributing to the inclusion of multiple antigens to activate DC targets (Xia et al., 2015), and guaranteeing the sustained release of antigens for a prolonged immune stimulation (Engelberth et al., 2014).

Literature reports only few examples of pre-clinical studies investigating the potential of nanotechnology-based platforms to improve the outcome of immunotherapeutic regimens

in OC. These include polymeric nanoparticles (Cubillos-Ruiz et al., 2009; Hanlon et al., 2011; Ortega et al., 2015; Teo et al., 2015), liposomes (Rajan et al., 2018; Turk, Waters, & Low, 2004), and lipid–polymer hybrids (Anwer et al., 2013).

Nanoplatforms for OC have been synthesized primarily to guide the delivery of RNA oligonucleotides to target cells, thus overcoming the current limitations related to the use of RNA therapeutics. Limitations include the low bioavailability, poor cellular uptake, cytotoxicity and the need to evade the phagocytic cellular components of the immune system (Kole et al., 2012). Polymeric nanostructures have been developed to provide additional control over drug release at tumor sites as they offer the advantage of being able to respond to specific stimuli provided by the tumor environment, such as pH and enzymatic activity (Uthaman, Huh, & Park, 2018). Among the many polymers available, polyethylenimine (PEI) is one of the most employed materials in OC treatment as it is considered a versatile gene carrier (Teo et al., 2013). PEI displays high efficacy for siRNA encapsulation and delivery, for both in vitro and in vivo purposes. Its cationic charge enables the loading of siRNA into nanocomplexes and protects it from enzymatic degradation (Höbel & Aigner, 2013; M. Zheng et al., 2011). The abundant presence of amine groups allows for the functionalization of the platform and favors further modifications of this polymer to improve the bioactive features, such as its targeting ability and cell specificity. Cubillo et al. have investigated PEI-siRNA nanoparticles uptake by tumor-associated DC and its effect in reprogramming their phenotype from immunosuppressive cells to efficient APC. The authors found that the changes induced in DC through the use of PEI-siRNA against immunosuppressive determinants consequently activated tumor-reactive human and murine lymphocytes and exerted a

direct tumoricidal activity in aggressive ovarian carcinoma–bearing murine models (Cubillos-Ruiz et al., 2009). The induced T cell–mediated tumor regression and prolonged survival were dependent upon the activation of the myeloid differentiation primary response gene 88 (MyD88). PEI alone was sufficient to mediate the upregulation of MHC-II, MHC-I and co-stimulatory molecules in tumor DC in vivo. This suggests that the intrinsic stimulation of the Toll Like receptors (TLR) 5 and 7 by PEI nanoparticles synergizes with the gene-specific silencing activity of the siRNA to transform tumor-infiltrating regulatory DC into cells capable of promoting therapeutic antitumor immunity. Cubillos at al. further optimized the platform to achieve the synthetic enhancement of the specific molecular pathway miR-155 signaling in DC. This pathway is responsible for boosting a potent antitumor immune response that abrogates the progression of established ovarian cancers (Cubillos-Ruiz et al., 2012). Other researchers have taken advantages of polymeric nanoparticles' capability to be functionalized, thus improving targeting and, consequently, the therapeutic outcome. By applying a different immunotherapy-based approach Teo PY et al. proposed various folic acid (FA)–functionalized PEI polymers to block PD-1/PD-L1 interactions by delivering PD-L1 siRNA to human epithelial ovarian cancer (EOC) cells (SKOV-3 line), and to sensitize them against T cells (Teo et al., 2015). With their hypothesis to target PD-L1, the authors responded to the need for a specific targeted delivery of PD-L1 siRNA to epithelial cancer tissues, as PD-L1 is also expressed on healthy tissues (Liang et al., 2003), including placenta and eyes. The polymer/siRNA nanocomplexes knocked-down PD-L1 on a luciferase expressing SKOV-3, enhancing the efficacy of T cell immunotherapy for the treatment of EOC compared to the respective PEI–FA and PEI–PEG–FA/scrambled siRNA treated controls. These data highlight the

potential use of PEI–FA as specific gene delivery carriers. The modification of PEI with FA or PEG–FA proved to be a valuable tool to reduce cytotoxicity while improving tumor cell targeting towards EOC cells and uptake, with a striking ≈40%–50% knockdown of PD-L1 expression. Ortega et al. have used click chemistry to produce nanoparticles based on 2-(dimethylamino)ethyl methacrylate (DMAEMA) polymer further functionalized with the mannose ligand (MnNP). This platform was meant to condense siRNA against the polyoma middle T (PyMT) oncogene and specifically target the mannose receptor (CD206) present on the surface of TAM (Ortega et al., 2015). MnNP has been demonstrated to be biocompatible both in *in vitro* and *in vivo* settings. MnNP is also able to efficiently incorporate and deliver functional siRNA into the cytoplasm of TAM. This study provides evidence that mannosylation is responsible for TAM selectivity in vivo following intraperitoneal injection with a 2-fold increase in TAM uptake compared to non-targeted particles and about 10-fold increase compared to non-myeloid cells. In this study, the spatial confinement of the MnNP within the peritoneal cavity enhances the opportunity for the interaction with immune cells associated to OC, and the biodegradability of the system ensures the persistence of the treatment for over 24 hours.

Poly(lactic-co-glycolic acid) nanoparticles (PLGA-NP) are biodegradable and their composition can be tuned to temporally control the release of the payload (Corradetti et al., 2012; Minardi et al., 2016). PLGA-NP have been employed as an alternative route to deliver whole WTA to DC since the injection of soluble antigens presents inherent limitations due to instability and poor internalization rates. These factors result in the transient and inefficient activation of T-cells (Hanlon et al., 2011). At the same time, PLGA-NPs protect antigens from enzymatic degradation and maintain their bioactivity,

leading to a more efficient presentation of MHC-peptide complexes by recipient cells following uptake and processing. In vitro studies confirmed the effectiveness of PLGA-NPs in the activation of a CD8+ cell response, characterized by a significant increase in the production of inflammatory cytokines, a greater expression of co-stimulatory molecules, and providing encouraging evidence for their potential clinical translation. Interestingly, the delivery of WTA through PLGA-NP appeared to facilitate the antigens access to the MHC class I compartment in the cytoplasm, providing a reservoir for a prolonged and enhanced Ag presentation.

Liposomes are small artificial spherical vesicles synthesized primarily from natural non-toxic phospholipids (Akbarzadeh et al., 2013). Their wide application as drug-delivery systems in biomedical settings is due to their biocompatibility, biodegradability, low toxicity, and capability to load both hydrophobic and hydrophilic drugs (Johnston et al., 2007). Moreover, liposomal encapsulation offers the advantage to effectively enhance the solubility of lipophilic and amphiphilic drugs, and to improve site-specific drug delivery to tumor tissues through surface functionalization (Corradetti et al., 2012; Hofheinz et al., 2005). The latter aspect is crucial to increase the retention time which can be modulated by drug-lipid interactions, and permit the accumulation of liposome-encapsulated chemotherapeutic agents at the tumor site (Deshpande et al., 2013).

Doxil is the first pegylated liposome-based drug to enter the market in 1995. The nanoformulation includes doxorubicin, a DNA intercalating agent used against a variety of cancers, including gynecological cancers (Howard, et al., 2016). While no significant differences were observed in terms of efficacy compared to the free drug, the liposomal formulation allowed to reduce cardiotoxicities related to the use of doxorubicin and to

preferentially accumulate the drug at the tumor site (Green & Rose, 2006). More recently, the FDA approved the use of RNAi-therapeutic delivered by lipid nanoparticles, the patisiran (D. Adams et al., 2018). While developed for the treatment of degenerative diseases, Patisiran shows promise as a new breakthrough in patient care as it heralds the arrival of an entirely new class of medicines to treat human diseases. Despite the wide interest in the use of liposomal formulations for OC treatment, however, only one group has tested liposomes as nanocarriers for immunotherapy. Turk et al. developed folate-conjugated liposomes to target intraperitoneal ovarian carcinoma cells as they overexpress the folate receptor (Turk et al., 2004). Data revealed that this formulation was also uptaken by TAM through a folate receptor-mediated internalization, with a 10-fold increase in the engulfment of macrophages compared to ascitic tumor cells *in vivo*, corroborating the need to develop combinatorial strategies aiming at modulating TAM and inhibiting cancer cells growth.

