Ovarian Cancer and the Immune Tumour Microenvironment

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Declaration

I, Tami Grunewald, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Epithelial ovarian cancer (EOC) is the leading cause of gynaecological cancer deaths worldwide. Most women present with advanced disease and whilst the majority of patients respond to primary treatment, most will relapse and eventually develop chemotherapy resistance. New treatment strategies are urgently needed.

I implemented a phase II clinical trial (PROMPT) exploring whether maintenance immunotherapy (pembrolizumab) improved progression free survival (PFS) in patients with platinum resistant ovarian cancer, who have stable disease / response to weekly paclitaxel. The 6-month PFS rate was 5.0%. Median PFS was 2.0 months and overall survival (OS) 9.8 months. The trial did not meet its primary end point and was closed early due to futility.

I interrogated the ovarian cancer immune microenvironment using blood and tumour samples from patients with high grade ovarian cancer treated at University College London Hospital (UCLH) between October 2020 and June 2021. Blood was collected at various timepoints, processed into peripheral blood mononuclear cells (PBMCs) and frozen. They were later analysed using flow cytometry; two panels exploring T- and myeloid cells. Where available, formalin-fixed paraffin-embedded (FFPE) tissue blocks were collected and analysed using three multiplex immunofluorescence (mIF) panels.

I found changes in circulating immune cells of patients; specifically increased numbers of regulatory T-cells (Tregs), with overexpression of TIM-3 and PD-1 when compared to age- and gender-matched healthy donors (HD). I also observed a reduction in circulating classical monocytes following chemotherapy, particularly in patients with worse outcomes. Within the tumour, I found a reduction in the number of CD4+ TIM-3+ +/- PD-1+ T-cells and increased numbers of macrophages in patients with a poorer prognosis.

These results highlight the complexity of the immune microenvironment in ovarian cancer. It is difficult to interpret the clinical implications of these findings, but this research provides a foundation for future work in identifying prognostic and predictive biomarkers in EOC.

Impact statement

Epithelial ovarian cancer (EOC) is the 6th most common cancer diagnosed in women and is the leading cause of death from gynaecological malignancies worldwide. High grade serous ovarian cancer (HGSC) is the most common histological subtype. It often presents with non-specific symptoms, resulting in the majority of women being diagnosed at an advanced stage. Most patients will respond to first line treatment, with optimal treatment consisting of surgery, platinum-based chemotherapy, often followed by maintenance treatment with either bevacizumab, poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi) or both. Despite this, a large proportion of patients will relapse and ultimately develop platinum-resistant disease. 5-year survival remains poor at less than 30%.

This research focuses on HGSC. Treatment for platinum-resistant HGSC has remained static over the years. Immunotherapy, in the form of immune checkpoint inhibitors (ICPIs) has transformed treatment and outcomes of numerous solid tumours. Its role in HGSC is still unknown and to date, trials investigating their use either alone or in combination with anti-angiogenics and/or PARPi, have not been successful. The reasons for this are poorly understood, with minimal published translational research despite the large numbers of patients enrolled in immunotherapy trials. There is an urgent need to further understand the complexity of the ovarian cancer immune microenvironment, the drivers of chemotherapy resistance and identify prognostic and predictive biomarkers.

The phase II trial of maintenance pembrolizumab following weekly paclitaxel for recurrent ovarian, fallopian or peritoneal cancer (PROMPT) is an important study, highlighting the poor prognosis of platinum-resistant disease. This study did not meet its primary end point of prolonging 6-month progression-free survival (PFS). It confirms that use of single agent ICPIs is unlikely to benefit patients with ovarian cancer and therefore publication of the results is vital to ensure this is not replicated. It also demonstrates the importance of translational research in all clinical trials, to provide insight into the biology of this cancer and to guide further research.

Ultimately, the main goal of my project was to gain insight into the diverse biology of ovarian cancer in order to aid future research and ultimately improve outcomes for patients. I have demonstrated the complexity of the ovarian cancer immune microenvironment. Firstly, how circulating immune cells differ to those seen in healthy donors, and secondly, the changes in these cells with treatment. I have demonstrated increased numbers of regulatory T-cells (Tregs), with overexpression of markers indicating dysfunction/exhaustion. I also identified the immune cells within the tumour microenvironment at diagnosis, such as dysfunctional CD4+ T-cells and macrophages and their potential contribution to outcomes and prognosis. These are important data as they open up the opportunity to develop new therapeutic targets.

My research emphasises the heterogeneity of the ovarian cancer immune microenvironment and its complexity. It provides a foundation on which to develop future translational research projects – allowing clinicians and scientists to work together to explore the ovarian cancer immune microenvironment and to drive development of effective treatments.

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Finally, this thesis is dedicated to my beautiful sister, Julie Cohn, who died from metastatic breast cancer in September 2023. She always encouraged me to follow my dreams and would have been so proud to have been with me at the end of this long and challenging journey.

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Abbreviations

AE	Adverse event
ACT	Adoptive T-cell therapy
ADC	Antibody drug conjugate
AGO	Arbeitsgemeinschaft Gynakologische Onkologie
AGO-OVAR12	Randomised placebo-controlled phase III trial of nintedanib combined with chemotherapy for newly diagnosed advanced ovarian cancer
AGO-OVAR16	A phase III study to evaluate the efficacy and safety of pazopanib monotherapy versus placebo in women who have not progressed after first-line chemotherapy for epithelial ovarian, fallopian tube, or primary peritoneal cancer
APC	Antigen presenting cell
ALT	Alanine aminotransferase
ASCO	American Society of Clinical Oncology
AURELIA	Avastin Use in Platinum Resistant Epithelial Ovarian Cancer
BCG	Bacillus Calmette-Guérin
BGTB	Barts Gynae Tissue Bank
BICR	Blinded independent central review
BRCA	Breast cancer related antigen
BRCA1	Breast cancer related antigen 1
BRCA2	Breast cancer related antigen 2
BRCAwt	BRCA wild-type
BRIP1	BRCA1-interacting protein 1
CA 125	Cancer antigen 125
CAR	Chimeric antigen receptor-modified
CCR7	Chemokine receptor 7
CD4+ Teff	CD4 T effector
cDC	Conventional dendritic cell

cDC1	Conventional dendritic cell 1
cDC2	Conventional dendritic cell 2
CHIVA study	A GINECO randomised double blind phase II trial of nintedanib versus placebo with the neo-adjuvant chemotherapy strategy for patients with advanced unresectable ovarian cancer
Clas	Classical
CM	Central memory
CNS	Central nervous system
CR	Complete response
CRR	Complete resection rate
CRS	Chemotherapy response score
CR UK	Cancer Research UK
CT	Chemotherapy
CTC	Cancer Trials Centre
CTL	Cytotoxic lymphocyte
CTLA-4	Cytotoxic T lymphocyte-associated antigen
DAMP	Danger-associated molecular patterns
DAPI	4', 6-diamidino-2-phenylindole
DC	Dendritic cell
DCR	Disease control rates
DCVAC/OvCa	Active cellular immunotherapy designed to stimulate an immune response against ovarian cancer
DDS	Delayed debulking surgery
DESKTOP III	Randomised phase III study to evaluate the impact of secondary cytoreductive surgery in recurrent ovarian cancer
DIs	Deconvolved images
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid

ECOG PS	Eastern Cooperative Oncology Group performance status
EDTA	Ethylenediaminetetraacetic acid
EM	Effector memory
EMA	European Medicines Agency
eMDSC	Early-stage myeloid derived suppressor cells
EnAd	Enadenotucirev
EOC	Epithelial ovarian cancer
ESGO	European Society of Gynaecological Oncology
ESMO	European Society for Medical Oncology
FACS	Flow cytometry
FBS	Fetal bovine serum
FC	Fold change
FcR	Fc receptor
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
FIGO	International Federation of Gynaecology and Obstetrics for ovarian cancer
FoxP3	Forkhead box P3
FRα	Folate receptor α
F/up	Follow-up
gBRCAm	Germline BRCA mutation
G	Grade
GCP	Good clinical practice
GI	Gastrointestinal
GOG-218	Gynecologic Oncology Group study 0218

GzmB	Granzyme B
H&E	Haematoxylin and Eosin
HD	Healthy donors
HGSC	High grade serous ovarian cancer
HR	Homologous recombination
HRD	Homologous recombination deficient/deficiency
HRR	Homologous recombination repair
HSPC	Haematopoietic stem and progenitor cells
ICON7	International Collaboration on Ovarian Neoplasms (ICON7) trial
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICPI	Immune checkpoint inhibitors
ICS	Interval cytoreductive surgery
IDO	Indoleamine 2,3 dioxygenase
IFN-γ	Interferon gamma
IgG4	Immunoglobulin G4
IL-2	Interleukin-2
IL-10	Interleukin-10
Int	Intermediate
IP	Intraperitoneal
irAE	Immune related adverse event
ITT	Intention-to-treat
LAG-3	Lymphocyte-activation gene 3
MCSF/CSF-1	Macrophage colony stimulating factor
mDCs	Myeloid dendritic cells
MDSC	Myeloid derived suppressor cell
MDT	Multidisciplinary team meeting

MEK	Mitogen-activated protein kinase
MFI	Median fluorescence intensity
MHC	Major histocompatibility complex
MHC-I/II	Major histocompatibility complex class I/II
mIF	Multiplex immunofluorescence
MIMOSA study	Abagovomab as maintenance therapy in patients with epithelial ovarian cancer: a phase III trial of the AGO OVAR, COGI, GINECO and GEICO
M-MDSC	Monocytic MDSC
MSI	Microsatellite instability
NACT	Neoadjuvant chemotherapy
NK	Natural killer
Non-clas	Non-classical
NSCLC	Non-small cell lung cancer
OC	Ovarian cancer
OCEANS study	A randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer
OCTAVE	Phase I clinical trial of Enadenotucirev given in combination with weekly paclitaxel in platinum resistant ovarian cancer
OK-432	Picibanil (a lyophilised mixture of group A streptococcus pyogenes)
ORR	Overall / objective response rate
OS	Overall survival
OV	Oncolytic virus
PALB2	Partner and localizer of BRCA2
PAMP	Pathogen-associated molecular patterns
PARP	Poly (ADP-ribose) polymerase
PARPi	Poly (ADP-ribose) polymerase inhibitors

PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PD	Progressive disease
PD-1	Programmed cell death protein 1
pDC	Plasmacytoid dendritic cell
PDGF	Platelet derived growth factor
PDGFR	Platelet derived growth factor receptor
PD-L1	Programmed death-ligand 1
PCS	Primary cytoreductive surgery
PFI	Platinum free interval
PFS	Progression free survival
PLD	Pegylated liposomal doxorubicin
PMN-MDSC	Polymorphonuclear myeloid derived suppressor cells
PR	Partial response
PRR	Pattern-recognition receptors
PROMPT	Phase II trial of maintenance pembrolizumab following weekly paclitaxel for recurrent ovarian, fallopian tube or peritoneal cancer
PSOC	Platinum sensitive ovarian cancer
QC	Quality control
RAD51	Radiation sensitive protein 51
RCC	Renal cell carcinoma
RCT	Randomised controlled trial
RECIST	Response Evaluation Criteria in Solid Tumours
RFS	Recurrence-free survival
ROC	Recurrent ovarian cancer
RoI	Regions of interest
RPMI	Cell culture medium used to culture mammalian cells

RT	Room temperature
SD	Stable disease
SigMa	Signature Multivariate Analysis
SOP	Standard Operating Procedure
STING	Stimulator of interferon genes
T	Timepoint
TAA	Tumour associated antigens
TAM	Tumour associated macrophage
Tcm	Central memory T-cells
TCR	T-cell receptor
Tem	Effector memory T-cells
TGF	Transforming growth factor
TILs	Tumour infiltrating lymphocytes
TIM-3	T-cell immunoglobulin and mucin domain 3
TLR	Toll-like receptors
TME	Tumour microenvironment
TMG	Trials Management Group
TNF	Tumour necrosis factor
TLS	Tumour adjacent lymphoid islets
TOX	Thymocyte selection-associated HMG BOX
TP53	Gene that provides instructions for making p53, a tumour suppressor protein
TRAE	Treatment related adverse events
Treg	Regulatory T-cell
TRUST study	Trial of Radical Upfront Surgical Therapy in advanced ovarian cancer
TSA	Tumour specific antigens
UCL	University College London

UCLH	University College London Hospital
UK	United Kingdom
VEGF	Vascular endothelial growth factor
VEGF-R	Vascular endothelial growth factor receptor
VITAL trial	Gemogenovatucel-T (Vigil) immunotherapy as maintenance in frontline stage III/IV ovarian cancer (VITAL): a randomized, double-blind, placebo-controlled, phase IIb trial
WSI	Whole slide image
WT	Wild-type

Chapter 1: Epithelial Ovarian Cancer

1.1 Introduction to Ovarian Cancer

1.1.1 Incidence

Epithelial ovarian cancer (EOC) is the 6th most common cancer diagnosed in women, with over 7,000 new cases diagnosed in the United Kingdom each year (1). It is the leading cause of death amongst gynaecological malignancies and is the 8th most common cause of death from cancer worldwide (2). It often presents with non-specific symptoms, resulting in the majority of women being diagnosed with advanced (stage III/IV) disease at the outset (3). Initial treatment consists of a combination of cytoreductive surgery and platinum-based chemotherapy. Whilst most women respond to first line treatment, approximately 80% will relapse, usually within 18 months, requiring further systemic therapy. 5-year survival is less than 30% (4), due to the eventual emergence of chemotherapy resistance.

1.1.2 Histological subtypes

EOC is an umbrella term for a group of diseases. There are five main histological subtypes; high-grade serous, low-grade serous, mucinous, endometrial and clear cell carcinoma. Whilst each of these subtypes has its own unique molecular profile and behaviour, transcoelomic spread is common across the board. High-grade serous ovarian cancer (HGSC) is the most common subtype, primarily thought to arise from the fallopian tubes, accounting for approximately 70% of all new cases of ovarian cancer and the majority of deaths from this disease (5). This thesis will focus on HGSC, which is a term that encompasses all sites of origin (fallopian tubes, ovaries and peritoneum).

Whilst surgery, chemotherapy and targeted therapies have improved progression free survival (PFS) in women with HGSC, overall, survival remains poor. There are multiple factors contributing to this, including advanced stage at diagnosis and lack of biomarkers predictive for a response to treatment.

1.1.3 Staging of ovarian cancer

Stage at diagnosis is a key prognostic factor. It is determined using the International Federation of Gynaecology and Obstetrics for ovarian cancer (FIGO) staging scheme as summarised in table 1.

Table 1: 2014 FIGO staging for cancer of the ovary, fallopian tube and peritoneum (6)

Stage I	**Tumour confined to ovaries or fallopian tube(s)**
IA	Tumour in 1 ovary (capsule intact) / fallopian tube. No tumour on ovarian or fallopian tube surface. No malignant cells in ascites or peritoneal washings
IB	Tumour in both ovaries (capsule intact)/fallopian tubes. No tumour on ovarian or fallopian tube surface. No malignant cells in ascites or peritoneal washings
IC	Tumour in 1 or both ovaries/fallopian tubes with any of the following: - IC1: Surgical spill - IC2: Capsule ruptured pre-surgery or tumour on ovarian/fallopian tube surface - IC3: Malignant cells in ascites/peritoneal washings
Stage II	**Tumour involves 1 or both ovaries/fallopian tube(s) with pelvic extension or peritoneal cancer**
IIA	Extension and/or implants on uterus and/or fallopian tubes and/or ovaries
IIB	Extension to other pelvic organs
Stage III	**Tumour involves 1 or both ovaries / fallopian tube(s) or peritoneal cancer, with confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lymph nodes**
IIIA1	Positive retroperitoneal lymph nodes only - IIIA1(i): Metastasis ≤10mm - IIIA1(ii): Metastasis >10mm
IIIA2	Microscopic disease within the peritoneum outside of the pelvis +/- positive retroperitoneal nodes
IIIB	Macroscopic peritoneal disease outside of the pelvis ≤2cm +/- positive retroperitoneal nodes
IIIC	Macroscopic peritoneal disease outside of the pelvis >2cm +/- positive retroperitoneal nodes, including extension of tumour to liver capsule or spleen without parenchymal involvement
Stage IV	**Distant metastases, excluding peritoneal disease**
IVA	Malignant pleural effusion
IVB	Metastases to extra-abdominal organs / parenchymal metastases, including inguinal lymph nodes and any nodes outside the abdominal cavity

Table 1: 2014 FIGO staging for cancer of the ovary, fallopian tube and peritoneum.
(Adapted from Berek et al, International Journal of Gynecology & Obstetrics. 2018;143:59–78).

1.2 Current Treatment of High Grade Serous Ovarian Cancer

1.2.1 First line treatment

First line treatment of ovarian cancer comprises a combination of surgery and platinum-based chemotherapy, routinely carboplatin, with the addition of paclitaxel and has been the standard of care for many years (7,8). Complete macroscopic resection is another key prognostic factor and strongly correlates with improved overall survival (9,10). If it is felt that this cannot be achieved upfront, patients will be offered neoadjuvant chemotherapy (NACT) followed by interval cytoreductive surgery (ICS), which has been shown to be non-inferior to primary cytoreductive surgery (PCS) (11,12). However, it is currently unknown whether a selection of patients who are deemed operable at diagnosis, may in fact benefit more from NACT and this is being investigated in the TRUST study (13).

1.2.2 Maintenance therapy

The aim of maintenance treatment is to attempt to kill off any residual cancer cells that may still be present following initial systemic therapy, and therefore prolong the platinum free interval (PFI) and PFS. There are several ways in which this can be done, either by removing the remaining slowly dividing cells, inhibiting cell turnover or through immunological manipulation (14). Prolonging the PFI may impact treatment decisions and overall response to subsequent lines of therapy upon progression. Whilst a number of different chemotherapeutic agents have been evaluated in the maintenance setting for ovarian cancer, the only two classes of drugs with proven efficacy in prolonging PFS are anti-angiogenics and poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi).

1.2.3 Anti-angiogenics

Angiogenesis plays a key role in the development, growth and metastasis of ovarian cancer through overexpression of growth factors. The main ones involved in this process are vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), angiopoietin-Tie2 receptor and fibroblast growth factor (FGF) (15).

Bevacizumab, a humanised recombinant anti-VEGF monoclonal antibody, is routinely used in first line treatment of ovarian cancer, following publication of two pivotal

randomised phase III trials; GOG-218 and ICON7 (16,17). Bevacizumab is commenced with chemotherapy and continued as maintenance therapy for a total of 18 cycles. Both studies reported that the addition of bevacizumab to carboplatin and paclitaxel significantly improved PFS compared to chemotherapy alone. Whilst an overall survival (OS) benefit was not observed in either trial (18), a subgroup analysis from ICON7 identified improved outcomes in 'high risk patients', defined as stage IV disease, inoperable stage III disease or the presence of >1cm residual disease following surgical debulking (19,20), although there are no predictive biomarkers. A retrospective analysis in GOG-218 mirrored these results and also showed an improved OS benefit in stage IV disease (18).

Nintedanib is an oral triple angiokinase inhibitor of VEGF receptor (VEGFR), PDGF receptor (PDGFR) and FGF receptor (FGFR). The AGO-OVAR12 study investigated its use in combination with first line chemotherapy (21). Pazopanib is an oral small molecule tyrosine kinase inhibitor (TKI) that inhibits VEGFR-1,2,3, PDGFR-α and β and c-kit (22). The AGO-OVAR16 study investigated the efficacy of pazopanib maintenance treatment after first-line chemotherapy in advanced ovarian cancer. Both studies showed an improvement in PFS, but no OS benefit (23,24).

NACT plus nintedanib, followed by maintenance nintedanib resulted in worse outcomes and increased toxicity (CHIVA trial) when compared to chemotherapy alone (25).

1.2.4 PARP inhibitors

Breast cancer-related antigen (BRCA) 1 and 2 are tumour suppressor genes that encode proteins involved in repair of double stranded deoxyribonucleic acid (DNA) breaks via homologous recombination (HR). Mutations in these genes result in a cell's inability to effectively repair DNA damage (26). Approximately 15% of HGSC are associated with a germline BRCA1/2 mutation (27) and somatic mutations found in up to 8% of cases (28). Additionally, almost half of all HGSC are homologous recombination deficient (HRD). HRD is a complex phenotype that is characterised by ineffective repair of DNA double-strand breaks within a cell by the homologous recombination repair (HRR) pathway (29). Causes of HRD include pathogenic BRCA mutations or defects in other HRR genes e.g. BRIP1, RAD51C, RAD51D and PALB2

(30). There are a variety of commercial assays available to detect HRD in HGSC (31). Both BRCA mutations and HRD are biomarkers predictive of response to PARPi (32).

The PARP enzymes play a role in repair of single strand DNA breaks. PARP inhibition prevents single strand DNA break repair and therefore results in double stand DNA breaks. This, combined with either a BRCA mutation or HRD, results in cell death by synthetic lethality. Synthetic lethality is best defined as a process where one genetic mutation does not affect cell function, however, when a second genetic or protein defect occurs, this particular cell is no longer viable (26). PARPi also interfere with DNA replication (33).

Olaparib was the first PARPi to be approved as maintenance therapy in patients with platinum sensitive relapse (34–37). Both Study-19 and SOLO-2 show long-term benefit with PARPi maintenance (35,38). Subsequently, the SOLO-1 study (39) reported a PFS benefit in the first line setting for BRCA mutated HGSC, with an extraordinarily sustained benefit seen at 7 years (40,41). Based on these results, olaparib is Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved for this indication.

Niraparib and rucaparib are also effective PARPi in prolonging PFS in both first-line and recurrent settings, regardless of HRD or BRCA status (33,42). Prolonged long-term benefits are seen, even beyond disease progression (43–45). In the UK, niraparib is approved in all settings regardless of BRCA or HRD status. Rucaparib (46–48) is available in the relapsed setting and has recently become licensed in the first line setting, but is not yet funded in the UK (49).

1.2.5 Combination maintenance therapies

Despite the advent and successes of maintenance treatment with anti-angiogenics and PARP inhibitors, they do not result in cure in the majority of women. However, the 7-year OS data from SOLO-1 suggests that maintenance olaparib in women with a BRCA mutation may result in cure, although longer term follow-up is required to make a definitive judgement on this (41). Combining anti-angiogenics with PARPi prolongs PFS further in relapsed disease, regardless of BRCA status (50–53) and following first line therapy (54).

The use of combination olaparib and bevacizumab maintenance therapy was approved in the UK in March 2021, and is available in the first line setting to all patients with HRD positive EOC, provided they have responded to platinum-based therapy (55).

1.3 Recurrent ovarian cancer

1.3.1 Treatment

Most women diagnosed with ovarian cancer will eventually relapse and require further systemic therapy. The choice of treatment in recurrent disease, and predicted outcome, was thought to be highly dependent on the PFI (56). Platinum sensitive disease was previously defined as relapse greater than 6 months following completion of platinum-based therapy (57). It is now considered to be more complex than this, with other factors required to determine the next line of treatment, such as the presence of a BRCA mutation/HRD, histology and prior use of maintenance therapy (58–60).

Re-challenge with platinum-based chemotherapy is offered to patients thought to have platinum sensitive ovarian cancer (PSOC). This can be given alone, but preferably given in combination with another drug. Whilst paclitaxel can be used again, it is often combined with a different agent, for example gemcitabine (61) or pegylated liposomal doxorubicin (PLD) (62). Decisions regarding treatment for relapsed disease are now only partly based on the PFI (58).

The role of secondary cytoreduction in relapsed platinum sensitive disease remains controversial, but may be beneficial in a carefully selected group of patients (33). The Arbeitsgemeinschaft Gynakologische Onkologie (AGO) score is a validated score predictive of complete cytoreduction in recurrent ovarian cancer. Resectability is more likely with the following 3 factors: Complete resection at primary surgery, good performance status (PS) and absence of ascites >500ml (63). The DESKTOP III study concluded that patients with a positive AGO score had a longer OS when compared to patients who underwent chemotherapy alone (64–66).

Platinum resistance encompasses patients who are not deemed eligible for further platinum-based chemotherapy, either due to progression during or within a few weeks

of treatment (58,60). Response rates to chemotherapy are poor (30-40%), with reported PFS rates of 3-4 months and median OS less than 12 months (67).

1.3.2 Targeted therapy in recurrent ovarian cancer

The addition of bevacizumab to chemotherapy in PSOC, when bevacizumab was not used in the first line setting, prolongs PFS (68) however, no OS benefit has been demonstrated (OCEANS study) (69,70). This also seems to be the case in patients already treated with bevacizumab at diagnosis (71).

In the event that PARPi were not used as maintenance therapy in the first line setting, they are offered to women with PSOC who have had a response to platinum-based chemotherapy (38,42,47).

Cediranib is an oral VEGF-R and c-KIT inhibitor, and early phase studies reported some activity in relapsed EOC (72). Whilst the subsequent phase III RCT (ICON6) showed improvement in PFS in the cediranib maintenance group (73), no OS benefit was demonstrated (74). It is not used routinely in clinical practice but is still being evaluated in clinical trials (75–77).

The AURELIA trial was the first randomised phase III study to combine bevacizumab with chemotherapy in platinum resistant ovarian cancer (PROC) and reported a significant improvement in PFS in the bevacizumab containing arm (78). Despite these findings, the use of bevacizumab in this setting is not licenced in the UK and a large proportion of patients will have already received bevacizumab as part of their first line treatment. More recently, Mirvetuximab soravtansine, an antibody drug conjugate (ADC) targeting folate receptor α (FRα), showed a significant benefit when compared to chemotherapy and is now approved in the US for the treatment of PROC (79). It is not yet available in the UK.

Standard of care treatment options and outcomes for PROC have remained static over the years and alternative therapeutic strategies are urgently needed. Targeting the immune system has been beneficial in multiple solid tumours, but its role in ovarian cancer is still unclear and the next section of this thesis will focus on this.

1.4 Ovarian cancer and the immune system

1.4.1 The immune system and cancer

Cancer development is described as a six-stage process – 'The Hallmarks of Cancer'. This consists of sustained growth, evading growth-inhibitory signals, avoidance of apoptosis, unlimited replicative potential, angiogenesis and tissue invasion and metastasis (80). However, over the years, it has become clear that it is more complex than this and there is increasing interest in the role of the immune system in cancer. The cancer immunity cycle (figure 1) summarises the steps involved in cancer progression, which include cancer antigen release and presentation by dendritic cells (DCs), priming and activation of peripheral immune cells, infiltration of T-cells to the tumour and immune-mediated cell death (81). Through the recognition of tumour-associated antigens (TAA) and tumour-specific antigens (TSA), immune cells are able to identify and kill neoplastic cells (82). However, tumours eventually manage to induce immune tolerance and escape immune destruction (83).

Figure 1: The Cancer Immunity Cycle (81)

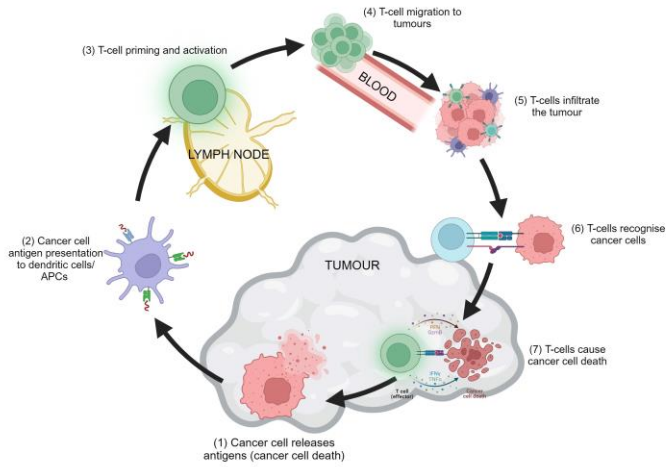


Figure 1: Cancer immunity is a cyclic process, consisting of a combination of immune-stimulatory factors that, in theory, should strengthen T-cell responses. Inhibitory factors are also involved, creating a negative feedback, thus preventing anti-tumour immunity. The 7 main stages are highlighted in this diagram, showing the location and the key cells involved in each step.

Image adapted from ref (81) and created in Biorender.com.

Abbreviations: APCs – antigen presenting cells

The immune system is divided into two separate, but interacting, entities; innate and adaptive immunity. The innate immune system is activated immediately when exposed to a foreign pathogen, and is comprised of multiple factors, including physical barriers such as skin and mucosa, various effector cells, for example Natural Killer (NK) T cells, monocytes/macrophages, and DCs, and humoral factors such as cytokines and complement. It recruits immune cells to sites of infection, activates complement and

identifies foreign substances. Its main roles are to prevent infection, eradicate pathogens and activate the adaptive immune response (84,85).