Lipid–polymer hybrid nanoparticles (LPN) are core–shell nanoparticle structures constituted by a polymeric core and a lipid shell. LPN have been considered by other researchers to confer a high degree of physical stability to the platform, resulting in a superior *in vivo* cellular delivery efficacy (Hadinoto et al., 2013) compared to polymeric and liposomal nanoparticles. The combination of the two LPN platforms formulated with a lipopolymer PEG-PEI-Cholesterol were employed as an effective tool to deliver an interleukin 12 (IL-12) plasmid at the tumor site. IL-12 was chosen for the therapeutic action it plays in OC, which relies on its potential to activate the anti-tumor immunity

(Whitworth & Alvarez, 2011). This approach proved to be safe and effective in platinum-sensitive OC patients treated with IV carboplatin and docetaxel (Anwer et al., 2013).

5. Physical and biological barriers challenging the treatment of OC

Innovative immunotherapeutic targeted strategies mediated by nanotechnology offer the promise of enhancing host anti-tumor responses which may improve clinical outcomes in women with OC. Although preclinical studies have demonstrated the induction of an anti-tumor response, there is no clinically effective nanomedicine-based immunotherapy available for OC patients. The biological barriers that physically and mechanically influence the processes involved in tumor spread and immune cell infiltration must be considered when developing new strategies for the treatment of OC. As mentioned above, one of the main mechanisms by which OC cells spread is through transcoelomic metastasis, which involves dissemination throughout the peritoneal cavity (Tan, Agarwal, & Kaye, 2006). Ascites formation is determined by the accumulation of cancer cells, growth factors and immunosuppressive ligands (VEGF and fibroblastic growth factor beta (FGF-β)), which increase peritoneal capillary permeability (Ahmed & Stenvers, 2013) and thus the leakage of plasma proteins (i.e albumin, fibrin and fibrinogen) from newly developed vessels (Stanojevic et al., 2004). The obstruction of lymphatic vessels by cancer cells also occurs, which leads to an impaired re-absorption of the physiological peritoneal fluid (Kipps et al., 2013). As a consequence of the compromised lymphatic drainage of the peritoneal cavity, fluid confinement in the peritoneum occurs contributing to the pathogenesis of malignant ascites. The environment that these biological and physical processes create impedes immune cell migration and infiltration within the

metastatic tumors (Cai & Jin, 2017), and induces a peripheral tolerance that attenuates their function (Kulshrestha et al., 2017). For instance, ascites proved to recruit and immunologically suppress a population of neutrophils through cell contact in a cohort of newly diagnosed OC patients (Singel et al., 2017). The release of macrophage migration inhibitory factor (MIF) from ascites-derived cancer cells has also been proposed to halt the tumour-killing ability of NK cells by transcriptionally down regulating the expression of the surface receptor NKG2D (Krockenberger et al., 2008). These findings confirm the proactive role of malignant ascitic fluid in physically supplying cells and chemical stimuli to favour an immune suppressed environment. Additionally, another physical barrier to immune cells penetration is represented by the tumour vascular endothelium (Motz & Coukos, 2013). In a physiological environment the presence of adhesion molecules such as intercellular cell adhesion molecule (ICAM) or vascular cell adhesion molecule (VCAM) allows T-cells to adhere to and travel through the endothelium. In the tumour milieu the release of angiogenic growth factors impedes T-cell to pass through by inhibiting the adhesion molecules expression (Bouzin et al., 2007).

The employment of nano-sized molecules/structures able not only to precisely target and accumulate at the site of interest, maintain the bioactivity of the drug while ensuring its release but also to overcome biological and physical barriers is pivotal in unveiling the mechanisms behind tumoral immune suppression. The development of approaches capable of capitalizing on the transport oncophysics of the peritoneal cavity will improve the delivery strategies for the treatment of metastatic OC (Nizzero et al., 2018).

6. Exosomes: an alternative tunable and nanoscopic strategy

Recently, biological nanoparticles (called exosomes) have also emerged as a powerful translational platform to be harnessed in the development of naturally inspired delivery systems. Exosomes are nanoscopic lipidic vesicles with a size range spanning from 30 to 150 nm that are released by cells and thus retain their bioactive moieties. Due to their small size and architecture exosomes can penetrate across the lymphatic vessels and tumor interstitium and reach target organs (S. Srinivasan et al., 2016). Their composition and cargo can be further modified by conditioning parental cells or by improving their natural potential with the addition of functional drugs, thus conferring them additional functions (Conlan et al., 2017). Exosomes play a crucial role in cell-to-cell communication and are characterized by a precise targeting potential that allows for the activation or repression of specific molecular cascades in targeted cells (Syn et al., 2017). Currently, their role in the exchange of information between the tumor and the surrounding microenvironment is being explored (Maia et al., 2018), as is their potential as delivery vessels for both therapeutic and imaging purposes (Luan et al., 2017; L.-M. Shen et al., 2018).

Recent advances in the field of immunotherapy unveiled the role of appropriately stimulated exosomes released from cancer cells as potent endogenous nanocarriers responsible for the suppression of T cells and the facilitation of tumor growth (Chen et al., 2018). Once injected for therapeutic purposes, exosomes are not susceptible to further modifications determined by the microenvironment, offering a great advantage over the use of CAR-T cells or DC, which are amenable to acquire a different phenotype (Yamashita et al., 2018).

Interestingly, they have been also proposed as useful tools to predict the patient response to immunotherapy. On the other hand, exosomes derived from immune cells, APC or TAM, are now at the forefront for the development of innovative vaccine strategies for cancer immunotherapy against tumor initiation and are the subject of current clinical trials for the treatment of other tumor types (Hong et al., 2017; Liu et al., 2018).

7. Conclusions and Perspectives

In this review we have discussed the widely recognized impact of immunotherapy in the treatment of cancers, highlighting the challenges researchers face in the effort to overcome the limitations provided by OC. These include its cold nature, determined by the immunosuppressive environment and the transport oncophysics, which urgently calls for the conception of alternative approaches to deliver immunotherapies. Ideally, these approaches are meant to preferentially accumulate the drug at the tumor site, sustain the temporal and spatial release of the payload thereby reducing cytotoxicity, and selectively target specific cell types to stimulate anti-tumor immunity (Figure 2). Nanotechnology offers advantageous drug delivery systems with demonstrated therapeutic efficacy, with a direct or indirect effect on cancer cells. However, the potential of nanomedicines for the treatment of OC has been limitedly harnessed. Although capable of identifying and targeting the cell population of interest, none of the nano-enabled strategies proposed have yet shown significant clinical benefits. Furthermore, literature lacks a comprehensive discussion about the *in vivo* biodistribution of the proposed nanoplatforms, reinforcing the concept that the drastic changes within the peritoneal cavity in terms of transport oncophysics and metastases heterogeneity, largely limit their capability to reach tumor

masses. A deep understanding of the role exosomes play in travelling and mediating cell interaction within the OC environment will successfully lead to the development of cutting-edge approaches to prime the body's immune system against tumor initiation. The continuous advancements in the field of nanotechnology will provide the tools needed to synthesize exosomes-resembling particles to be used as alternative immunotherapy treatment for OC. Another approach may include the coupling of naturally derived exosomes with established multistage vectors (MSV), demonstrated to achieve efficient delivery of chemotherapeutics to metastatic breast cancer (Xu et al., 2016) and ovarian tumor tissues (H. Shen et al., 2013). The possibility to exploit the physical properties of the ascitic fluid and the geometry of the peritoneal cavity during metastatic OC to tailor the architecture of MSV paves the way for the fabrication of nanotechnology-based immunotherapies to accomplish the challenge of boosting the anti-cancer immune system and minimizing tumor relapse.

7. Acknowledgement

Mauro Ferrari gratefully acknowledges support through NIH/NCI center grant U54CA210181, NIH/NCI NIH/NCI R01CA222959, DoD Breast Cancer Research Breakthrough Level IV Award W81XWH-17-1-0389, and his Ernest Cockrell Jr. Presidential Distinguished Chair at Houston Methodist Research Institute. Mauro Ferrari serves on the Board of Directors of Arrowhead Pharmaceuticals. Bruna Corradetti acknowledges support through the Sêr Cymru II programme, funded by the European Commission through the Horizon 2020 Marie Skłodowska-Curie Actions (MSCA)

COFUND scheme and the Welsh European Funding Office (WEFO) under the European Regional Development Fund (ERDF). Simone Pisano is sponsored by the Swansea University (UK)/Houston Methodist Research Institute (US) joint PhD Initiative. The authors gratefully acknowledge Ms. Federica Ferrari and Mr. Oscar Velascquez for the editorial assistance and Mr Matthew Landry for the graphical support.

8. Authorship Contributions

Contributed to the writing of the manuscript: Corradetti, Pisano, Conlan, and Ferrari.