The adaptive immune response is activated within a few days of antigen exposure and is highly antigen specific and generates immune memory, enabling quick responses in the event of re-exposure to that specific antigen (85). It destroys invading pathogens and their associated toxic molecules, predominantly through B- and T-lymphocytes (86). B-cells produce antibodies, which bind to pathogens, and block them from binding to receptors on host cells (87). There are two types of T-cells, CD8+ cytotoxic T cells (CTLs) and CD4+ T-helper cells, which are involved in cell-mediated immunity (88). Each individual T-cell has a unique T-cell receptor (TCR), which, when activated, can multiply quickly (89). Antigen presenting cells (APCs), usually DCs, are required to activate T-cells. They break down the specific antigen and present it to the T-cell bound to its major histocompatibility complex (MHC), which leads to the production of CD4+ and CD8+ T-cells. CD8+ cytotoxic T-cells destroy virus-infected cells and antigen expressing tumour cells. They are short lived and tend to die once they have successfully eradicated the infected cells, only leaving behind a small number of memory effector cells in the event of antigen re-exposure. CD4+ T-helper cells do not have cytotoxic capabilities, but rather 'help' the immune response via multiple mechanisms, such as cytokine secretion and activation of B-cells and macrophages (88,89).

Ordinarily, the immune system is able to recognise and remove 'foreign' cancer cells from the body – this is known as 'immune surveillance' (90). Uncontrolled tumour growth is caused by a shift in this 'equilibrium' between tumour cells and the immune system. This is known as 'immune escape', where the tumour has lower immunogenicity and inhibits the anti-tumour immune response (91) (figure 2).

Figure 2: Cancer Immunoediting (90)

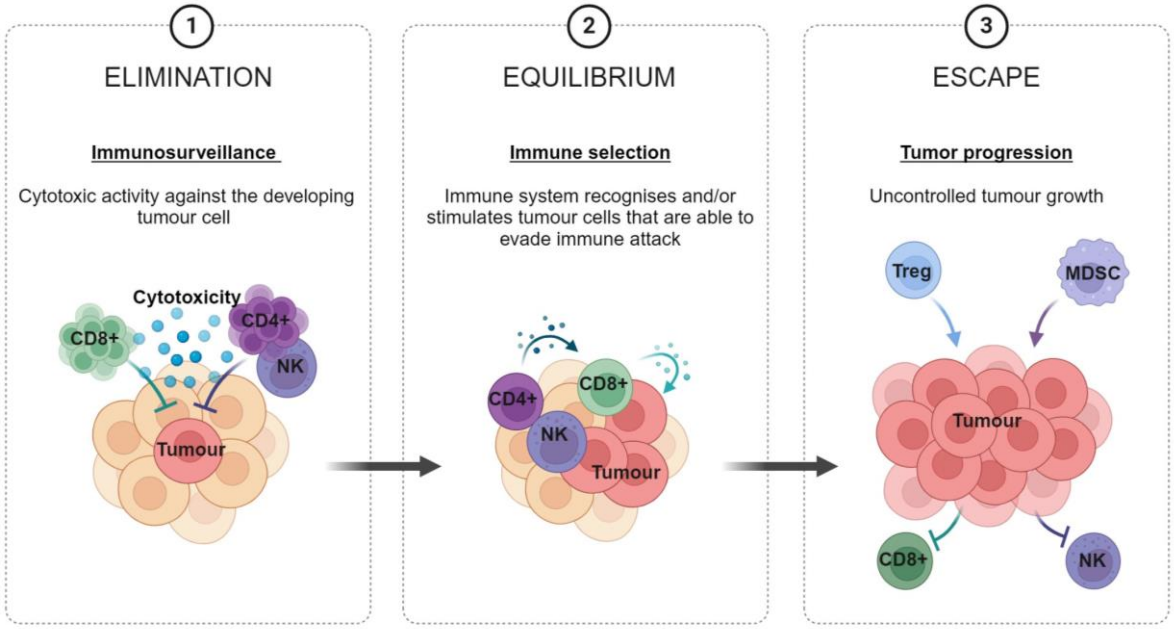


Figure 2: Cancer immunoediting consists of 3 stages 1) Elimination is immunosurveillance. 2) Equilibrium, where the immune system picks and/or stimulates tumour cell variants that have the ability to evade immune attack. 3) Escape is when uncontrolled tumour growth occurs.
Image adapted from ref (90) and created in Biorender.com.

Tumour growth and metastasis is heavily influenced by the tumour microenvironment (TME). The TME is complex, comprising of numerous cells, including malignant cells, immune cells, fibroblasts, blood vessels and cytokines and inflammatory mediators (92–94). Achieving an effective immune response against a tumour entails neoantigen presentation to T-cells, followed by activation of the T-cells present within the tumour (95). There are constant changes within the TME that impact tumour development, occurring due to interactions between cancer and host cells, as well as interactions between innate and adaptive immune cells (93). The constant immune pressure induces immunosuppression through a variety of mechanisms, including loss of TAAs and MHC molecules. The accumulation of regulatory T-cells (Tregs) results from persistent activation of T-cells and the presence of immunosuppressive factors such as indoleamine 2,3 dioxygenase (IDO) and transforming growth factor β (TGF-β). Tregs are a subpopulation of predominantly CD4+ T-cells, which suppress the immune system and play a major role in preventing autoimmunity (96). Pro-inflammatory chemokines draw myeloid derived suppressor cells (MDSCs) and monocytes into the

tumour. The monocytes subsequently differentiate into tumour associated macrophages (TAMs), further potentiating immunosuppression within the TME (97).

1.4.2 The ovarian cancer tumour microenvironment

Ovarian cancer is thought to be an 'immunologically cold' tumour (98). Over the last 20 years, there have been advances in the understanding of ovarian cancer immunogenicity (99). In 2003, Zhang et al demonstrated that the presence of CD3+ tumour infiltrating lymphocytes (TILs) correlates with improved survival in EOC (100). A couple of years later, Sato et al reported that improved survival is associated with the presence of intraepithelial CD8+ TILs and a high CD8+/Treg ratio (101). In contrast to this, increased numbers of CD4+ CD25+ FoxP3+ Tregs within the TME are associated with poor outcomes (83), as they reduce anti-tumour immunity and augment angiogenesis, leading to cancer progression (102). Little is known about CD8+ Tregs, although there have been reports of the presence of CD8+ T-cells within the ovarian cancer TME with a phenotype consistent with Tregs (103). It is also believed that the TME is heterogeneous, with numerous, distinct, immune microenvironments found within an individual patient (104). This is particularly relevant in EOC as the TME of the primary tumour may differ substantially to the metastatic TME, the most common site being the peritoneum/omentum (105).

The majority of HGSC have a TP53 mutation, which has been reported to be associated with an inflammatory TME due to raised levels of proinflammatory chemokines (106). However, the relevance of these chemokines as biomarkers predictive of prognosis is unclear (107).

T-cells and macrophages can be found in both the centre of the tumour and at the invasive margins. Other cells, mainly located at the invasive margins, include natural NK cells, MDSCs, mast cells and neutrophils. B-lymphocytes and DCs are generally found in tumour-adjacent lymphoid islets (TLSs) (108).

Macrophages enter the TME in response to growth factors and chemokines, commonly secreted by tumour cells. They are found within the tumour centre and invasive margins and/or tumour stroma and are known as tumour-associated macrophages (TAMs) (109). They account for the largest proportion of immune cells within the ovarian cancer TME (92,110). An increased macrophage density correlates

with a poor prognosis (111). There are two main functional types of macrophages; ‘M1-like’ (classical) and ‘M2-like’ (alternative) phenotype. The ‘M2-like’ phenotype is immunosuppressive (111) and promotes tumour progression in a number of different ways, including expression of chemokines, cytokines and growth factors involved in tumour development and invasion, immunosuppression and angiogenesis (112) .

Genome-wide expression profiling has shown the TAMs found in patients with HGSC have phenotypic similarity to both M1 and M2 macrophages; with up-regulation of CD163 and interleukin (IL)-10, typical M2 markers, as well us up-regulation of the M1 markers, CD86 and TNF (113–117). The M2-like CD163+ TAMs are associated with worse survival in ovarian cancer (118). TAMs secrete CCL18, a chemokine involved in Treg recruitment into the TME. In contrast to this, better outcomes are seen with a high M1/M2 macrophage ratio (106). Wanderley et al demonstrated that paclitaxel can transform the M2 TAM into an M1 phenotype, thereby promoting anti-tumour immunity (119).

Macrophage colony stimulating factor (M-CSF or CSF-1) is produced by monocytes, macrophages and ovarian cancer tumour cells and stimulates the production of M2-macrophages (120). Overexpression of CSF-1 has been reported in a number of solid tumours, including ovarian cancer, and is a predictor of poor prognosis (111,121).

Whilst a small proportion of EOC tumour cells express Programmed death-ligand 1 (PD-L1), it has been reported that a large number of TAMs in HGSC express PD-L1 (122). PD-L1 expression, together with high levels of B7H4, a co-inhibitory molecule, enables inhibition of cytotoxic T-cell immunity and development of T-cell exhaustion (123). T-cell dysfunction/exhaustion promotes tumour progression by preventing effective T-cell responses to tumour antigens. Exhaustion markers include, but are not limited to, Programmed cell death protein 1 (PD-1), Lymphocyte-activation gene 3 (LAG-3), T-cell immunoglobulin and mucin domain 3 (TIM-3) and Thymocyte selection-associated HMG BOX (TOX) (124). TIM-3, another inhibitory checkpoint (92,125), can be found on TILs. Its co-expression on T-cells with PD-1 indicates ‘very’ exhausted/dysfunctional T-cells (126). High levels of CD8+ TIM-3+ T-cells within the TME have been shown to predict poor outcomes in a number of solid tumours (127) .

There are some data that suggest that immune infiltrates in the ovarian TME may be predictive of response to NACT. As expected, increased numbers of Tregs were associated with a poor response; chemotherapy response score (CRS 1), whilst patients who demonstrated moderate or near-complete/complete responses (CRS 2 and 3) had an abundance of CD8+ T-cells and CD20+ B cells in their post-NACT tumour samples (128).

1.5 Immune cells within the peripheral blood

Whilst it is possible to examine the TME and characterise the numerous immune cells within it, obtaining sequential tumour tissue is invasive, cumbersome and it is often difficult to get adequate tissue for analysis (129). Immune cells within the peripheral blood of cancer patients may also play a role in immune evasion and cancer progression and may be an easier way to identify predictive and prognostic biomarkers. Blood tests are routinely taken both during treatment and throughout follow up. The immune cell profile within peripheral blood mononuclear cells (PBMCs) may provide useful information, although it lacks the heterogeneity seen within the tumour.

Effector cells are often predictive of a good response to immunotherapy, whereas the presence of immunosuppressive cells, such as Tregs and MDSCs have the opposite effect and are associated with a worse prognosis (130) (figure 3).

Figure 3: Key immune cells found in peripheral blood associated with a clinical response to immunotherapy (130)

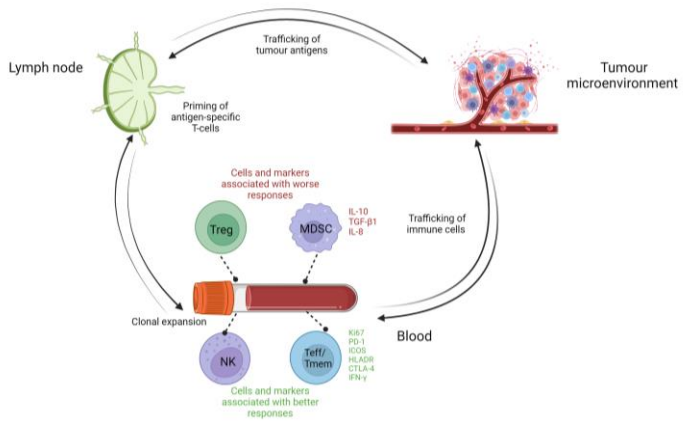


Figure 3: Image depicting the main immune cells found in the peripheral blood of patients with cancer. They can predict response to immunotherapy

Key: Green text – cells and markers associated with better response; Red text – cells and markers associated with worse responses; MDSC – myeloid-derived suppressor cell; NK – natural killer; Teff – effector T cell; Tmem – memory T cell; Treg – regulatory T-cell

Image adapted from ref 130 and created in Biorender.com.

In healthy adults, approximately 5-10% of circulating CD4+ T-cells are Tregs (CD25+) (130). Increased levels of CD4+ CD25+ T-regs have been found in patients with ovarian cancer, when compared to bloods taken from healthy donors (HD) (131). Their presence can prevent activated T-cells from functioning normally and may also predict early relapse (132). TIM-3+ T-cells play an important role in immune tolerance and their presence in peripheral blood may also influence disease progression. Higher levels of CD4+ TIM3+ and CD8+ TIM3+ T-cells have been found in women with ovarian cancer, compared to healthy controls. These correlated with higher tumour grade and were more frequently seen in recurrent disease (103). Ye et al explored peripheral blood immune cell populations in patients with ovarian cancer and compared these results to participants with borderline or benign adnexal lesions. They found that patients with ovarian cancer had an increased number of circulating Tregs and fewer CD3+ CD8+ (inhibitory) and CD8+ CD28+ (defined as cytotoxic) T-cells when compared to the controls. Additionally, levels of activated CD8+ CD28+ cells dropped post-PCS, when compared to pre-operative levels. Whilst the role of B-cells in ovarian cancer is unclear, this study demonstrated that reduced numbers of circulating B-cells were seen in patients with clear cell carcinoma, advanced stage and platinum resistant disease. This may be predictive of more aggressive disease; however further work needs to be done before making any definitive conclusions (133).

Immune checkpoint inhibitors are effective in producing durable responses in renal cell carcinoma (RCC). Analysis of peripheral blood taken from patients with RCC pre-operatively revealed high PD-1 expression on a variety of different immune cells, including CD14+ myeloid cells, effector T-cells and NK cells. Its expression was linked to disease stage and appreciably reduced following removal of the primary tumour (134). High numbers of PD-L1+ monocytes and PD-1+ T-cells have been identified in blood of women with ovarian cancer, when compared to blood sampled from women with benign ovarian disease (135). Further research is required to determine whether the identification of PD-1+ cells within the blood predicts response to anti-PD-1 therapy (136).

There are few data published looking at the changes of circulating immune cells after chemotherapy and/or surgery. A study looking at the effect of NACT on various

immune cell subsets in patients with triple negative breast cancer (TNBC) involved the analysis of blood taken at 3 different timepoints; At baseline, after initial chemotherapy with nab-paclitaxel and at the time of surgery and after a further 8 weeks of epirubicin/cyclophosphamide chemotherapy. Chemotherapy appeared to increase the number of monocytes, T-cells and Tregs. Initially, chemotherapy caused a fall in the number of CD4+ central memory (CM) T-cells, however bloods taken at the time of surgery, showed a reduction in the number of naïve CD4+ T-cells, resulting in a higher number of effector and effector memory (EM) T-cells. Changes in CD8+ T-cells were less marked, however, chemotherapy induced greater expression of CD38 and TIM-3, which may be indicative of immune activation (137).

Although monocytes were increased after chemotherapy, the study was unable to form any conclusions about the myeloid cells as MDSC markers or HLADR expression were not examined (137). When compared to HD bloods, the proportion of circulating plasmacytoid DCs (pDCs) was reduced in blood taken from cancer patients. However, the clinical relevance of this is unknown (138).

A meta-analysis of 40 studies found that elevated levels of pre-treatment circulating MDSCs in solid tumours are predictive of a poor prognosis (139). The significance of MDSCs in EOC is unclear (135,136). High proportions of circulating MDSCs, macrophages and monocytes, together with fewer DCs, have been found in women with ovarian cancer when compared to samples taken from HD, and these findings appear to be associated with poorer outcomes (140,141). There have been reports in melanoma that the number of circulating CD14+ CD16- HLA-DR^{hi} monocytes pre-treatment predicts response to immune checkpoint blockade (142).

The presence of a germline BRCA mutation may also impact the number of circulating T-cells and MDSCs. A higher number of CD8+ T-cells and fewer MDSCs were found in the peripheral blood of women with BRCA-associated ovarian cancer, compared to those with BRCA wild-type (BRCAwt), suggesting these women may do better in the early stages of their disease (143).

1.6 Immunotherapy in Ovarian Cancer

1.6.1 Background

Immunotherapy is a therapeutic approach that employs the host immune system to fight cancer (144). Examples include immune checkpoint inhibitors, cancer vaccines, cytokines, adoptive T-cell transfer and chimeric antigen receptor-modified (CAR) T- cells (145). The efficacy of cancer immunotherapy relies on it being able to produce cancer-specific anti-tumour T-cell responses, recognize TAAs and create memory responses (102,146).

Immunotherapeutic approaches in treating cancer were first explored in the 19th century, where a small number of spontaneous remissions of haematological malignancies were seen following infection with naturally occurring viruses, such as influenza (146).

The first reports of immunotherapy in ovarian cancer appeared in 1987. Iodine (^{131}I-) labelled monoclonal (anti-human milk fat globulin-1) antibodies directed against tumour-associated antigens were administered intraperitoneally to patients with advanced ovarian cancer, with some benefit noted in patients with persistent small volume disease following surgery and chemotherapy (147). Further studies with intraperitoneal ^{90}yttrium-labeled anti-human milk fat globulin-1 antibodies suggested possible, but limited, efficacy in ovarian cancer (148).

1.6.2 BCG

Following this, immunotherapy in the form of non-specific bacterial or synthetic adjuvants was considered for treatment of ovarian cancer. Bacillus Calmette-Guérin (BCG) can help the immune system respond to specific tumour antigens or enhance non-specific immunity (149–152). However, the addition of BCG to chemotherapy did not add any additional survival benefit (153).

1.6.3 Interferon

Interferon-γ (IFN- γ) is produced by activated T-cells and NK cells and has been shown to have anti-proliferative effects on ovarian cancer cells in vitro. Initially, intraperitoneal (IP) IFN-γ was shown to be effective in patients with residual disease after first line chemotherapy (154,155). A subsequent randomised phase III study of systemic

IFN-γ combined with cisplatin and cyclophosphamide showed a significant improvement in PFS in the IFN-γ group compared with the control group (chemotherapy alone) (156). However, this study was terminated early due to a change in the standard of care chemotherapy for first line ovarian cancer to carboplatin and paclitaxel. The addition of IFN-γ to carboplatin/paclitaxel shortened OS compared to chemotherapy alone, concluding that IFN-γ had no role in the first line treatment of ovarian cancer (157). Another randomised phase III study of IFN-α as first line maintenance treatment was also negative, with no progression or overall survival benefit seen (158).

1.6.4 Interleukin-2

Interleukin-2 (IL-2) is a cytokine, produced by activated T-cells and its main function is to stimulate the proliferation of immune cells (159). It was first shown to be effective in the treatment of cancer in the late 1980's, where clinically significant and durable responses were documented in patients with metastatic melanoma (160,161) and renal cell carcinoma (162,163), as well as demonstrating some efficacy in other solid tumours, including ovarian cancer (164).

Advanced ovarian cancer tends to be confined to the peritoneal cavity. Therefore, the preferred route of administration of IL-2 was by IP infusion, and the majority of published studies focussed on treating women with platinum resistant/refractory disease (165). However, IP administration is difficult, requiring placement of an IP catheter, increasing the risk of infection and catheter related complications. Patients require close observation and are also at risk of direct toxicities from the drug itself. Documented toxicities of IL-2 include hypotension, renal, CNS, GI toxicities, fever and allergies (166,167).

Another small retrospective study looked at the combination of picibanil (OK-432) (a lyophilised mixture of group A streptococcus pyogenes with anti-cancer activity), IL-2 and platinum/paclitaxel chemotherapy compared to standard chemotherapy alone in the adjuvant setting. The theory behind this study was that the immunotherapy component would provide a boost to the immune system prior to administering chemotherapy. No firm conclusions were made regarding efficacy or toxicity of this regimen, due to the retrospective nature of the study, the fact that histology was not

limited to high grade ovarian cancer and only a small number of patients were enrolled (168).

Whilst many early phase clinical trials demonstrated both tolerability and efficacy of IP IL-2 in the treatment of ovarian cancer, it did not materialise as a standard treatment option.

1.6.5 Oncolytic viruses

The use of viruses to treat cancer was first reported in the 1950s. The concept behind oncolytic viruses (OV) for the treatment of cancer consists of the use of either native or genetically modified viruses that target specific tumour cells for infection, induce cell death, and infect neighbouring tumour cells, resulting in a more widespread anti-tumour immune response. The mechanism by which cell death occurs depends on the type of virus and its dose (169). OVs also have the capacity to infect normal cells, but can be detected and destroyed by the anti-viral response mechanism present in healthy cells. This mechanism is either defective, or perhaps absent, from tumour cells, allowing the virus to replicate unchecked (170,171), resulting in malignant cell death. Once infected, the tumour cells themselves stimulate anti-tumour immunity by releasing tumour associated antigens, viral pathogen-associated molecular patterns (PAMPs), danger-associated molecular patter signals (DAMPs) and a variety of cytokines, leading to APC stimulation and eventual activation of T-cell responses (170).

OVs are typically administered either by intratumoural or intravenous injections. There have been a number of early phase clinical trials investigating the use of OVs in EOC. Whilst some have shown moderate anti-tumour activity, there are no approved OV therapies approved for ovarian cancer (172). The OCTAVE study, a phase I study of Enadenotucirev (EnAd), an oncolytic group B adenovirus, given in combination with weekly paclitaxel in PROC has completed recruitment. Preliminary results from the intravenous dose expansion group were presented at the ESMO congress in 2019, concluding that this combination has a manageable toxicity profile, with a disease control rate that warrants further investigation (171).

Despite some evidence of success and a vast amount of research, the use of OVs in the treatment of cancer has not become part of normal practice (173).

1.6.6 Toll-like Receptor 8 agonists

Toll-like receptors (TLRs) are endosomal receptors, one of many pattern-recognition receptors (PRRs), involved in innate immunity. They are expressed on both immune and non-immune cells and are crucial in identifying and removing pathogens, through recognition of PAMPs and DAMPs, and subsequent activation of the adaptive immune response (174). TLR8 is found on myeloid dendritic cells (mDCs), monocytes and NK cells. When activated, it stimulates inflammatory mediators, including the maturation of mDCs (175–177).

In general, chemotherapy induces tumour cell death through apoptosis and does not impact anti-tumour immunity (176). However, anthracyclines differ from other chemotherapeutic agents and have been shown to cause immunogenic cell death, through a variety of mechanisms. In particular increasing uptake of tumour antigens by mDCs, resulting in production of tumour-specific CD8+ T-cells (178). However, this process may be thwarted by the immunosuppressive factors in the TME.

PLD is an anthracycline, commonly used in the treatment of recurrent ovarian cancer. Motolimod is a selective agonist of TLR8 and was hypothesised to work synergistically with PLD to engage the anti-tumour immune response in ovarian cancer. However, this was disproven in a phase II randomised placebo-controlled study in women with relapsed ovarian cancer, which demonstrated no increase in either PFS or OS when compared with PLD alone (176,179,180).

1.6.7 IDO inhibitors

Indoleamine-2,3 dioxygenase (IDO) is the enzyme involved in tryptophan metabolism. Tryptophan is an amino acid that is crucial for protein synthesis and cell survival. IDO is activated by pro-inflammatory cytokines (181). It breaks down tryptophan into multiple metabolites, such as kynurenine, which is toxic to T-lymphocytes. The subsequent immunosuppression occurs due to a number of different mechanisms, including the prevention of NK cell function (182), suppression of cytotoxic lymphocytes (183–185), increases the number of Tregs (186) and stimulates production of MDSCs (184,187,188).

IDO is upregulated in a number of cancers, including HGSC. Its overexpression has been shown to correlate with decreased levels of CD8+ TILs, chemotherapy resistance and a poor prognosis (189–191).

IDO inhibitors may be another possible strategy in which to reverse the immunosuppression occurring within the TME. IDO inhibitors are in the early stages of development, and their role in the clinical setting is yet to be clarified. It is thought that they may be most successful when used in combination with immune checkpoint inhibitors (187) .

1.6.8 Adoptive T-cell therapies

Adoptive T-cell therapy (ACT) is a personalised form of immunotherapy made up of an individual patient's immune cells. Tumour or circulating immune cells are collected from a patient, expanded and manipulated ex-vivo and then re-infused into the patient following a course of lymphodepleting chemotherapy (192). In order to be effective, this therapy requires a high level of T-cell infiltration (193)

Several types of ACTs are available; those using expanded natural TILs, TCR engineered T-cells and Chimeric-antigen receptor (CAR)-modified T-cells. The ovarian cancer non-profit organisation, OvaCure, has a number of early phase clinical trials looking at the use of ACTs in ovarian cancer (194).

1.6.9 Dendritic cell vaccines

DCs are APCs, involved in both innate and adaptive immunity. They play a vital role in anti-tumour immunity by activating T-cells when exposed to TAAs (195). There are multiple ways in which DCs produce anti-tumour responses; through direct antigen presentation via MHCI/II molecules, their ability to migrate between lymphoid and non-lymphoid tissues and regulation of cytokines and chemokines to control inflammation (196). Cancer cells secrete anti-inflammatory cytokines, e.g. IL-10, and produce immunosuppressive signals, thereby preventing DCs from exerting their anti-tumour activity within the TME.

DC vaccines are another form of personalised therapy. Their production is complex, involving multiple steps. Patient blood is collected by apheresis and monocytes/haematopoietic stem and progenitor cells (HSPCs) are isolated from

peripheral blood. These cells are then treated with a combination of cytokines, toll-like receptor agonists and other activators, to stimulate differentiation of immature DCs. These DCs are then exposed to TAAs and these mature, TAA loaded DCs are then injected back into the patient (196,197), resulting in anti-tumour T-cell activity (198).

DC immunotherapy was first described in the early 1990's, with the first DC vaccine trial set up in 1996 (197). Since then, DC based treatment has been increasingly popular in numerous solid tumours, although despite being safe, clinical response rates have been variable (196). In 2010, Kantoff et al published the results from a phase III study, showing that Sipuleucel-T prolonged OS in men with metastatic castration-resistant prostate cancer (199). This was the first DC vaccine to be approved by the FDA.

Various trials of DC vaccines in EOC have shown favourable outcomes. SOV02, an open-label randomised phase II study demonstrated activity of the DC vaccine DCVAC/OvCa in recurrent ovarian cancer, when given together with platinum-based chemotherapy. PFS was not better when compared to chemotherapy alone, however, the exploratory analysis showed a statistically significant improvement in OS (200). SOV01, a randomised phase II trial presented at American Society of Clinical Oncology (ASCO) Conference 2021 showed an improvement in PFS and OS in patients with stage III EOC, who received adjuvant chemotherapy with the addition of DCVAC/OvCA. The largest benefit was seen in patients with immunologically 'cold' tumours, defined as those with low CD8+ T-cells counts in their tumour samples (201).

Despite these promising results, DCs are not used to treat ovarian cancer. Further work needs to be done to explore their full potential (197).

1.6.10 Monoclonal antibodies for cancer immunotherapy

Ca125 is a mucin-like glycoprotein expressed on epithelial ovarian cancer cells. The majority of patients with advanced EOC have high levels of circulating Ca125, and it is therefore a good marker to assess response to treatment.

Early phase studies using Abagovomab, an anti-idiotypic antibody which imitates Ca125, were promising, with evidence of a measurable immune response (202,203). However, the subsequent phase III study, MIMOSA did not demonstrate

improvements in recurrence-free survival (RFS) or OS when used as maintenance therapy in women with EOC (204).

Oregovamab is a murine monoclonal antibody (mAb), used as a therapeutic vaccine. It specifically binds to Ca125, resulting in an immunogenic complex. These complexes are subsequently processed by DCs and macrophages, which then triggers a Ca125-specific T- and B-cell anti-tumour immune response (205).

1.6.11 Autologous tumour cell vaccines

Transforming growth factor (TGF)-β is involved in a number of different cellular processes, including immunosuppression. Gemogenvatucle-T (Vigil) is a personalised immunotherapy autologous tumour cell vaccine made from harvested tumour tissue. It acts by reducing the expression of TGF-β1 and TGF-β2, causing inhibition of various immunosuppressive cells involved in cancer development. The VITAL trial, a phase IIb study, was designed to determine whether this vaccine was safe and effective when used as maintenance therapy in women with a clinical complete response after surgery and first-line chemotherapy. The primary end point of recurrence free survival in all patients was not met. However, several of the secondary end points were met, with significant improvements in recurrence-free and overall survival in BRCAwt and homologous recombination proficient (HRP) tumours (206,207). The combination of Vigil with immune checkpoint inhibitors (atezolizumab/durvalumab) in advanced gynaecological cancers are currently being investigated (208,209).

1.7 Immune checkpoint inhibitors

1.7.1 ICPIs in ovarian cancer

Immune checkpoint inhibitors (ICPIs) are drugs that have shown the most promise in the treatment of solid tumours. Immune checkpoints play an integral role in preventing autoimmunity and protecting surrounding tissue when the immune system responds to infection (180). One mechanism by which cancer cells avoid immunosurveillance is through the activation of these immune checkpoint pathways, which interfere with the anti-tumour immune response (210). Responses to ICPIs are thought to be attributed to the presence of a high mutational load and neoantigen burden (211).