9. References

Abe, Y., Kobayashi, H., Akizawa, Y., Ishitani, K., Hashimoto, K., & Matsui, H. (2018). Possible Application of Ascites-infiltrating Gamma-delta T Cells for Adoptive Immunotherapy. *Anticancer Research*, *38*(7), 4327–4331. https://doi.org/10.21873/anticanres.12732

Adams, D., Gonzalez-Duarte, A., O'Riordan, W. D., Yang, C.-C., Ueda, M., Kristen, A. V., … Suhr, O. B. (2018). Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. *New England Journal of Medicine*, *379*(1), 11–21. https://doi.org/10.1056/NEJMoa1716153

Adams, M., Kerby, I. J., Rocker, I., Evans, A., Johansen, K., & Franks, C. R. (1989). A comparison of the toxicity and efficacy of cisplatin and carboplatin in advanced ovarian cancer. The Swons Gynaecological Cancer Group. *Acta Oncologica (Stockholm, Sweden)*, *28*(1), 57–60. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2650722

Ahmed, N., & Stenvers, K. L. (2013). Getting to Know Ovarian Cancer Ascites: Opportunities for Targeted Therapy-Based Translational Research. *Frontiers in Oncology*, *3*, 256. https://doi.org/10.3389/fonc.2013.00256

Akbarzadeh, A., Rezaei-Sadabady, R., Davaran, S., Joo, S. W., Zarghami, N., Hanifehpour, Y., … Nejati-Koshki, K. (2013). Liposome: Classification, preparation, and applications. *Nanoscale Research Letters*. https://doi.org/10.1186/1556-276X-8-102

Alexis Dunkle, Craig Blanchette, Tyler Boone, Michele Corzett, Nicholas Fischer, Paul Hoeprich, A. D. and A. R. (2013). *Co-delivery of adjuvant and subunit antigens via a nanoparticle platform induces tissue-associated and systemic adaptive immune responses (P4409)*. *The Journal of Immunology* (Vol. 190). Williams & Wilkins. Retrieved from http://www.jimmunol.org/content/190/1_Supplement/205.14

Anwer, K., Kelly, F. J., Chu, C., Fewell, J. G., Lewis, D., & Alvarez, R. D. (2013). Phase i trial of a formulated IL-12 plasmid in combination with carboplatin and docetaxel chemotherapy in the treatment of platinum-sensitive recurrent ovarian cancer. *Gynecologic Oncology*. https://doi.org/10.1016/j.ygyno.2013.07.081

Barnett, J. C., Bean, S. M., Whitaker, R. S., Kondoh, E., Baba, T., Fujii, S., … Berchuck, A. (2010). Ovarian cancer tumor infiltrating T-regulatory (Treg) cells are associated with a metastatic phenotype. *Gynecologic Oncology*. https://doi.org/10.1016/j.ygyno.2009.11.020

Bast, R. C., Feeney, M., Lazarus, H., Nadler, L. M., Colvin, R. B., & Knapp, R. C. (1981). Reactivity of a monoclonal antibody with human ovarian carcinoma. *The Journal of Clinical Investigation*. https://doi.org/10.1172/JCI110380

Berek, J. S., Edwards, R. P., Parker, L. P., DeMars, L. R., Herzog, T. J., Lentz, S. S., … Krasner, C. N. (2014). Catumaxomab for the Treatment of Malignant Ascites in Patients With Chemotherapy-Refractory Ovarian Cancer. *International Journal of Gynecological Cancer*, *24*(9), 1583–1589. https://doi.org/10.1097/IGC.0000000000000286

Berek, J., Taylor, P., McGuire, W., Smith, L. M., Schultes, B., & Nicodemus, C. F. (2009). Oregovomab Maintenance Monoimmunotherapy Does Not Improve Outcomes in Advanced Ovarian Cancer. *Journal of Clinical Oncology*, *27*(3), 418–425. https://doi.org/10.1200/JCO.2008.17.8400

Blanco, E., Shen, H., & Ferrari, M. (2015). Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nature Biotechnology*, *33*(9), 941–951. https://doi.org/10.1038/nbt.3330

Bolis, G., D'Incalci, M., Gramellini, F., & Mangioni, C. (1978). Adriamycin in ovarian cancer patients resistant to cyclophosphamide. *European Journal of Cancer*, *14*(12), 1401–1402. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/738344

Bouzin, C., Brouet, A., Vriese, J. De, DeWever, J., & Feron, O. (2007). Effects of Vascular Endothelial Growth Factor on the Lymphocyte-Endothelium Interactions: Identification of Caveolin-1 and Nitric Oxide as Control Points of Endothelial Cell Anergy. *The Journal of Immunology*, *178*(3), 1505–1511. https://doi.org/10.4049/JIMMUNOL.178.3.1505

Cai, D. L., & Jin, L.-P. (2017). Immune Cell Population in Ovarian Tumor Microenvironment. *Journal of Cancer*, *8*(15), 2915–2923. https://doi.org/10.7150/jca.20314

Chambers, C. A., Krummel, M. F., Boitel, B., Hurwitz, A., Sullivan, T. J., Fournier, S., … Allison, J. P. (1996). The role of CTLA-4 in the regulation and initiation of T-cell responses. *Immunological Reviews*, *153*, 27–46. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9010718

Cheever, M. A., & Higano, C. S. (2011). PROVENGE (Sipuleucel-T) in Prostate Cancer: The First FDA-Approved Therapeutic Cancer Vaccine. https://doi.org/10.1158/1078-0432.CCR-10-3126

Chen, G., Huang, A. C., Zhang, W., Zhang, G., Wu, M., Xu, W., … Guo, W. (2018). Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. *Nature*, *560*(7718), 382–386. https://doi.org/10.1038/s41586-018-0392-8

Chou, H.-H., Wang, K.-L., Chen, C.-A., Wei, L.-H., Lai, C.-H., Hsieh, C.-Y., … Yen, M.-S. (2006). Pegylated liposomal doxorubicin (Lipo-Dox®) for platinum-resistant or refractory epithelial ovarian carcinoma: A Taiwanese gynecologic oncology group study with long-term follow-up. *Gynecologic Oncology*, *101*(3), 423–428. https://doi.org/10.1016/J.YGYNO.2005.10.027

Conlan, R. S., Pisano, S., Oliveira, M. I., Ferrari, M., & Mendes Pinto, I. (2017). Exosomes as Reconfigurable Therapeutic Systems. *Trends in Molecular Medicine*. https://doi.org/10.1016/j.molmed.2017.05.003

Corradetti, B., Freile, P., Pells, S., Bagnaninchi, P., Park, J., Fahmy, T. M., & de Sousa, P. A. (2012). Paracrine signalling events in embryonic stem cell renewal mediated by affinity targeted nanoparticles. *Biomaterials*, *33*(28), 6634–6643. https://doi.org/10.1016/j.biomaterials.2012.06.011

Coward, J. I., Middleton, K., & Murphy, F. (2015). New perspectives on targeted therapy in ovarian cancer. *International Journal of Women's Health*, *7*, 189–203. https://doi.org/10.2147/IJWH.S52379

Cubillos-Ruiz, J. R., Baird, J. R., Tesone, A. J., Rutkowski, M. R., Scarlett, U. K., Camposeco-Jacobs, A. L., … Conejo-Garcia, J. R. (2012). Reprogramming tumor-associated dendritic cells in vivo using miRNA mimetics triggers protective immunity against ovarian cancer. *Cancer Research*. https://doi.org/10.1158/0008-5472.CAN-11-3160

Cubillos-Ruiz, J. R., Engle, X., Scarlett, U. K., Martinez, D., Barber, A., Elgueta, R., … Conejo-Garcia, J. R. (2009). Polyethylenimine-based siRNA nanocomplexes reprogram tumor-associated dendritic cells via TLR5 to elicit therapeutic antitumor immunity. *Journal of Clinical Investigation*. https://doi.org/10.1172/JCI37716

Cubillos-Ruiz, J. R., Silberman, P. C., Rutkowski, M. R., Chopra, S., Perales-Puchalt, A., Song, M., … Glimcher, L. H. (2015). ER Stress Sensor XBP1 Controls Anti-tumor Immunity by Disrupting Dendritic Cell Homeostasis. *Cell*. https://doi.org/10.1016/j.cell.2015.05.025

Dal Molin, G. Z., Omatsu, K., Sood, A. K., & Coleman, R. L. (2018). Rucaparib in ovarian cancer: an update on safety, efficacy and place in therapy. *Therapeutic Advances in Medical Oncology*, *10*, 1758835918778483. https://doi.org/10.1177/1758835918778483