The B7 family of immune-regulatory ligands includes activating and inhibitory co-stimulatory molecules. Under normal circumstances, the inhibitory molecules control immune responses to prevent tissue and organ damage (94). CTLA-4 is a member of the CD28:B7 immunoglobulin superfamily. It is a co-receptor on T-cells, involved in T-cell responses to antigens (212,213). When naïve T cells are stimulated through the TCR, CTLA-4 is upregulated and competes with CD28 for B7 on APCs. It has stronger binding affinity than B7, and in contrast to CD28, is an inhibitory molecule, thereby suppressing T cell activity through cell cycle arrest and decreased effector function (214). CTLA-4 inhibitors prevent this T-cell suppression and act to stimulate anti-tumour immunity (215).

PD-1 is expressed on chronically stimulated T-cells, Tregs, activated T cells and NK cells and is seen in instances of high T cell stimulation, such as cancer (216). It regulates immunity at multiple phases of the immune response, resulting in a co-inhibitory pathway. Its interaction with its ligands, PD-L1 and PD-L2, generates dysfunctional/exhausted T-cells and production of IL-10, a pro-tumour cytokine, preventing T cell anti-tumour activity (94). Both ligands, but particularly PD-L1, are expressed on many cancer cells (217). In EOC, worse outcomes are seen with PD-L1+ tumour cells, which have been associated with a reduced number of TILs. In addition, PD-L1+ tumour-associated dendritic cells within the TME inhibit T-cell function (94). ICPIs preclude the tumour from activating these checkpoint proteins on the surface of T-cells, thereby preventing immune escape and allowing the immune system to generate an anti-tumour response (218).

In ovarian cancer, PD-L1 is most commonly expressed by TILs, particularly TAMs, but is also found on tumour cells (217). There are conflicting data regarding whether PD-L1 expression is associated with better survival outcomes. Improved survival is seen in non-small cell lung cancer (NSCLC) (219,220) and has also been reported in HGSC (221). However, other studies have shown this to be associated with poorer outcomes in ovarian cancer (220,222). PD-L1 expression may be associated with response in some solid tumours (219), but does not necessarily predict response to anti-PD-1 therapy in all circumstances (223).

Ipilimumab is a fully human monoclonal antibody targeting CTLA-4 and was the first ICPI to be approved for metastatic melanoma in 2011, after showing improved overall

survival in this patient population (224,225). Ipilimumab causes a number of significant immune related adverse events (irAEs), most commonly affecting bowels, skin, liver and endocrine glands (226,227). Anti-PD-1 antibodies such as Nivolumab, a fully human immunoglobulin G4 (IgG4) monoclonal antibody and pembrolizumab, a humanised IgG4 monoclonal antibody have proven to be as effective as ipilimumab. Several anti- PD-L1 monoclonal antibodies are also in development. Common toxicities seen with nivolumab and pembrolizumab are fatigue, rash and diarrhoea (226,228). ICPIs are now routinely used in the treatment of a number of different solid tumours, including melanoma, head and neck cancer, non-small cell lung cancer, renal cell carcinoma, Merkel cell carcinoma and urothelial carcinoma (91).

A number of early phase clinical trials using single agent ICPIs in recurrent ovarian cancer (ROC) have been published. These patients are often heavily pre-treated and reported response rates range between 6 and 15%, although disease control rates (DCR) have been shown to be as high as 45% (229). High mutation rate and neoantigen burden appears to influence response to ICPIs. However, there is little understanding of the ovarian cancer TME prior to commencing treatment, making it difficult to identify why the majority of patients do not respond to these agents (230) and there are currently no known predictive biomarkers for response (146).

A phase II study of nivolumab in PROC reported an overall response rate (ORR) of 15% and DCR 45% (231). Keynote-028 was a multicohort, phase Ib study, assessing the efficacy and safety of pembrolizumab in patients with advanced biomarker-positive solid tumours and included a cohort of patients with PD-L1 positive advanced ovarian cancer. Pembrolizumab was well tolerated and the reported ORR for patients with ovarian cancer was 11.5%, with durable responses (≥20.5+ months). However, the dose used was weight based (10mg/kg every 2 weeks), which differs from the current standard dose of 200mg every 3 weeks. This trial was lacking specific information about the histological subtype of ovarian cancer, microsatellite instability (MSI) and BRCA status and it is impossible to make any definitive conclusions with regards to PD-L1 expression and response to pembrolizumab from this study alone (225).

The Keynote-100, phase II study treated 2 patient cohorts with ROC with pembrolizumab and reported an ORR of 8%. Median OS was 18.7 months. Higher PD-L1 expression on ovarian cancer cells, measured as combined positive score

(CPS) ≥10%, showed a trend towards better response and OS rates (232). This is the first study to document PD-L1 expression using an established PD-L1 antibody.

Avelumab, a human immunoglobulin G1 monoclonal antibody with a wild-type Fc region that blocks PD-L1 was trialled in two randomised phase III studies in combination with chemotherapy, Javelin 200 and Javelin 100. Javelin Ovarian 200 was a multicentre, randomised phase III study, in which patients with platinum resistant/refractory ovarian cancer were randomised to avelumab alone versus avelumab plus PLD. The primary end points of superior OS or PFS for one or both avelumab arms versus PLD were not met (233). Javelin Ovarian 100 was designed to evaluate avelumab with and/or following platinum-based chemotherapy in previously untreated patients with EOC. The primary objectives to demonstrate superior PFS for one or both avelumab-based treatment regimens were also not met. PD-L1 expression, CD8 and BRCA did not predict benefit from avelumab (234).

The NEOPEMBROV study is a non-comparative randomised phase II study designed to investigate whether the addition of pembrolizumab to standard NACT increased the rates of optimal debulking, assessed by complete resection rate (CRR), after ICS. The primary objective was met, with a higher CRR in the pembrolizumab group. Survival data and translational research, including PD-L1 status, are still immature and will be reported at a later date (235).

Clear cell ovarian cancer (CCOC) is a less common subtype of high grade EOC. The biology of clear cell carcinoma differs to that of HGSC. Response rates to standard chemotherapy are lower and identification of alternative therapeutic strategies is essential (236). A small group of patients with CCOC were enrolled in the nivolumab and KEYNOTE-100 studies and analysis showed activity of ICPIs in these patients, suggesting that they may be more effective in CCOC. This highlights the importance of assessing each histological subtype individually, rather than classifying all EOC as one disease.

The PEACOCC study, presented at ESMO 2022, shows efficacy in heavily pre-treated patients with advanced clear cell gynaecological cancers (237). This is an exciting development in these individuals with limited treatment options and a poor prognosis.

Table 2 summarises the main studies of ICPIs in EOC, either as single agents or in combination with chemotherapy.

Table 2: Main studies of ICPIs in EOC

Study	Drug	Phase	Clinical setting	No of pts	ORR	PFS (months)	OS (months)
NCT01375842 (51)	Atezolizumab	I	ROC	9	22.2%	2.9	11.3
Keynote-028 (NCT02054806)(232)	Pembrolizumab	Ib	ROC	26	11.5%	1.9	13.8
O'Cearbhaill et al (NCT02431559) (238)	Durvalumab & PLD	II	PROC	40	22.5	5.5	NE
Keynote-100 (NCT02674061) (231,239)	Pembrolizumab	II	ROC	376	7.4% 9.9%	2.1	18.7
INSPIRE (ovarian cohort) (NCT02644369) (240)	Pembrolizumab	II	ROC	18	NE	1.9	NE
UMIN000005714 (225)	Nivolumab	II	PROC	20	15%	3.5	20
Lee et al (241)	Pembrolizumab & PLD	II	PROC	26	26.1%	5.6	NE
NINJA (Japanese study) (242)	Nivolumab Gemcitabine OR PLD	III	PROC	316	7.6% 13.2%	2.0 3.8	10.1 12.1
Javelin Ovarian 200 (NCT02580058) (234)	Avelumab Ave & PLD PLD	III	PROC	566	3.7% 13.3% 4.2%	1.9 3.7 3.5	11.8 15.7 13.1
Javelin Ovarian 100 (NCT02718417) (243)	CT alone CT & Ave-> Ave maintenance CT-> Ave maintenance	III	Newly diagnosed stage III-IV EOC	998	30.4% 36% 30.4%	NE 18.1 16.8	NR NR NR
NEOPEMBROV (NCT03275506) (235)	NACT & Pembrolizumab NACT alone	II	Newly diagnosed stage III-IV HGSC	91	76% 61%	NE	NR
PEACOCC (237)	Pembrolizumab	II	Advanced CCGC	4	25%	12.2w	71.0w
MOCCA (NCT03405454)(244)	Durvalumab (MEDI-4736) Physician's choice chemotherapy	II	Recurrent CCOC	47	10.7% 18.8%	7.4w 14.0w	NE

Table 2: Main studies of ICPIs in EOC Abbreviations: No of pts – number of evaluable patients; ORR – objective response rate; PFS – median progression free survival (months); OS – median overall survival (months); Pembrolizumab - anti-PD-1 antibody; Nivolumab – anti PD-1 antibody; ROC – recurrent ovarian cancer; PROC – platinum resistant ovarian cancer; EOC – epithelial ovarian cancer; CT – chemotherapy (carboplatin/paclitaxel); Ave – Avelumab (anti- PD-L1 antibody); PLD – pegylated liposomal doxorubicin; NE – Not evaluated; NR – Not reached; NACT – neoadjuvant chemotherapy (carboplatin/paclitaxel); CRR – complete resection rate; CCGC – clear cell gynaecological cancers; COCC – ovarian clear cell carcinoma

1.7.2 Enhancing the benefits of ICPIs in ovarian cancer

It is believed that anti-cancer therapies, such as chemotherapy and targeted agents, influence anti-tumour immunity (245). As well as being pro-angiogenic, VEGF has been reported to promote immunosuppression within the TME (246). Therefore, combining ICPI's with anti-angiogenic agents, for example bevacizumab, may produce favourable results. A phase Ib study, using a combination of atezolizumab (anti- PD-L1 antibody) and bevacizumab in recurrent ovarian cancer showed an ORR of 15%, DCR 55%, median PFS 4.9 months and median OS 10.2 months (243).

PARP is an important enzyme involved in DNA damage repair and genome stability. In tumours that are HRD positive, PARPi inhibit DNA repair pathways and through synthetic lethality, result in cancer cell death (247). BRCA mutations and HRD are associated with increased mutational load, immunogenicity and higher expression of PD-1/ PD-L1 (248). PARPi have been associated with increased PD-L1 expression via a number of different mechanisms and immunomodulatory properties (249). PARPi result in DNA fragments accumulating in the cytoplasm, and in turn, neo-antigens accumulate on the cell surface. These neo-antigens are subsequently recognised by APCs, which then causes activation of the immune response and the Stimulator of interferon genes (STING) pathway (250). This results in the upregulation of pro-inflammatory cytokines and chemokines, and anti-tumour immunity (251). These immunomodulatory properties are behind the rationale of combining ICPIs with PARPi, in an attempt to enhance the immune response in EOC.

The TOPACIO/KEYNOTE-162 study explored the combination of niraparib plus pembrolizumab in breast and relapsed ovarian cancer. A total of 62 patients with ovarian cancer were enrolled. In the integrated efficacy analysis, ORR was 18% and DCR 65%. Among the 60 evaluable patients, 3 (5%) patients achieved a complete response (CR) and 8 (13%) had a partial response (PR), regardless of BRCA mutation, HRD or tumour PD-L1 expression. This study demonstrated promising activity of this particular combination of therapy in patients with very limited treatment options (252).

Immunogenic profiling was performed on tumour samples taken from patients enrolled in the TOPACIO study. Samples from chemotherapy naïve and chemotherapy-treated

patients were analysed. BRCA/HRD status, PD-L1 expression or tumour mutational burden, were not predictive of response with this combination; therefore, more advanced analyses were performed on the patient samples. Signature Multivariate Analysis (SigMa) is a relatively new computational tool, which is able to identify mutational signatures in tumours with a low mutational burden. Signature 3, a specific mutational signature, correlates with HRD (253) and was associated with response (32).

Amongst the patients who had prior chemotherapy, there were a higher number of exhausted CD8+ T-cells found in responding patients, when compared to chemotherapy naïve tumour samples. Single cell sequencing revealed fewer tumour cells and a higher proportion of immune and stromal cells, the majority of which were macrophages, CD8+ and CD4+ T-cells. The data also suggests that response was associated with a close interaction between PD-L1+ macrophages/tumour cells and exhausted CD8+ T-cells (32).

The MEDIOLA study evaluated the combination of olaparib given together with durvalumab (anti- PD-L1 antibody) as maintenance therapy in patients with PSOC bearing a germline BRCA mutation (gBRCAm), and shows promising results with 7 patients achieving a CR. The median PFS and duration of response in the cohort of patients who had received fewer lines of chemotherapy has not yet been reached, suggesting the benefit from this combination is greater in less heavily pre-treated patients. Biomarker endpoints were PD-L1 expression and presence of TILs, the results of which have not yet been published (254,255). Further evaluation of the non-gBRCAm patients revealed that the doublet combination did not meet its prespecified target for disease control rate, however, results for the triplet combination (olaparib, durvalumab and bevacizumab) are awaited (256).

The IMagyn050 study looked at the addition of atezolizumab (anti- PD-L1 antibody) to first line therapy with carboplatin/paclitaxel/bevacizumab in patients with advanced EOC. No PFS benefit was seen in the experimental arm, regardless of BRCA/HRD status or PD-L1 expression, and the majority of patients (97%) had a low tumour mutational burden (TMB) (257). However, a post-hoc exploratory analysis indicated that high PD-L1 expression on immune cells (defined as ≥5%) may improve PFS,

although further assessment of this is required as this cut off was only positive in a small proportion of patients enrolled in this study (258).

The ATALANTE/ENGOT-ov26, a placebo-controlled, double blinded randomised phase III study combining atezolizumab with bevacizumab and platinum-based chemotherapy for PSOC did not meet either of its primary endpoints; PFS in the intention-to-treat (ITT) or PD-L1- positive populations (259).

The PEMBOV study, a French multicentre, open-label, phase Ib study, designed to evaluate the safety and efficacy of the combination of PLD with bevacizumab and pembrolizumab in women with PROC. This combination was well tolerated, with 74% of patients having clinical benefit and 53% of patients with a durable response of >6 months (260).

The ANITA trial, a phase III randomised, double blinded trial of platinum-based chemotherapy with or without atezolizumab followed by niraparib +/- atezolizumab maintenance treatment. Patients had PSOC with a PFI >6 months. This trial did not meet its primary end point, with no significant benefit seen with the addition of atezolizumab to chemotherapy and maintenance niraparib in ROC (261).