Damjanov, N., Fishman, M. N., Steinberg, J. L., Fetterly, G. J., Haas, A., Grahn, A., … Rubin, E. H. (2005). Final results of a Phase I study of Liposome Entrapped Paclitaxel (LEP-ETU) in patients with advanced cancer. *Journal of Clinical*

Oncology, *23*(16_suppl), 2048–2048. https://doi.org/10.1200/jco.2005.23.16_suppl.2048

Das, P. M., Bast, R. C., & Jr. (2008). Early detection of ovarian cancer. *Biomarkers in Medicine*, *2*(3), 291–303. https://doi.org/10.2217/17520363.2.3.291

Daudi, S., Eng, K. H., Mhawech-Fauceglia, P., Morrison, C., Miliotto, A., Beck, A., … Odunsi, K. (2014). Expression and immune responses to MAGE antigens predict survival in epithelial ovarian cancer. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0104099

Deshpande, P. P., Biswas, S., & Torchilin, V. P. (2013). Current trends in the use of liposomes for tumor targeting. *Nanomedicine (London, England)*, *8*(9), 1509–1528. https://doi.org/10.2217/nnm.13.118

Ducie, J., Dao, F., Considine, M., Olvera, N., Shaw, P. A., Kurman, R. J., … Levine, D. A. (2017). Molecular analysis of high-grade serous ovarian carcinoma with and without associated serous tubal intra-epithelial carcinoma. *Nature Communications*, *8*(1), 990. https://doi.org/10.1038/s41467-017-01217-9

Dzhandzhugazyan, K. N., Guldberg, P., & Kirkin, A. F. (2018). Adoptive T cell cancer therapy. *Nature Materials*, *17*(6), 475–477. https://doi.org/10.1038/s41563-018-0094-5

Eitan, R., Fishman, A., Meirovitz, M., Goldenberg, H., Amit, A., Koren, C., … Safra, T. (2014). Liposome-encapsulated doxorubicin citrate (Myocet) for treatment of recurrent epithelial ovarian cancer. *Anti-Cancer Drugs*, *25*(1), 101–105. https://doi.org/10.1097/CAD.0000000000000023

Engelberth, S. A., Hempel, N., & Bergkvist, M. (2014). Development of Nanoscale

Approaches for Ovarian Cancer Therapeutics and Diagnostics. *Critical Reviews in Oncogenesis*. https://doi.org/10.1615/CritRevOncog.2014011455

Essel, K. G., & Moore, K. N. (2018). Niraparib for the treatment of ovarian cancer. *Expert Review of Anticancer Therapy*, *18*(8), 727–733. https://doi.org/10.1080/14737140.2018.1490180

Fan, Y., & Moon, J. (2015). Nanoparticle Drug Delivery Systems Designed to Improve Cancer Vaccines and Immunotherapy. *Vaccines*, *3*(3), 662–685. https://doi.org/10.3390/vaccines3030662

Gaillard, S. L., Secord, A. A., & Monk, B. (2016). The role of immune checkpoint inhibition in the treatment of ovarian cancer. *Gynecologic Oncology Research and Practice*. https://doi.org/10.1186/s40661-016-0033-6

Gjerstorff, M. F., Andersen, M. H., & Ditzel, H. J. (2015). Oncogenic cancer/testis antigens: prime candidates for immunotherapy. *Oncotarget*, *6*(18), 15772–15787. https://doi.org/10.18632/oncotarget.4694

Gordinier, M. E., Kudelka, A. P., Kavanagh, J. J., Wharton, J. T., & Freedman, R. S. (2002). Thiotepa in combination with cisplatin for primary epithelial ovarian cancer: a phase II study. *International Journal of Gynecological Cancer : Official Journal of the International Gynecological Cancer Society*, *12*(6), 710–714. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12445247

Green, A. E., & Rose, P. G. (2006). Pegylated liposomal doxorubicin in ovarian cancer. *International Journal of Nanomedicine*, *1*(3), 229–239. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17717964

Haanen, J. B. A. G. (2017). Converting Cold into Hot Tumors by Combining

Immunotherapies. *Cell*. https://doi.org/10.1016/j.cell.2017.08.031

Hadinoto, K., Sundaresan, A., & Cheow, W. S. (2013). Lipid–polymer hybrid nanoparticles as a new generation therapeutic delivery platform: A review. *European Journal of Pharmaceutics and Biopharmaceutics*, *85*(3), 427–443. https://doi.org/10.1016/j.ejpb.2013.07.002

Handolias, D., Quinn, M., Foo, S., Mileshkin, L., Grant, P., Dutu, G., & Rischin, D. (2016). Oral cyclophosphamide in recurrent ovarian cancer. *Asia-Pacific Journal of Clinical Oncology*, *12*(1), e154–e160. https://doi.org/10.1111/ajco.12074

Hanlon, D. J., Aldo, P. B., Devine, L., Alvero, A. B., Engberg, A. K., Edelson, R., & Mor, G. (2011). Enhanced Stimulation of Anti-Ovarian Cancer CD8+ T Cells by Dendritic Cells Loaded with Nanoparticle Encapsulated Tumor Antigen. *American Journal of Reproductive Immunology*. https://doi.org/10.1111/j.1600-0897.2010.00968.x

Hasan, J., & Jayson, G. C. (2003). Oral melphalan as a treatment for platinum-resistant ovarian cancer. *British Journal of Cancer*, *88*(12), 1828–1830. https://doi.org/10.1038/sj.bjc.6601044

Höbel, S., & Aigner, A. (2013). Polyethylenimines for siRNA and miRNA delivery in vivo. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, *5*(5), 484–501. https://doi.org/10.1002/wnan.1228

Hofheinz, R. D., Gnad-Vogt, S. U., Beyer, U., & Hochhaus, A. (2005). Liposomal encapsulated anti-cancer drugs. *Anti-Cancer Drugs*. https://doi.org/10.1097/01.cad.0000167902.53039.5a

Hong, C.-S., Sharma, P., Yerneni, S. S., Simms, P., Jackson, E. K., Whiteside, T. L., & Boyiadzis, M. (2017). Circulating exosomes carrying an immunosuppressive cargo

interfere with cellular immunotherapy in acute myeloid leukemia. *Scientific Reports*, *7*(1), 14684. https://doi.org/10.1038/s41598-017-14661-w

Howard, D., Garcia-parra, J., Healey, G. D., Amakiri, C., Margarit, L., Francis, L. W., … Conlan, R. S. (2016). Antibody – drug conjugates and other nanomedicines : the frontier of gynaecological cancer treatment. *Interface Focus*, *6*(6), 6–12. https://doi.org/10.1098/rsfs.2016.0054

Howard, D., Garcia-Parra, J., Healey, G. D., Amakiri, C., Margarit, L., Francis, L. W., … Conlan, R. S. (2016). Antibody-drug conjugates and other nanomedicines: The frontier of gynaecological cancer treatment. *Interface Focus*. https://doi.org/10.1098/rsfs.2016.0054

Jiménez-Sánchez, A., Memon, D., Pourpe, S., Veeraraghavan, H., Li, Y., Vargas, H. A., … Miller, M. L. (2017). Heterogeneous Tumor-Immune Microenvironments among Differentially Growing Metastases in an Ovarian Cancer Patient. *Cell*, *170*(5), 927–938.e20. https://doi.org/10.1016/j.cell.2017.07.025

Jindal, V., Arora, E., Gupta, S., Lal, A., Masab, M., & Potdar, R. (2018). Prospects of chimeric antigen receptor T cell therapy in ovarian cancer. *Medical Oncology*, *35*(5), 70. https://doi.org/10.1007/s12032-018-1131-6

Johnston, M. J. W., Semple, S. C., Klimuk, S. K., Ansell, S., Maurer, N., & Cullis, P. R. (2007). Characterization of the drug retention and pharmacokinetic properties of liposomal nanoparticles containing dihydrosphingomyelin. *Biochimica et Biophysica Acta - Biomembranes*. https://doi.org/10.1016/j.bbamem.2007.01.019

Kaldawy, A., Segev, Y., Lavie, O., Auslender, R., Sopik, V., & Narod, S. A. (2016). Low-grade serous ovarian cancer: A review. *Gynecologic Oncology*, *143*(2), 433–438.

https://doi.org/10.1016/j.ygyno.2016.08.320

Kandalaft, L. E., Chiang, C. L., Tanyi, J., Motz, G., Balint, K., Mick, R., & Coukos, G. (2013). A Phase I vaccine trial using dendritic cells pulsed with autologous oxidized lysate for recurrent ovarian cancer. *Journal of Translational Medicine*. https://doi.org/10.1186/1479-5876-11-149

Kather, J. N., Suarez-Carmona, M., Charoentong, P., Weis, C.-A., Hirsch, D., Bankhead, P., … Halama, N. (2018). Topography of cancer-associated immune cells in human solid tumors. *ELife*, *7*. https://doi.org/10.7554/eLife.36967

Khanna, C., Rosenberg, M., & Vail, D. M. (2015). A Review of Paclitaxel and Novel Formulations Including Those Suitable for Use in Dogs. *Journal of Veterinary Internal Medicine*, *29*(4), 1006–1012. https://doi.org/10.1111/jvim.12596

Kipps, E., Tan, D. S. P., & Kaye, S. B. (2013). Meeting the challenge of ascites in ovarian cancer: new avenues for therapy and research. *Nature Reviews Cancer*, *13*(4), 273–282. https://doi.org/10.1038/nrc3432

Kline, J. B., Kennedy, R. P., Albone, E., Chao, Q., Fernando, S., McDonough, J. M., … Nicolaides, N. C. (2017). Tumor antigen CA125 suppresses antibody-dependent cellular cytotoxicity (ADCC) via direct antibody binding and suppressed Fc-γ receptor engagement. *Oncotarget*, *8*(32), 52045–52060. https://doi.org/10.18632/oncotarget.19090

Kole, R., Krainer, A. R., & Altman, S. (2012). RNA therapeutics: beyond RNA interference and antisense oligonucleotides. *Nature Reviews. Drug Discovery*, *11*(2), 125–140. https://doi.org/10.1038/nrd3625

Krockenberger, M., Dombrowski, Y., Weidler, C., Ossadnik, M., Hönig, A., Häusler, S.,

… Wischhusen, J. (2008). Macrophage migration inhibitory factor contributes to the immune escape of ovarian cancer by down-regulating NKG2D. *Journal of Immunology (Baltimore, Md. : 1950)*, *180*(11), 7338–7348. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18490733

Kulshrestha, D., Yeh, L.-T., Chien, M.-W., Chou, F.-C., & Sytwu, H.-K. (2017). Peripheral Autoimmune Regulator Induces Exhaustion of CD4+ and CD8+ Effector T Cells to Attenuate Autoimmune Diabetes in Non-Obese Diabetic Mice. *Frontiers in Immunology*, *8*, 1128. https://doi.org/10.3389/fimmu.2017.01128

Leach, D. R., Krummel, M. F., & Allison, J. P. (1996). Enhancement of antitumor immunity by CTLA-4 blockade. *Science*. https://doi.org/10.1126/science.271.5256.1734

Lee, S.-W., Kim, Y.-M., Kim, Y. T., & Kang, S. B. (2017). An open-label, multicenter, phase I trial of a cremophor-free, polymeric micelle formulation of paclitaxel combined with carboplatin as a first-line treatment for advanced ovarian cancer: a Korean Gynecologic Oncology Group study (KGOG-3016). *Journal of Gynecologic Oncology*, *28*(3), e26. https://doi.org/10.3802/jgo.2017.28.e26

Liang, S. C., Latchman, Y. E., Buhlmann, J. E., Tomczak, M. F., Horwitz, B. H., Freeman, G. J., & Sharpe, A. H. (2003). Regulation of PD-1, PD-L1, and PD-L2 expression during normal and autoimmune responses. https://doi.org/10.1002/eji.200324228

Liao, J. B., & Disis, M. L. (2013). Therapeutic vaccines for ovarian cancer. *Gynecologic Oncology*, *130*(3), 667–673. https://doi.org/10.1016/j.ygyno.2013.06.023

Lipson, E. J., & Drake, C. G. (2011). Ipilimumab: An anti-CTLA-4 antibody for metastatic

melanoma. *Clinical Cancer Research*. https://doi.org/10.1158/1078-0432.CCR-11-1595

Liu, H., Chen, L., Peng, Y., Yu, S., Liu, J., Wu, L., … Liu, T. (2018). Dendritic cells loaded with tumor derived exosomes for cancer immunotherapy. *Oncotarget*, *9*(2), 2887–2894. https://doi.org/10.18632/oncotarget.20812

Long, H. J., Bundy, B. N., Grendys, E. C., Benda, J. A., McMeekin, D. S., Sorosky, J., … Gynecologic Oncology Group Study. (2005). Randomized Phase III Trial of Cisplatin With or Without Topotecan in Carcinoma of the Uterine Cervix: A Gynecologic Oncology Group Study. *Journal of Clinical Oncology*, *23*(21), 4626–4633. https://doi.org/10.1200/JCO.2005.10.021

Lorusso, D., Di Stefano, A., Fanfani, F., & Scambia, G. (2006). Role of gemcitabine in ovarian cancer treatment. *Annals of Oncology*, *17*(suppl_5), v188–v194. https://doi.org/10.1093/annonc/mdj979

Luan, X., Sansanaphongpricha, K., Myers, I., Chen, H., Yuan, H., & Sun, D. (2017). Engineering exosomes as refined biological nanoplatforms for drug delivery. *Acta Pharmacologica Sinica*, *38*(6), 754–763. https://doi.org/10.1038/aps.2017.12

Mah, A. Y., & Cooper, M. A. (2016). Metabolic Regulation of Natural Killer Cell IFN-γ Production. *Critical Reviews in Immunology*, *36*(2), 131–147. https://doi.org/10.1615/CritRevImmunol.2016017387

Maia, J., Caja, S., Strano Moraes, M. C., Couto, N., & Costa-Silva, B. (2018). Exosome-Based Cell-Cell Communication in the Tumor Microenvironment. *Frontiers in Cell and Developmental Biology*, *6*, 18. https://doi.org/10.3389/fcell.2018.00018

Mills, C. D., Lenz, L. L., & Harris, R. A. (2016). A breakthrough: Macrophage-directed

cancer immunotherapy. *Cancer Research*. https://doi.org/10.1158/0008-5472.CAN-15-1737

Minardi, S., Corradetti, B., Taraballi, F., Sandri, M., Martinez, J. O., Powell, S. T., … Tasciotti, E. (2016). Biomimetic Concealing of PLGA Microspheres in a 3D Scaffold to Prevent Macrophage Uptake. *Small*, *12*(11), 1479–1488. https://doi.org/10.1002/smll.201503484

Mirzaei, H. R., Rodriguez, A., Shepphird, J., Brown, C. E., & Badie, B. (2017). Chimeric Antigen Receptors T Cell Therapy in Solid Tumor: Challenges and Clinical Applications. *Frontiers in Immunology*, *8*, 1850. https://doi.org/10.3389/fimmu.2017.01850

Monneret, C. (2011). Platinum anticancer drugs. From serendipity to rational design. *Annales Pharmaceutiques Françaises*, *69*(6), 286–295. https://doi.org/10.1016/J.PHARMA.2011.10.001

Moore, K., Colombo, N., Scambia, G., Kim, B.-G., Oaknin, A., Friedlander, M., … DiSilvestro, P. (2018). Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. *New England Journal of Medicine*, *379*(26), 2495–2505. https://doi.org/10.1056/NEJMoa1810858

Motz, G. T., & Coukos, G. (2013). Deciphering and Reversing Tumor Immune Suppression. *Immunity*, *39*(1), 61–73. https://doi.org/10.1016/J.IMMUNI.2013.07.005

Nair, S., & Dhodapkar, M. V. (2017). Natural Killer T Cells in Cancer Immunotherapy. *Frontiers in Immunology*, *8*, 1178. https://doi.org/10.3389/fimmu.2017.01178

Nezhat, F. R., Apostol, R., Nezhat, C., & Pejovic, T. (2015). New insights in the

pathophysiology of ovarian cancer and implications for screening and prevention. *American Journal of Obstetrics and Gynecology*, *213*(3), 262–267. https://doi.org/10.1016/j.ajog.2015.03.044

Nizzero, S., Ziemys, A., & Ferrari, M. (2018). Transport Barriers and Oncophysics in Cancer Treatment. *Trends in Cancer*, *4*(4), 277–280. https://doi.org/10.1016/j.trecan.2018.02.008

Ortega, R. A., Barham, W. J., Kumar, B., Tikhomirov, O., McFadden, I. D., Yull, F. E., & Giorgio, T. D. (2015). Biocompatible mannosylated endosomal-escape nanoparticles enhance selective delivery of short nucleotide sequences to tumor associated macrophages. *Nanoscale*. https://doi.org/10.1039/c4nr03962a

Owens, G. L., Price, M. J., Cheadle, E. J., Hawkins, R. E., Gilham, D. E., & Edmondson, R. J. (2018). Ex vivo expanded tumour-infiltrating lymphocytes from ovarian cancer patients release anti-tumour cytokines in response to autologous primary ovarian cancer cells. *Cancer Immunology, Immunotherapy*, *67*(10), 1519–1531. https://doi.org/10.1007/s00262-018-2211-3

Patankar, M. S., Jing, Y., Morrison, J. C., Belisle, J. A., Lattanzio, F. A., Deng, Y., … Clark, G. F. (2005). Potent suppression of natural killer cell response mediated by the ovarian tumor marker CA125. *Gynecologic Oncology*. https://doi.org/10.1016/j.ygyno.2005.07.030

Pfirschke, C., Siwicki, M., Liao, H.-W., & Pittet, M. J. (2017). Tumor Microenvironment: No Effector T Cells without Dendritic Cells. *Cancer Cell*, *31*(5), 614–615. https://doi.org/10.1016/J.CCELL.2017.04.007

Pham, E., Birrer, M. J., Eliasof, S., Garmey, E. G., Lazarus, D., Lee, C. R., … Kerbel, R.