The MOONSTONE study is an open-label, single-arm phase II study evaluating the efficacy and safety of niraparib plus dostarlimab in PROC. The primary endpoint of investigator assessed ORR was not met and PD-L1 status did not predict response (262).

KGOG3046/TRU-D, a phase II study of durvalumab and tremelimumab (anti-CTLA-4 antibody) combined with first-line NACT in patients with advanced-stage EOC, is the first study to report promising activity of dual immune checkpoint blockade in the neoadjuvant setting. Median follow up duration was 29.2 months, with 12-month, 24-month and 30-month PFS rates of 63.6%, 45.0% and 40.0% respectively (263).

Table 3 summarises the published studies to date of combination ICPIs with other targeted therapy in EOC.

Table 3: Studies using combination of ICPIs and other agents in EOC

Study	Drugs	Phase	Clinical setting	No of pts	ORR	PFS	OS
BP28179 (NCT01688206) (264)	Vanucizumab & Atz	I	PROC	17	24%	6-mth PFS: 65%	NE
TOPACIO /Keynote 162 (252)	Niraparib & pembro	I/II	PROC	60	18%	3.4	NR
MEDIOLA Doublet cohort (NCT02734004) (254,255)	Olaparib & durvalumab	I/II	gBRCAm PSOC	32/34	71.9%	11.1/NR	NR
MEDIOLA (NCT02734004) (256)	Olaparib & durvalumab Olaparib/durvalumab/ bev	II	Non-gBRCAm PSOC	32 31	31.3% 77.4%	5.5 14.7	NE NE
Moroney et al, 2020 (129)	Atezolizumab & Bevacizumab	Ib	PROC	20	15%	4.9	10.2
IMagyn050/GOG 3015/ENGOT-OV29 (NCT03038100) (258,265)	CT/Bev/placebo CT/Bev/Atz	III	Newly diagnosed stage III-IV EOC	1301	NE	18.4 19.5	NR
PEMBOV (NCT03596281) (260)	CT/Bev/pembro	Ib	PROC	22	32%	NE	NE
ATALANTE (NCT02891824) (266)	CT/bev/Atz CT/bev/placebo	III	PSOC	614	NE	13.5 11.2	35.4 30.6
ANITA (NCT03598270) (261)	CT/Atz x6 vs CT/placebo x6 If response: Niraparib/placebo vs Niraparib/Atz	III	PSOC	417 306	45% 43%		 5.3 6.7
MOONSTONE (NCT03955471) (262)	Niraparib/dostarlimab	II	PROC	41	29.3%	2.1	NE

Table 3: Combination ICPIs and other agents in EOC; Abbreviations: Pembro – pembrolizumab; PROC – platinum resistant ovarian cancer; gBRCAm – germline BRCA mutation; PSOC – platinum sensitive ovarian cancer; ORR – overall response rate; PFS – median progression free survival (months); OS – median overall survival (months); NR – not reached; NE – not evaluated; No of pts – total number of evaluable patients; Atezolizumab – anti-PDL1 antibody; Bevacizumab (Bev) – VEGF inhibitor; Vanucizumab – bi-specific human IgG1 antibody, blocking Ang-2 and VEGF-A, niraparib – PARP inhibitor; Pembrolizumab – anti-PD-1 antibody; Olaparib – PARP inhibitor; Durvalumab – anti- PD-L1 antibody; CT – chemotherapy; Atz – Atezolizumab

There are now multiple ongoing studies of combination ICPIs with targeted therapies (267–271).

The ATHENA (GOG-3020/ENGOT-ov45) study, a randomised phase III trial evaluating rucaparib maintenance therapy after first line platinum-based chemotherapy for ovarian cancer, consists of 2 parts; ATHENA-MONO, results of which have been published (272), leading to the approval of rucaparib maintenance therapy in the first-line setting. ATHENA-COMBO, investigating the effects of combination rucaparib and nivolumab as first-line maintenance therapy vs rucaparib alone (273) did not meet its primary end-point (274).

The DUO-O study is a randomised phase III study of durvalumab (durva), chemotherapy (carboplatin/paclitaxel) and bevacizumab (bev) followed by maintenance durva, bev and olaparib in the first line setting. Patients were randomised 1:1:1 to 3 different arms: Arm 1: chemotherapy/bev/placebo followed by maintenance bev/placebo; Arm 2: chemotherapy/bev/durva followed by maintenance bev/durva/placebo; Arm 3: chemotherapy/bev/durva followed by bev/durva/olaparib. Results from the pre-specified interim analysis demonstrate an improvement in PFS in Arm 3 compared to Arm 1 (hazard ratio (HR) 0.49 and 0.63 in the HRD positive and ITT populations). A non-statistically significant improvement in PFS between Arms 2 vs Arm 1 in the ITT population (275). Of note, the DUO-O study did not include an olaparib/placebo or olaparib/bev arm, making it impossible to conclude whether the addition of durva improves the PFS beyond what has already been reported in the PAOLA1 study (54).

The BEACON study a phase II single arm study of cobimetinib, a mitogen-activated protein kinase (MEK) inhibitor combined with bevacizumab and atezolizumab in women with PROC was presented at the ASCO annual meeting 2024. Preliminary results are promising, showing an overall ORR of 21% at week 24, with a further 28% of patients achieving stable disease (SD). 5 patients (17%) remain on treatment for ≥52 weeks to data, suggesting durable responses in a handful of patients (276).

The LEAP-005 study is a phase II, multicohort, open-label trial evaluating the combination of pembrolizumab and Lenvatinib (oral multikinase inhibitor), which included an advanced ovarian cancer cohort. Patients had received 3 prior lines of

therapy and anti-tumour activity was seen, with median PFS by blinded independent central review (BICR) 6.2 (4.0-8.5) months and OS 21.3 (11.7-32.3) months (277).

1.8 Conclusion

There remains a great deal to discover about the immune profile of ovarian cancer and whether an immunotherapy strategy will become established as a treatment for this disease. Based on the published data to date, there is minimal evidence to support single agent ICPIs in the upfront treatment of HGSC There is evidence to support ICPIs as an effective therapeutic approach for CCOC (237). Data from studies using combination approaches of ICPIs with active agents, such as anti-angiogenics and PARPi, have thus far been disappointing. There may be a role for immune checkpoint inhibition in the maintenance setting, attempting to build on a response to chemotherapy.

Despite the numerous trials investigating the use of ICPIs in EOC, there has been minimal translational research performed on patient samples. If immunotherapy is going to work in HGSC, we need to understand the underlying mechanisms of response and resistance. In order to do this, more in-depth exploration of the ovarian cancer TME and circulating immune cells is required. Were we to better understand the ovarian cancer TME better, could we select those patients likely to respond to immunotherapy with predictive biomarkers? To date, there are no identified predictive biomarkers.

1.9 Aims of this project

- **To establish whether maintenance pembrolizumab improves PFS in patients with PROC who respond or have stable disease after treatment with weekly paclitaxel within a phase II clinical trial maintenance pembrolizumab following weekly paclitaxel for recurrent ovarian, fallopian tube or peritoneal cancer (PROMPT trial).**
- **Collection of samples from patients enrolled in the PROMPT trial for translational research.**
- **Collection of samples from patients treated at University College London Hospital (UCLH) to evaluate the circulating immune cells and the tumour microenvironment of high-grade ovarian cancer and whether there are any changes associated with treatment.**

Chapter 2: Materials and Methods

2.1 Human samples

For the translational component of this project, collection of human samples was approved by the Institutional review board of the Barts/UCLH Gynaecological Tissue Biobank (BGTB) (REF042_E_Grunewald). Biological material was obtained, and clinical data stored for each patient.

2.1.1 Patient identification and consent

Eligible patients were identified during the weekly gynae-oncology pre-clinic meetings. All patients were treated at University College London Hospital (UCLH), London, UK. When seen in clinic, they were given a copy of the patient information sheet (Version 12: 21st January 2020) and had an opportunity to ask questions. All patients gave written informed consent (BGTB patient consent form version 11.1: 21/01/2020).

2.1.2 Healthy donors

Age and gender matched healthy volunteers were approached and sent a volunteer information sheet (Version 6: 21st January 2020). All healthy donors (HD) had an opportunity to ask questions and gave written informed consent (BGTB volunteer consent version 6.1: 21st January 2020).

2.1.3 Sample collection

Peripheral blood was taken at various timepoints from patients as specified in the ethics application. Healthy donors provided one sample of blood (2x10ml EDTA tubes (BD) at a time that was convenient for them. Fresh peripheral blood was transported at room temperature (RT) to the laboratory, stored at RT and processed within 24 hours of collection. All samples were logged in the laboratory 'Samples Logbook', including date of receipt, sample ID, timepoint, brief description of sample and name of biobank. Tissue processing sheet for each sample were completed, as per the laboratory standard operating procedure (SOP).

If available, FFPE blocks from biopsy and/or surgery were requested and reviewed by an expert gynae-oncology pathologist. If a suitable FFPE block was available, this was then cut into seven 2µm sections. The first section was stained with haemotoxylin and

eosin (H&E), with a further three sections stained with the designated multiplex immunofluorescence (mIF) panels.

2.2 Preparation of human samples for immunological analysis

All materials and reagents used for flow cytometry are detailed in table 4. All processing of samples was conducted in Class II biosafety cabinets (hoods). Flow cytometry antibodies are listed in tables 5 and 6.

Table 4: Materials and reagents

Material	Company
Plain RPMI	Merck Life Science Limited, Sigma, Cat: R0883 – 500ml
Fetal bovine serum (FBS)	PAN-Biotech, cat nr P40-37500
L-glutamine - 100ml	Merck Life Science Limited, cat nr G7513-100ML
Penicillin-Streptomycin - 100ml	Merck Life Science Limited, cat nr P0781-100ML
Human Ficoll-Paque Plus	Merck Life Science Limited, cat no 17-1440-03
ACK Lysing Buffer	Thermo Fisher Scientific (Life Technologies), cat no. A1049201
15ml / 50ml conical tubes	Sarstedt, cat nr 62.554.502 / cat nr 62.547.254
Trypan Blue	Merck Life Science Limited, Cat nr: T8154-100ML
Sarstedt, Microtest Plate, V Well, Polystyrene, With Lid, Sterile (V bottomed 96-well plate)	Sarstedt 82.1583001
Haematocytometer with CE Neubauer Improved	VWR, cat nr 1080339
1.8ml Cryogenic Vial, Int. Thread, Skirted (Sterile) 10 x50 packs	Starlab, cat nr E3110-6122
Dimethyl sulfoxide (DMSO)	Merck Life Science Limited, cat nr D2650-100ml
CoolCell™ and/or Nalgene™ Mr Frosty Freezing Containers	Fisher Scientific, cat nr 432138
DNase I grade II	Merck Life Science Limited, cat nr 10104159001 Stock: 7mg/ml
Heparin sodium salt from porcine intestine	Merck Life Science Limited, cat nr H3393-100KU
Dulbecco's Phosphate Buffered Saline 500ml (DPBS) - 10x500ml	Thermo Fisher Scientific (Life Technologies), cat nr 14190-169
EDTA 500mM	Merck Life Science Limited, cat nr 03690-100ML
Thawing media: RPMI, 20% FBS, 1% Penicillin-streptomycin 20,000 IU Heparin	Cat no: P4333, Sigma-Aldrich Sigma, Cat: H3393 – 10KU
Human Fc Receptor Binding Inhibitor	Thermo Fisher Scientific (Life Technologies), cat nr 14-9161-73
FOXP3 staining buffer set	Thermo Fisher Scientific (Life Technologies), cat nr 00-5523-00
Titretubes – 1000 pack	Bio-Rad, cat nr 223-9391
UltraComp eBeads™ Compensation Beads 5.0ml	Thermo Fisher Scientific (Life Technologies), cat nr 01-2222-42
ArC™ Amine Reactive Compensation Bead Kit (for use with LIVE/DEAD™ Fixable dead cell stain kits)-1 kit	Thermo Fisher Scientific (Life Technologies), cat nr A10346
Falcon 5mL Round Bottom Polystyrene Test Tube without Cap 12 x 75mm Sterile	VWR, cat nr 352052

Table 4: List of materials and reagents used for translational research in this project.

2.2.1 Peripheral blood mononuclear cell (PBMC) isolation

Peripheral blood was collected in 2 x 10ml EDTA tubes and transferred into a 50ml conical tube. The blood vacutainers were rinsed with an equal amount of room temperature plain RPMI to collect any remaining cells. Using a glass pipette, the blood and RPMI were underlaid with 10ml Human Ficoll-Paque Plus and centrifuged for 10 minutes at room temperature and a speed of 750 x g, without a brake.

Following centrifugation, the excess media was aspirated and the 'buffy coat' layer containing PBMCs at the interface of the Ficoll and media was collected. The PBMCs were resuspended in cold (4°C) complete RPMI (2% FBS, penicillin-streptomycin, L-glutamine). This was mixed briefly and centrifuged at 450 x g for 5 minutes in order to wash the cells thoroughly. Once washed, the media was aspirated, and the pellet of cells was disturbed to ensure no cells were stuck to the side of the tube.

The cells were then resuspended in 10mls of complete RPMI and kept on ice in order to improve viability. The cell suspension was vortexed and 10µl cell suspension was mixed with 10µl Trypan Blue in one well of a 96-well plate. The mixture was left for one minute to penetrate the cells and then transferred into a haematocytometer. The cells were visualised under the light microscope. In order to calculate the average cell count, the total number of cells in a designated number of squares (e.g. 4) were counted with an RS Pro-Counter. Dead cells appeared blue and were also counted. The mean number of cells was calculated by dividing the total number of cells counted by the number of squares. The total number of live cells in the cell suspension was then calculated using the following formula:

$$Average\ cell\ count$$

$$= \left(\frac{Total\ cell\ count - number\ of\ dead\ cells}{number\ of\ squares\ counted}\right) x\ dilution\ x\ total\ volume\ cell\ suspension\ x\ 10^4$$

2.2.2 Freezing PBMCs

Prior to freezing, temporary labels were printed using the label printer, documenting the sample ID, timepoint, sample type, cell count, date processed and affixed onto the appropriate number of cryovials. The cell suspension was then centrifuged at 450 x g

for 5 minutes at 4°C prior. Freezing media consisted of 50% FBS and 50% of FBS+20% DMSO, giving a final concentration of 10% DMSO.

Following centrifugation, the supernatant was aspirated, and pellet resuspended in the appropriate amount of FBS. Whilst the cell suspension remained on ice and gently swirling the tube, the appropriate amount of 20% DMSO in FBS was added drop by drop. The PBMCs were frozen down in aliquots of 4-5million cells per vial and aliquoted into 1ml freezing media. Once the DMSO was added to the cells, the cryovials were placed in 'Mr Frosties' and promptly placed in the -80C freezer. They slowly froze over a 24-hour period, at which point they were transferred to a -80 HTA freezer for storage until they were required for analysis.

2.2.3 'Slow' thawing of PBMCs

50ml aliquots of thawing media (RPMI, 20% FBS, 1% Penicillin-streptomycin 20,000 IU Heparin) were pre-warmed in the water bath for a minimum of 15 minutes and 250µl DNAse was added to each 50ml aliquot at a final concentration of 35µg/ml. Vials of cells to be thawed were retrieved from the freezer and quickly transferred into the water bath at 37°C until the ice pellet was completely thawed. The cells were transferred slowly to a pre-labelled 15ml conical tube and 15ml thawing media was added very slowly to the tube.

Once all the vials were thawed, the cells were left to sit at room temperature for 5 minutes and then spun at 250g for 15 minutes at 20°C. The supernatant was then aspirated, and the pellet resuspended in 400µl flow cytometry (FACS) buffer (PBS, 2% FBS, 2mM EDTA) in V-bottomed 96-well plates for flow cytometry staining.

2.3. Multi-parametric flow cytometry

2.3.1 Flow cytometry panels

Two flow cytometry panels were designed and optimised for PBMC analysis for the patient samples (tables 5 & 6).

Table 5: T-cell panel

Marker	Fluorophore	Supplier	Clone	Cat #	Dilution
Ki67	BUV395	BD	B56	564071	1/100
CD8	BUV496	BD	RPA-T8	612942	1/100
CD45RA	BUV563	BD	HI100	612926	1/100
CD38	BUV615	BD	HIT2	751138	1/100
HLADR	BUV661	BD	G46-6	612980	1/100
CD4	BUV737	BD	SK3	612748	1/100
CD3	BUV805	BD	SK7	612893	1/100
CD27	BV510	BioLegend	O323	302824	1/100
CD25	BV650	BioLegend	M-A251	356120	1/100
PD-1	BV711	BD	EH12.1	563245	1/50
TIM-3	BV750	BD	7D3	565564	1/100
CCR7	BV785	BioLegend	G043H7	353254	1/20
FoxP3	AF488	Thermofisher	PCH101	53-4776-42	1/100
EOMES	PerCPCy5	eBioscience	WD1928	46-4877-42	1/50
TCF7	PE	Cell Signalling Technology	C63D9	14456S	1/50
CD39	PE-CF594	BD	TU66	563678	1/100
CD57	PE-Cy5	BioLegend	HNK-1	359624	1/100
T-bet	PE-CY7	BD	4B10	7208782	1/100
GzmB	AF700	BD	GB11	560213	1/100
Viability	APC-e780	eBioscience	-	65-0865-14	1/1000

Table 5: Anti-human flow cytometry antibodies used in the experiments, with corresponding clone, fluorophore, supplier and volume of antibody used per test. The grey highlighted antibodies indicates intra-cellular staining; CCR7 staining (highlighted in blue) was done separately prior to all other antibody staining.

Table 6: Myeloid panel

Marker	Fluorophore	Supplier	Clone	Cat #	Dilution
CD45	BUV395	BD	HI30	563792	1/200
CD16	BUV496	BD	3G8	612944	1/200
CD56	BUV563	BD	NCAM16.2	612928	1/200
CD19	BUV615	BD	SJ25C1	612989	1/200
CD15	BUV661	BD	W6D3	741660	1/200
PDL1 (CD274)	BUV737	BD	MIH1	741881	1/200
CD3	BUV805	BD	SK7	612893	1/200
CD11B	BV510	BioLegend	ICRF44	301334	1/200
CD64	BV650	BD	10.1	740580	1/200
CD141	BV711	BD	1A4	563155	1/200
CD14	BV750	BioLegend	63D3	367136	1/200
CD40	BV785	BioLegend	5C3	334340	1/200
CD163	AF488	Thermofisher	GHI/61	53-1639-42	1/200
CD68	PerCPCy5	BioLegend	Y1/82A	333814	1/200
CD123	PE	Thermofisher	6H6	12-1239-42	1/200
CD86	PE-CF594	BioLegend	IT2.2	305434	1/200
CD11C	PE-Cy5	Thermofisher	3.9	15-0116-42	1/100
CD1C	PE-CY7	Thermofisher	L161	25-0015-42	1/200
HLADR	AF700	BioLegend	LN3	327014	1/200
Viability	APC-e780	eBioscience	-	65-0865-14	1/1000

Table 6: Anti-human flow cytometry antibodies used in the experiments, with corresponding clone, fluorophore, supplier and volume of antibody used per test. The blue highlighted antibodies indicate Fc receptor (FcR) staining and grey highlighted antibodies indicate intra-cellular staining.

2.3.2 Extracellular staining for T-cell panel

Following thawing and spinning, the cells were resuspended in 20µl FcR block to prevent non-specific staining and incubated in the fridge for 20 minutes. Each well of cells was initially stained with 50µl of the CCR7 mastermix and incubated for 15 minutes at 37°C. Following this, the wells were topped up to 180µl of FACS buffer and

spun at 800g for 2 minutes at 4°C. This was repeated twice more prior to resuspending the cells in 50µl of the extracellular mastermix. This was left to incubate in the fridge for 30 minutes, followed by a further 2 and a half washes with FACS buffer, prior to intracellular staining.

2.3.3 Extracellular staining for myeloid panel

Following thawing and spinning, the cells were resuspended in 50µl CD16/CD64 mastermix and incubated in the fridge for 30 minutes. The cells were then washed 3 times with 180µl of FACS buffer and centrifuged at 800g for 2 minutes at 4°C between each wash. Each well of cells was then stained with 50µl of the remaining extracellular mastermix and incubated for another 30 minutes in the fridge. This was followed by a further 3 washes with FACS buffer.

2.3.4 Intracellular staining for T-cell and myeloid panels

The same protocol was used for intranuclear permeabilization and intracellular staining for both panels. The cells were resuspended with 100µl Fix Perm and left to incubate in the fridge for 2 hours. These were then washed three times with Perm Wash and spun at 800g for 2 minutes at 4°C after each wash. The cells were then stained with 50µl intracellular staining mastermix, and incubated in the fridge for 2 hours, followed by three final washes with Perm Wash. The cell pellets were then resuspended in 100µl PBS, and kept at 4°C in fridge, until acquisition the following day.

2.3.5 Preparation of compensation beads

Each titre tube was labelled with a fluorophore. After gently mixing the beads, 20µl beads were pipetted into each titre tube followed by 1µl antibody. These were then incubated at 4 °C for 10 minutes and topped up with 180µl PBS.

The live/dead bead was prepared using ArC reactive and negative beads. After gently mixing the ArC reactive beads with the vortex, one drop was added to a pre-labelled FACS tube and let to sit at room temperature for 5 minutes followed by the addition of 1µl eF780 viability dye. This was then covered and incubated at room temperature for 30 minutes. It was then washed with 3ml PBS and spun in the centrifuge at 300 x g for 5 minutes. The supernatant was carefully poured off and the bead pellet was

resuspended in 500µl FACS buffer. Finally, one drop of ArC negative beads was added and mixed thoroughly.

2.4 Flow cytometry data acquisition

All flow cytometry data acquisition was performed on the BD FACSymphony (BD Biosciences).

2.5 Flow cytometry data analysis

Flow cytometry data analysis was performed in FlowJo version 10.8.1 (Tree Star Inc.). Statistical analyses were performed in Prism 9 (GraphPad Software, USA.). P-values were calculated using the Ordinary one-way Anova test if data was normally distributed (based on the Shapiro-Wilk (W) and D'Agostino-Pearson omnibus (K2) tests). If the data set did not pass these normality tests, P-values were calculated using Kruskall-Wallis analysis of variance. Statistical significance was determined by p-value <0.05.

The error bars on each graph represent the mean values with standard deviation, unless otherwise indicated.

2.6 Multiplex Immunofluorescence (mIF)

Each FFPE block was cut into seven sequential 2µm sections. The first section was stained with H&E and subsequent sections numbered 2-7 for mIF staining. Each H&E section was reviewed by an experienced Consultant Gynae-Oncology Histopathologist, who annotated the Regions of Interest (RoI). For each experiment, a stained tonsil FFPE section was included as a positive control.

2.6.1 Multiplex IF staining

Tumours were stained with three individual panels comprising six fluorophore-labelled markers and DAPI as a nuclear counterstain. The antibodies and staining conditions are shown in tables 7-9. Automated staining was performed on the Leica Bond RX autostainer.

Table 7: Panel 1: Staining conditions for mIF for T-myeloid panel

Marker	Species	Supplier	Product	Antibody dilution	Fluorophore	Fluorophore dilution
CD4	Mouse Monoclonal	Leica biosystems	NCL-L-CD4-368	1/50	Opal 520	1/100
FOXP3	Mouse Monoclonal	Abcam Plc.	Ab20034	1/200	Opal 540	1/100
CD14	Rabbit Monoclonal	Abcam Plc.	Ab183322	1/50	Opal 570	1/100
CD15	Mouse Monoclonal	Agilent Technologies, Inc.	M3515	1/50	Opal 620	1/200
CD11b	Rabbit Monoclonal	Abcam Plc.	Ab133357	1/6000	Opal 650	1/200
CD8	Mouse Monoclonal	Leica biosystems	NCL-L-CD8-4B11	1/200	Opal 690	1/100

Table 8: Panel 2: Staining conditions for mIF for dysfunctional T-cell panel

Marker	Species	Supplier	Product	Antibody dilution	Fluorophore	Fluorophore dilution
CD4	Mouse Monoclonal	Leica biosystems	NCL-L-CD4-368	1/50	Opal 520	1/100
TCF1	Mouse Monoclonal	Santacruz biotechnology Inc.	Sc-271453	1/80	Opal 540	1/100
TIM3	Rabbit Monoclonal	Cell signalling technology	#45208	1/100	Opal 570	1/200
GzmB	Mouse Monoclonal	Leica Biosystems	NCL-L-GRAN-B	1/40	Opal 620	1/200
PD1	Mouse Monoclonal	Abcam Plc.	Ab52587	1/350	Opal 650	1/200
CD8	Mouse Monoclonal	Leica biosystems	NCL-L-CD8-4B11	1/200	Opal 690	1/100

Table 9: Panel 3: Staining conditions for mIF for macrophage panel

Marker	Species	Supplier	Product	Antibody dilution	Fluorophore	Fluorophore dilution
CD68	Mouse Monoclonal	Agilent Technologies	M0876	1/100	Opal 480	1/50
CD163	Mouse Monoclonal	Leica biosystem	CD163-L-CE	1/100	Opal 690	1/100
CD86	Rabbit Monoclonal	Cell signalling Technologies	#91882	1/200	Opal 570	1/200
MHC-II	Mouse Monoclonal	Abcam Plc.	ab17101;	1/250	Opal 520	1/100
PD-L1	Mouse Monoclonal	Agilent Technologies	SK005	RTU	Opal 620	1/100
PAX-8	Mouse Monoclonal	Sigma Aldrich	363M-15	1/200	Opal 780	1/25

Tables 7-9: Multiplex immunofluorescence (mIF) panels and staining conditions

2.6.2 Multiplex IF image acquisition

Following staining of the first two panels, a Vectra 3 Automated Quantitative Pathology Imaging System (Akoya Biosciences) was used to scan the whole slide at 4x magnification. The third panel was scanned on Phenomager HT as the Vectra 3 was out of order. This whole slide image was then loaded onto Phenochart (Akoya Biosciences Inc.) for manual annotation for all the RoI, which were then scanned at 20x magnification. The images were then loaded into inForm image analysis software (Akoya Biosciences Inc.).

2.6.3 Multiplex IF data analysis

The multiplex immunofluorescence (mIF) images were processed by DeepMIF (278) to map locations of diverse T cells, cancer cells and myeloid cells. DeepMIF is a deep-learning-based pipeline for identifying cell phenotypes based on the combined expression of markers detected on mIF. Channels of the mIF image were first extracted into deconvolved images (DIs), each representing the expression of an individual marker. Each DI was processed by the cell detection model in DeepMIF (279) to identify pixels with high possibility of being a cell centre. A cell classifier was then implemented on the detections to discern positive or negative marker expression. To improve the accuracy of classification, distinct cell classifiers were applied for DIs of nuclear and non-nuclear markers respectively (Table 10).

Table 10. List of nuclear and non-nuclear markers in each panel.

Panels	Nuclear markers	Non-nuclear markers
T-myeloid	FoxP3	CD4, CD8, CD11b, CD14, CD15
Dysfunctional T	TCF1	CD4, CD8, GzmB, TIM3, PD1
Macrophage	PAX8	CD86, MHCII, CD163, CD68, PDL1

Table 10: List of nuclear and non-nuclear markers in each multiplex IF (mIF) panel

Cell phenotypes were characterised by the co-expression of markers. A cell is deemed to co-express multiple markers if positive detections on the corresponding DIs overlap (located within a radius of 5µm). The co-expression was evaluated for each pair of markers included in the cell phenotype definition. Coordinates of a co-expressing cells were determined as the centre of positive detections for each respective marker. Finally, cells with expression of negative markers were excluded. This pipeline gave rise to an average of 25611, 34758, and 68355 cells identified for the dysfunctional T panel, T-myeloid panel, and macrophage panel respectively. A breakdown of cell type densities are summarised in table 11.

Plots and statistical analyses were conducted using the ggpubr library in R version 4.3.2. For comparisons between two independent patient groups, Wilcoxon rank-sum test was used. For comparisons between paired samples from the same patient, Wilcoxon signed-rank test was used. All statistical tests were two-sided. Statistical significance was determined by a p-value <0.05.

Table 11. Average densities of cell phenotypes identified on mIF panels.