S. (2015). Translational Impact of Nanoparticle-Drug Conjugate CRLX101 with or without Bevacizumab in Advanced Ovarian Cancer. *Clinical Cancer Research*, *21*(4), 808–818. https://doi.org/10.1158/1078-0432.CCR-14-2810

Pisano, C., Cecere, S. C., Di Napoli, M., Cavaliere, C., Tambaro, R., Facchini, G., … Pignata, S. (2013). Clinical trials with pegylated liposomal Doxorubicin in the treatment of ovarian cancer. *Journal of Drug Delivery*, *2013*, 898146. https://doi.org/10.1155/2013/898146

Preston, C. C., Goode, E. L., Hartmann, L. C., Kalli, K. R., & Knutson, K. L. (2011). Immunity and immune suppression in human ovarian cancer. *Immunotherapy*. https://doi.org/10.2217/imt.11.20

Rajan, R., Sabnani, M. K., Mavinkurve, V., Shmeeda, H., Mansouri, H., Bonkoungou, S., … La-Beck, N. M. (2018). Liposome-induced immunosuppression and tumor growth is mediated by macrophages and mitigated by liposome-encapsulated alendronate. *Journal of Controlled Release*. https://doi.org/10.1016/j.jconrel.2017.12.023

Rauh-Hain, J. A., Krivak, T. C., Del Carmen, M. G., & Olawaiye, A. B. (2011). Ovarian cancer screening and early detection in the general population. *Reviews in Obstetrics & Gynecology*, *4*(1), 15–21. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21629494

Ries, C. H., Cannarile, M. A., Hoves, S., Benz, J., Wartha, K., Runza, V., … Rüttinger, D. (2014). Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. *Cancer Cell*. https://doi.org/10.1016/j.ccr.2014.05.016

Rossi, L., Verrico, M., Zaccarelli, E., Papa, A., Colonna, M., Strudel, M., … Tomao, F. (2017). Bevacizumab in ovarian cancer: A critical review of phase III studies. *Oncotarget*, *8*(7), 12389–12405. https://doi.org/10.18632/oncotarget.13310

Russell, M. R., Graham, C., D'Amato, A., Gentry-Maharaj, A., Ryan, A., Kalsi, J. K., … Graham, R. L. J. (2017). A combined biomarker panel shows improved sensitivity for the early detection of ovarian cancer allowing the identification of the most aggressive type II tumours. *British Journal of Cancer*, *117*(5), 666–674. https://doi.org/10.1038/bjc.2017.199

Sabado, R. L., Balan, S., & Bhardwaj, N. (2017). Dendritic cell-based immunotherapy. *Cell Research*. https://doi.org/10.1038/cr.2016.157

Sabbatini, P., Harter, P., Scambia, G., Sehouli, J., Meier, W., Wimberger, P., … Jacobus Pfisterer. (2013). Abagovomab as maintenance therapy in patients with epithelial ovarian cancer: A phase III trial of the AGO OVAR, COGI, GINECO, and GEICO-the MIMOSA study. *Journal of Clinical Oncology*. https://doi.org/10.1200/JCO.2012.46.4057

Sallusto, F., & Lanzavecchia, A. (2002). The instructive role of dendritic cells on T-cell responses. *Arthritis Research*, *4 Suppl 3*(Suppl 3), S127-32. https://doi.org/10.1186/ar567

Sapiezynski, J., Taratula, O., Rodriguez-Rodriguez, L., & Minko, T. (2016). Precision targeted therapy of ovarian cancer. *Journal of Controlled Release*. https://doi.org/10.1016/j.jconrel.2016.10.014

Sarojini, S., Tamir, A., Lim, H., Li, S., Zhang, S., Goy, A., … Suh, K. S. (2012). Early detection biomarkers for ovarian cancer. *Journal of Oncology*.

https://doi.org/10.1155/2012/709049

Scarlett, U. K., Rutkowski, M. R., Rauwerdink, A. M., Fields, J., Escovar-Fadul, X., Baird, J., … Conejo-Garcia, J. R. (2012). Ovarian cancer progression is controlled by phenotypic changes in dendritic cells. *The Journal of Experimental Medicine*. https://doi.org/10.1084/jem.20111413

Seiden, M. V, Muggia, F., Astrow, A., Matulonis, U., Campos, S., Roche, M., … Barrett, E. (2004). A phase II study of liposomal lurtotecan (OSI-211) in patients with topotecan resistant ovarian cancer. *Gynecologic Oncology*, *93*(1), 229–232. https://doi.org/10.1016/j.ygyno.2003.12.037

Seifi-Alan, M., Shamsi, R., & Ghafouri-Fard, S. (2018). Application of cancer-testis antigens in immunotherapy of hepatocellular carcinoma. *Immunotherapy*, *10*(5), 411–421. https://doi.org/10.2217/imt-2017-0154

Shen, H., Rodriguez-Aguayo, C., Xu, R., Gonzalez-Villasana, V., Mai, J., Huang, Y., … Lopez-Berestein, G. (2013). Enhancing chemotherapy response with sustained EphA2 silencing using multistage vector delivery. *Clinical Cancer Research*. https://doi.org/10.1158/1078-0432.CCR-12-2764

Shen, H., Sun, T., Hoang, H. H., Burchfield, J. S., Hamilton, G. F., Mittendorf, E. A., & Ferrari, M. (2017). Enhancing cancer immunotherapy through nanotechnology-mediated tumor infiltration and activation of immune cells. *Seminars in Immunology*, *34*, 114–122. https://doi.org/10.1016/j.smim.2017.09.002

Shen, L.-M., Quan, L., & Liu, J. (2018). Tracking Exosomes in Vitro and in Vivo To Elucidate Their Physiological Functions: Implications for Diagnostic and Therapeutic Nanocarriers. *ACS Applied Nano Materials*, *1*(6), 2438–2448.

https://doi.org/10.1021/acsanm.8b00601

Siegel, R. L., Miller, K. D., & Jemal, A. (2018). Cancer statistics, 2018. *CA: A Cancer Journal for Clinicians*. https://doi.org/10.3322/caac.21442

Singel, K. L., Khan, A. N. H., Moysich, K. B., Odunsi, K., & Segal, B. H. (2017). Ovarian cancer ascites induces a T cell suppressive phenotype in mature neutrophils: a potential barrier to anti-tumor immunity. *The Journal of Immunology*, *198*(1 Supplement).

Srinivasan, K. N., Rauthan, A., & Gopal, R. (2014). Combination Therapy of Albumin-Bound Paclitaxel and Carboplatin as First Line Therapy in a Patient with Ovarian Cancer. *Case Reports in Oncological Medicine*, *2014*, 1–5. https://doi.org/10.1155/2014/940591

Srinivasan, S., Vannberg, F. O., & Dixon, J. B. (2016). Lymphatic transport of exosomes as a rapid route of information dissemination to the lymph node. *Scientific Reports*, *6*, 24436. https://doi.org/10.1038/srep24436

Stanojevic, Z., Rancic, G., Radic, S., Potic-Zecevic, N., Djordjevic, B., Markovic, M., & Todorovska, I. (2004). Pathogenesis of malignant ascites in ovarian cancer patients. *Archive of Oncology*, *12*(2), 115–118. https://doi.org/10.2298/AOO0402115S

Syn, N. L., Wang, L., Chow, E. K.-H., Lim, C. T., & Goh, B.-C. (2017). Exosomes in Cancer Nanomedicine and Immunotherapy: Prospects and Challenges. *Trends in Biotechnology*, *35*(7), 665–676. https://doi.org/10.1016/j.tibtech.2017.03.004

Tan, D. S., Agarwal, R., & Kaye, S. B. (2006). Mechanisms of transcoelomic metastasis in ovarian cancer. *The Lancet Oncology*, *7*(11), 925–934.

https://doi.org/10.1016/S1470-2045(06)70939-1

Tanyi, J. L., Bobisse, S., Ophir, E., Tuyaerts, S., Roberti, A., Genolet, R., … Kandalaft, L. E. (2018). Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer. *Science Translational Medicine*. https://doi.org/10.1126/scitranslmed.aao5931

Tayama, S., Motohara, T., Narantuya, D., Li, C., Fujimoto, K., Sakaguchi, I., … Katabuchi, H. (2017). The impact of EpCAM expression on response to chemotherapy and clinical outcomes in patients with epithelial ovarian cancer. *Oncotarget*, *8*(27), 44312–44325. https://doi.org/10.18632/oncotarget.17871

Teo, P. Y., Yang, C., Hedrick, J. L., Engler, A. C., Coady, D. J., Ghaem-Maghami, S., … Yang, Y. Y. (2013). Hydrophobic modification of low molecular weight polyethylenimine for improved gene transfection. *Biomaterials*, *34*(32), 7971–7979. https://doi.org/10.1016/j.biomaterials.2013.07.005

Teo, P. Y., Yang, C., Whilding, L. M., Parente-Pereira, A. C., Maher, J., George, A. J. T., … Ghaem-Maghami, S. (2015). Ovarian Cancer Immunotherapy Using PD-L1 siRNA Targeted Delivery from Folic Acid-Functionalized Polyethylenimine: Strategies to Enhance T Cell Killing. *Advanced Healthcare Materials*. https://doi.org/10.1002/adhm.201500089

Terry, K. L., Schock, H., Fortner, R. T., Hu sing, A., Fichorova, R. N., Yamamoto, H. S., … Kaaks, R. (2016). A Prospective Evaluation of Early Detection Biomarkers for Ovarian Cancer in the European EPIC Cohort. *Clinical Cancer Research*, *22*(18), 4664–4675. https://doi.org/10.1158/1078-0432.CCR-16-0316

Timmermans, M., Sonke, G. S., Van de Vijver, K. K., van der Aa, M. A., & Kruitwagen,

R. F. P. M. (2018). No improvement in long-term survival for epithelial ovarian cancer patients: A population-based study between 1989 and 2014 in the Netherlands. *European Journal of Cancer*. https://doi.org/10.1016/j.ejca.2017.10.030

Torre, L. A., Trabert, B., DeSantis, C. E., Miller, K. D., Samimi, G., Runowicz, C. D., … Siegel, R. L. (2018). Ovarian cancer statistics, 2018. *CA: A Cancer Journal for Clinicians*. https://doi.org/10.3322/caac.21456

Turk, M. J., Waters, D. J., & Low, P. S. (2004). Folate-conjugated liposomes preferentially target macrophages associated with ovarian carcinoma. *Cancer Letters*. https://doi.org/10.1016/j.canlet.2003.12.028

Tyler, C., Kapur, A., Felder, M., Belisle, J. A., Trautman, C., Gubbels, J. A. A., … Patankar, M. S. (2012). The mucin MUC16 (CA125) binds to NK cells and monocytes from peripheral blood of women with healthy pregnancy and preeclampsia. *American Journal of Reproductive Immunology (New York, N.Y. : 1989)*, *68*(1), 28–37. https://doi.org/10.1111/j.1600-0897.2012.01113.x

Uthaman, S., Huh, K. M., & Park, I.-K. (2018). Tumor microenvironment-responsive nanoparticles for cancer theragnostic applications. *Biomaterials Research*, *22*, 22. https://doi.org/10.1186/s40824-018-0132-z

Ventola, C. L. (2017). Cancer Immunotherapy, Part 1: Current Strategies and Agents. *P & T : A Peer-Reviewed Journal for Formulary Management*, *42*(6), 375–383. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/28579724

Whitworth, J. M., & Alvarez, R. D. (2011). Evaluating the role of IL-12 based therapies in ovarian cancer: a review of the literature. *Expert Opinion on Biological Therapy*,

11(6), 751–762. https://doi.org/10.1517/14712598.2011.566854

Xia, X., Mai, J., Xu, R., Perez, J. E. T., Guevara, M. L., Shen, Q., … Shen, H. (2015). Porous Silicon Microparticle Potentiates Anti-Tumor Immunity by Enhancing Cross-Presentation and Inducing Type I Interferon Response. *Cell Reports*, *11*(6), 957–966. https://doi.org/10.1016/j.celrep.2015.04.009

Xu, R., Zhang, G., Mai, J., Deng, X., Segura-Ibarra, V., Wu, S., … Shen, H. (2016). An injectable nanoparticle generator enhances delivery of cancer therapeutics. *Nature Biotechnology*, (February), 1–7. https://doi.org/10.1038/nbt.3506

Yaari, Z., da Silva, D., Zinger, A., Goldman, E., Kajal, A., Tshuva, R., … Schroeder, A. (2016). Theranostic barcoded nanoparticles for personalized cancer medicine. *Nature Communications*, *7*, 13325. https://doi.org/10.1038/ncomms13325

Yadav, R. K., Chae, S.-W., Kim, H.-R., & Chae, H. J. (2014). Endoplasmic Reticulum Stress and Cancer. *Journal of Cancer Prevention*. https://doi.org/10.15430/JCP.2014.19.2.75

Yamashita, T., Takahashi, Y., & Takakura, Y. (2018). Possibility of Exosome-Based Therapeutics and Challenges in Production of Exosomes Eligible for Therapeutic Application. *Biological and Pharmaceutical Bulletin*, *41*(6), 835–842. https://doi.org/10.1248/bpb.b18-00133

Zhang, B.-L., Qin, D.-Y., Mo, Z.-M., Li, Y., Wei, W., Wang, Y.-S., … Wei, Y.-Q. (2016). Hurdles of CAR-T cell-based cancer immunotherapy directed against solid tumors. *Science China Life Sciences*, *59*(4), 340–348. https://doi.org/10.1007/s11427-016-5027-4

Zhang, L., Conejo-Garcia, J. R., Katsaros, D., Gimotty, P. A., Massobrio, M., Regnani,

G., … Coukos, G. (2003). Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *The New England Journal of Medicine*. https://doi.org/10.1056/NEJMoa020177

Zheng, M., Zhong, Y., Meng, F., Peng, R., & Zhong, Z. (2011). Lipoic acid modified low molecular weight polyethylenimine mediates nontoxic and highly potent in vitro gene transfection. *Mol Pharm*. https://doi.org/10.1021/mp2003797

Zheng, P.-P., Kros, J. M., & Li, J. (2018). Approved CAR T cell therapies: ice bucket challenges on glaring safety risks and long-term impacts. *Drug Discovery Today*, *23*(6), 1175–1182. https://doi.org/10.1016/j.drudis.2018.02.012

Zhu, X., Cai, H., Zhao, L., Ning, L., & Lang, J. (2017). CAR-T cell therapy in ovarian cancer: from the bench to the bedside. *Oncotarget*, *8*(38), 64607–64621. https://doi.org/10.18632/oncotarget.19929

Zhu, Y., Knolhoff, B. L., Meyer, M. A., Nywening, T. M., West, B. L., Luo, J., … De Nardo, D. G. (2014). CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. *Cancer Research*. https://doi.org/10.1158/0008-5472.CAN-13-3723

Zitvogel, L., Mayordomo, J. I., Tjandrawan, T., DeLeo, A. B., Clarke, M. R., Lotze, M. T., & Storkus, W. J. (1996). Therapy of murine tumors with tumor peptide-pulsed dendritic cells: dependence on T cells, B7 costimulation, and T helper cell 1-associated cytokines. *The Journal of Experimental Medicine*. https://doi.org/10.1084/jem.183.1.87

JPET Fast Forward. Published on February 8, 2019 as DOI: 10.1124/jpet.118.254979
This article has not been copyedited and formatted. The final version may differ from this version.

Figure Legend

Figure 1. Schematic of the current immunotherapies for Ovarian Cancer. Arrows show the interactions between immune system players (Dendritic Cell, T cells, Macrophages, and Monoclonal antibodies) and ovarian cancer cells. Each specific immune cell type can be employed to deliver specific therapies that can differently alter the immune system towards a more efficient activity rate.

Figure 2. Schematic representation of intraperitoneal injection (IP) of nanoparticles (NP) able to follow the ascetic fluid movement (green arrows) and reach metastatic sites. Tumor spreading from the ovaries is also shown.

Tables

Table 1. List of current therapies for Ovarian cancer. Chemotherapies and targeted therapies are FDA approved. Some of the nanotherapies mentioned are already used in clinics, but the majority of them is still undergoing clinical trials.

JPET Fast Forward. Published on February 8, 2019 as DOI: 10.1124/jpet.118.254979
This article has not been copyedited and formatted. The final version may differ from this version.

Gold standard chemotherapeutic			
Drug name	**Drug class**	**FDA approved**	**Reference**
Doxorubicin	Antibiotics/antineoplastics	1995	(Bolis et al., 1978)
Carboplatin	Alkylating agents	1989	(M. Adams et al., 1989)
Paclitaxel	Mitotic inhibitors	1998	(Khanna et al., 2015)
Cyclophosphamide	Alkylating agents	1959	(Handolias et al., 2016)
Gemcitabine	Antimetabolites	2006	(Lorusso et al., 2006)
Melphalan	Alkylating agents	2001	(Hasan & Jayson, 2003)
Cisplatin	Alkylating agents	1978	(Monneret, 2011)
Topotecan	Miscellaneous antineoplastics	1996	(Seiden et al., 2004)
Etoposide	Mitotic inhibitors	1998	(Long et al., 2005)
Thiotepa	Alkylating agents	2001	(Gordinier et al., 2002)
Targeted therapies			
Drug name	**Drug class**	**FDA approved (yr)**	**Reference**
Bevacizumab	VEGF/VEGFR inhibitors	2004	(Rossi et al., 2017)
Olaparib	PARP inhibitors	2017	(Moore et al., 2018)
Niraparib	PARP inhibitors	2017	(Essel & Moore, 2018)
Rucaparib	PARP inhibitors	2018	(Dal Molin et al., 2018)

Current nanotechnology treatments and ongoing trials				
Drug name	**Drug class**	**Formulation**	**FDA approval / Clinical trial phase**	**Reference**
Doxil	Antibiotics/antineoplastics	Pegylated Liposomal doxorubicin	1999	(Pisano et al., 2013)

JPET Fast Forward. Published on February 8, 2019 as DOI: 10.1124/jpet.118.254979
This article has not been copyedited and formatted. The final version may differ from this version.

Lipodox	Antibiotics/antineoplastics	Pegylated Liposomal doxorubicin	2012	(Chou et al., 2006)
Genexol-PM	Mitotic inhibitors	PEG-PLA polymeric micellar Paclitaxel	Phase II	(Lee et al., 2017)
LEP-ETU	Mitotic inhibitors	Liposomal Paclitaxel	Phase I	(Damjanov et al., 2005)
Paclical	Mitotic inhibitors	Paclitaxel micelles	Phase III	NCT00989131
OSI-211	Antineoplastics	Liposomal Lurtotecan	Phase II	(Seiden et al., 2004)

Table 2. FDA-approved immunotherapeutics since the beginning of 2018.

Drugs	Mechanism of action	Targeted disease	Release date
Durvalumab (IMFINZI®, AstraZeneca)	checkpoint immunotherapy targeting PD-1/PD-L1 pathway	Stage III non-small cell lung cancer	Feb 16, 2018
Brentuximab vedotin (Adcetris, Seattle Genetics, Inc.)	antibody-drug conjugate targeting the CD30 receptor	Untreated classical Hodgkin lymphoma	Mar 20, 2018
Blinatumomab (Blincyto, Amgen Inc.)	bispecific T cell-engaging antibody targeting CD19 receptor	B-cll precursor acute lymphoblastic leukemia	Mar 29, 2018
Nivolumab (Opdivo, Bristol-Myers Squibb) + Ipilimumab (Yervoy, Bristol-Myers Squibb)	checkpoint immunotherapy targeting PD-1 and CTLA-4	Advanced renal cell carcinoma	Apr 16, 2018
Tisagenlecleucel (Kymriah, Novartis)	CAR T cell immunotherapy targeting CD19 receptor	Relapsed / refractory large B cell lymphoma	May 01, 2018
Pembrolizumab (Keytruda, Merck)	checkpoint immunotherapy targeting PD-1	Cervical cancer	Jun 12, 2018
Pembrolizumab (Keytruda, Merck)	checkpoint immunotherapy targeting anti-PD-1	Adult and pediatric primary mediastinal large B-cell lymphoma	Jun 13, 2018
Nivolumab (Opdivo,l Bristol-Myers Squibb) + Ipilimumab (Yervoy, Bristol-Myers Squibb)	checkpoint immunotherapy targeting PD-1 and CTLA-4	Relapsed colorectal cancer with high microsatellite instability or deficient DNA mismatch repair	Jul 10, 2018

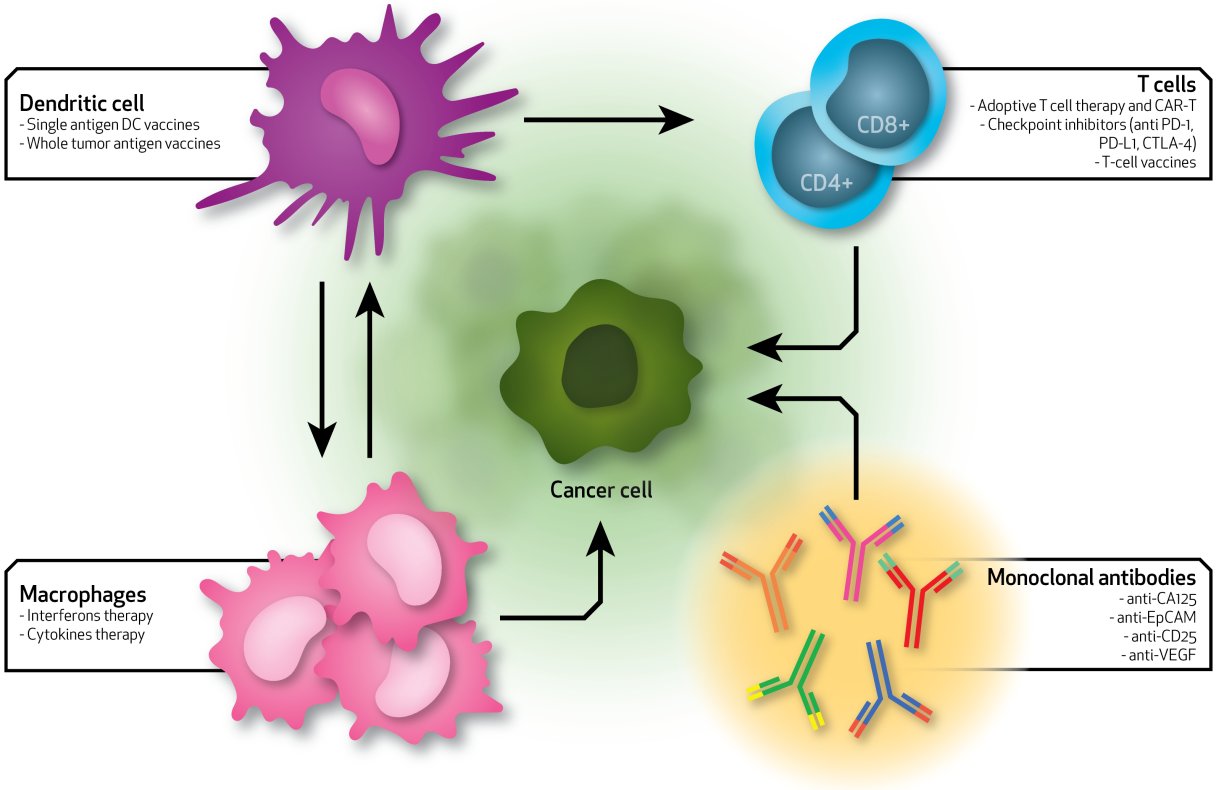
JPET Fast Forward. Published on February 8, 2019 as DOI: 10.1124/jpet.118.254979
This article has not been copyedited and formatted. The final version may differ from this version.

Nivolumab (Opdivo, Bristol-Myers Squibb)	checkpoint immunotherapy targeting PD-1	Metastatic small cell lung cancer	Aug 17, 2018

JPET Fast Forward. Published on February 8, 2019 as DOI: 10.1124/jpet.118.254979
This article has not been copyedited and formatted. The final version may differ from this version.

Figures

Figure 1.



JPET Fast Forward. Published on February 8, 2019 as DOI: 10.1124/jpet.118.254979
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 2.