Panels	Cell types	Markers	Subtypes	Cell counts/mm^2
T-myeloid	CD4+ T cells (T-myeloid panel)	FoxP3-	Th	236.75
		FoxP3+	Treg	22.86
	CD8+ (T-myeloid panel)	CD8+	CD8+ T	226.40
	MDSC (CD11b+)	CD15+/CD14-	PMN-MDSC (polymorphonuclear or granulocytic)	23.53
		CD14+/CD15-	M-MDSCs (monocytic MDSCs)	90.98
Macrophage	Macrophage (CD68+)	MHCII-/CD86+/CD163-	M1 macrophages	4.41
		MHCII-/CD86+/CD163+	CD86+/CD163+ macrophages	4.23
		MHCII-/CD86-/CD163+	M2 macrophages	27.99
		MHCII+/CD86+/CD163-	MHCII+ M1 macrophages	1.04
		MHCII+/CD86+/CD163+	MHCII+ CD86+/CD163+ macrophages	1.61
		MHCII+/CD86-/CD163+	MHCII+ M2 macrophages	4.44
	monocytes (CD163+CD68-)	MHCII+/PDL1+	MHCII+PDL1+ monocyte	2.56
		MHCII-/PDL1-	MHCII-PDL1- monocyte	421.04
	Tumour cells (PAX8+)	MHCII-/PDL1+	MHCII-PDL1+ tumour cells	14.41
		MHCII+/PDL1+	MHCII+PDL1+ tumour cells	1.44
		MHCII-/PDL1-	MHCII-PDL1- tumour cells	714.15
		MHCII+/PDL1-	MHCII+PDL1- tumour cells	40.77
Dysfunctional-T	CD4+ T cell	PD1-/TIM3-/TCF1-	CD4+ T cell	125.13
		PD1+/TIM3-/TCF1-	PD1+CD4+ T cell	19.05
		PD1-/TIM3+/TCF1-	TIM3+CD4+ T cell	17.47
		PD1+/TIM3+/TCF1-	PD1+TIM3+CD4+ T cell	1.34
		PD1-/TIM3-/TCF1+	TCF1+CD4+ T cell	46.48

	CD8+ T cell	PD1-/TIM3-/TCF1-/GzmB-	CD8+ T cell	141.43
		PD1-/TIM3+/TCF1-/GzmB-	TIM3+CD8+ T cell	10.02
		PD1+/TIM3-/TCF1-/GzmB-	PD1+CD8+ T cell	21.24
		PD1+/TIM3+/TCF1-/GzmB-	PD1+TIM3+CD8+ T cell	2.79
		PD1-/TIM3-/TCF1-/GzmB+	Active CD8 T cell	10.20
		PD1-/TIM3-/TCF1+/GzmB-	Naïve CD8 T cell	16.10

Table 11: Average densities of cell phenotypes identified on the mIF panels

Chapter 3: The Phase II trial of maintenance pembrolizumab following weekly paclitaxel for recurrent ovarian, fallopian tube or peritoneal cancer (PROMPT)

3.1 Introduction

As discussed in detail in the Introduction (Chapter 1), EOC is the most lethal gynaecological malignancy, with more than 300,000 women worldwide diagnosed each year, resulting in 200,000 deaths per year (280). Whilst most patients respond to first line treatment, the majority will relapse, with a median PFS of 18 months. At relapse, if patients are deemed to have a good chance of responding to further platinum-based chemotherapy, they would be rechallenged with a platinum-combination regimen. Patients who progress fairly soon after completing platinum-based chemotherapy have fewer options. Weekly paclitaxel is often used in the first instance for platinum-resistant relapse. Response rates have been reported to be between 30-40% (281)and median PFS approximately 3-4 months (282). Prognosis is poor, with a median OS of 12 months (283) Improvements in the treatment of platinum-resistant ovarian cancer are needed and as presented in chapter 1, immunotherapy has thus far not been shown to be effective.

Most studies enrolled heavily pre-treated patients and reported response rates range between 6 and 15%, although DCR have been shown to be as high as 45% (229). High mutation rate and neoantigen burden appears to influence response to ICPIs. However, there is little understanding of the ovarian cancer TME prior to commencing treatment, making it difficult to identify why the majority of patients do not respond to these agents (230) and there are currently no known predictive biomarkers (146).

Chapter 1, Introduction, summarises the results from numerous clinical trials using ICPIs as treatment either in combination with chemotherapy or as a single agent, which have mostly been negative studies. Despite the number of trials, there is minimal published translational work performed on samples taken from patients with EOC being treated with immunotherapy. The KEYNOTE-100 study was the first study to document PD-L1 expression using an established PD-L1 antibody. This showed a trend towards better response and OS rates in patients with higher- PD-L1 expression (8).

The Phase II trial of maintenance pembrolizumab following weekly paclitaxel for recurrent ovarian, fallopian tube or peritoneal cancer (PROMPT) was designed to

explore whether maintenance pembrolizumab in patients who have been treated with weekly paclitaxel and either responded or have stable disease after a minimum of 4 cycles can prolong the PFS and collect samples for more in depth translational research. Weekly paclitaxel is the preferred treatment choice in PROC and response rates are generally higher than other agents (283). Although overall response rates to ICPIs were low, DCR in some studies were more encouraging (284). The rationale for PROMPT was to explore whether maintenance pembrolizumab can build on this response/stability to weekly paclitaxel, as beyond this, there are very few effective treatments.

This study was designed to delay progression in patients with a poor prognosis and collect samples for translational research to better understand the ovarian immune microenvironment and what impact ICPIs have on this. The rationale behind this was that pembrolizumab may build on the response to paclitaxel and perhaps induce a better microenvironment for immunotherapy to be effective. There are some data to suggest that certain chemotherapy agents induce changes within the TME through a number of different mechanisms (285). Although the main mechanism of action of paclitaxel is prevention of microtubule depolymerisation, resulting in cell cycle arrest at G2/M phase of the cell cycle and ultimate cell death (286), it has also been shown to regulate various immune cells within the TME. This includes the inhibition of Tregs, thereby preventing tumour immune escape, as well as increasing the rate of apoptosis in cancer cells, release of tumour antigens and enhancing phagocytosis of APCs. However, further research is required to confirm whether these immunomodulatory effects influence responses to ICPIs (287).

3.2 Methods

3.2.1 Study design and participants

PROMPT was a phase II single arm, multicentre study, using maintenance pembrolizumab in patients who received weekly paclitaxel for recurrent ovarian cancer. Eligible patients were 18 years or older, with histologically confirmed high grade recurrent ovarian, fallopian tube or primary peritoneal cancer, who had relapsed within six months of platinum-based chemotherapy and were treated with weekly paclitaxel as first line treatment for platinum resistant disease, defined as relapsing

within 6 months of platinum-based chemotherapy. All patients needed to have demonstrated either SD/PR or CR to paclitaxel to be eligible for the study. They could have had up to 3 lines of platinum-based chemotherapy prior to starting weekly paclitaxel for their first platinum resistant relapse. Patients must have been treated with a minimum of 4 cycles weekly paclitaxel, with at least SD according to Response Evaluation Criteria in Solid Tumours (RECIST) version (v) 1.1 on imaging, with measurable and biopsiable disease. Eastern Cooperative Oncology Group performance status (ECOG PS) must be 0-1.

3.2.2 Treatment

Patients with PROC with at least SD or response to weekly paclitaxel received pembrolizumab 200mg IV every 3 weeks until disease progression or toxicity, for a maximum of 24 months. Imaging was performed at baseline, pre-cycle 4 and pre-cycle 7, then every 4 cycles (figure 4). Adverse events were graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

3.2.3 Outcomes

The trial objective was to demonstrate a clinically meaningful extension of PFS using maintenance pembrolizumab. The primary endpoint was 6-month PFS, measured from the start of maintenance pembrolizumab. There were a number of secondary endpoints; PFS at 6 months, measured from the start of weekly paclitaxel, OS, disease response (RECIST v1.1), toxicity and compliance. Exploratory end points included immunological analysis of both blood and tumour and further treatment following disease progression.

3.2.4 Ethical approval and trial conduct

The PROMPT trial gained Ethical and MHRA approvals on 22nd October 2018. The trial was funded by a grant from MSD/Merck and also supported by the core grant of the Cancer Research UK (CR UK) and University College London (UCL) Cancer Trials Centre (CTC). The trial was sponsored by UCL (sponsor reference number UCL/17/0629) and was registered in a public database, clinicaltrials.gov (reference NCT03430700) and conducted by the CR UK and UCL CTC.

The trial was conducted according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for Good Clinical Practice (GCP). All patients provided written informed consent prior to any trial activity.

3.2.5 Statistical analysis

For this trial to be deemed clinically worthwhile, the aim was to detect an increase in the 6-month PFS rase to 65%, based on the AURELIA trial (288). Using A'Hern's single stage phase II design (Sample Size Tables for Clinical Studies Software Program), with a one-sided 5% significance level and 80% power, a sample size of 28 patients was needed, with at least 16 alive and progression-free at 6 months to warrant further investigation. The aim was to recruit 28 patients within an 18-month period. Kaplan-Meier estimates were used to analyse PFS. The number and percentage of patients who are progression-free and alive at 6 months will be presented with both one-sided (lower confidence limit) and two-sided 95% confidence intervals. Analysis was based on an ITT basis for all eligible patients.

Figure 4: Overview of the PROMPT trial

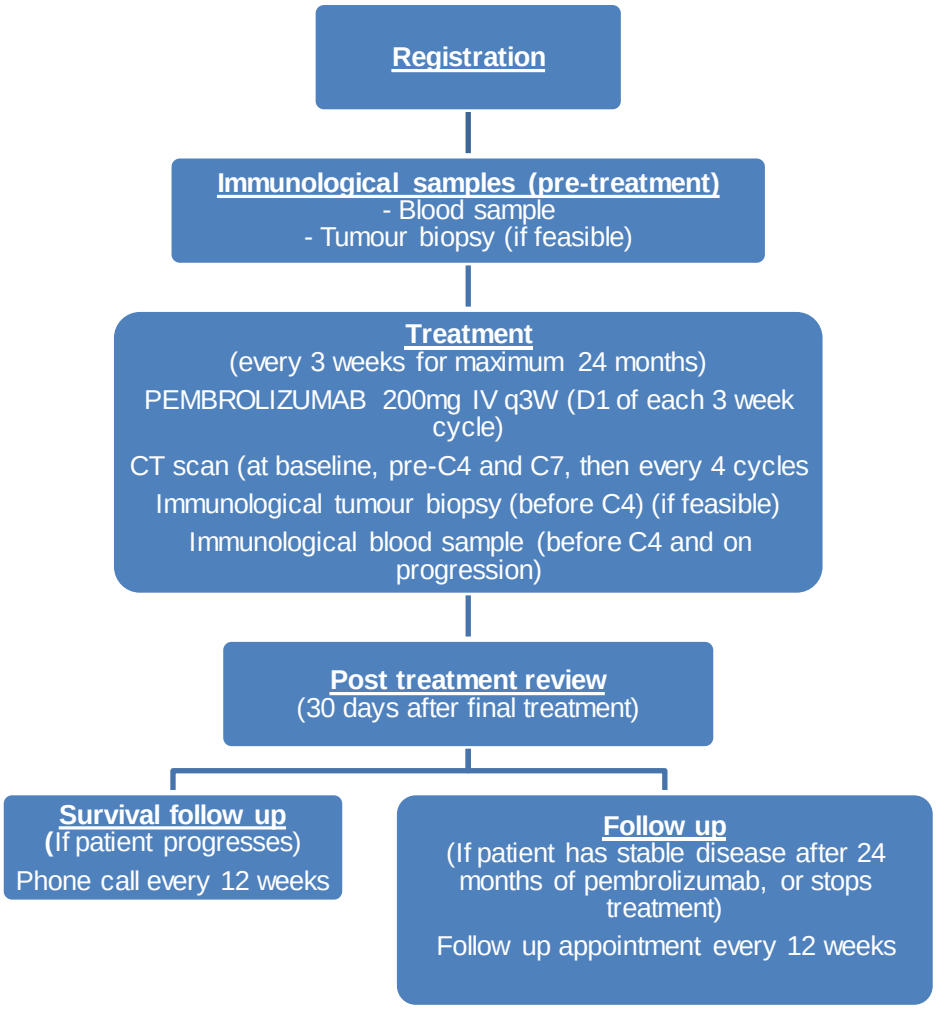


Figure 4: Overview of the PROMPT trial

3.2.6 Protocol modifications

Trial recruitment was slower than anticipated, with only 10 patients recruited between trial opening in 2018 and review on 17th March 2021. Reasons for this included the Covid-19 pandemic, the mandatory requirement for measurable and biopsiable disease and the poor prognosis associated with PROC. The majority of patients screened progressed before completion of the full 6 cycles of planned weekly paclitaxel. A handful of patients responded so well, they did not have measurable and biopsiable disease on their screening CT and were therefore ineligible for the study. In August 2021, a protocol amendment was approved. This made the requirement for

measurable and biopsiable optional, rather than mandatory, and patients were allowed to be registered to the trial after 4 cycles of weekly paclitaxel, rather than 6. These changes led to an improvement in recruitment, with a further 10 patients recruited. In October 2022 the Independent Data Monitoring Committee (IDMC) recommended that the trial stop recruiting due to an overall lack of efficacy with pembrolizumab. There were no safety concerns, and an urgent closure was not required. Recruitment stopped in December 2022 with a total of 20/28 patients enrolled. At this time, 5 patients remained on treatment. The IDMC recommendation was for site investigators to with patients whether they wished to continue treatment if they were deemed to be deriving clinical benefit. These patients remained on study treatment until disease progression. All patients were subsequently followed up in line with the protocol.

3.3 Results

3.3.1 Patients

Between September 2019 and November 2022, 20 patients were enrolled from 4 centres within the UK. Patients received at least one infusion of pembrolizumab. All patients were evaluable and included in the analysis. Demographic and baseline characteristics are summarized in table 1. The median age was 61 years, all had high grade serous histology and ECOG PS of 0/1 (50%/50%).

Patients received a median of 3 lines of prior platinum-based chemotherapy (range 1-3) and a median number of 5.5 cycles of weekly paclitaxel (range 4-17). 13 (65%) patients had stable SD following treatment with weekly paclitaxel, with 7 (35%) achieving a PR.

At the time of analysis, all patients had stopped pembrolizumab, completing a median of 3.5 cycles (range 2-18). 18 patients stopped due to radiological disease progression (PD) and 1 with clinical progression. This patient continued pembrolizumab beyond progression until PD was confirmed on imaging a few months later. One patient stopped due to an adverse event (AE) related to treatment. In total, 7 infusions in 4 patients were delayed or omitted. Reasons for this included patients being unwell (testing positive for COVID-19, grade 3 (G3) chest infection and possible progression), patient choice, patient holiday, capacity issues in the chemotherapy suite and an administrative error.

Table 12: Demographic and baseline patient characteristics

Baseline characteristics		Total (N=20)
Age (years)	Median (range)	61 (41-78)
ECOG Performance status	0 1	10 (50%) 10 (50%)
FIGO stage at diagnosis	I II III IV	2 (10%) 1 (5%) 14 (70%) 3 (15%)
Previous surgery	Yes No	20 (100%) 0 (0%)
Previous radiotherapy	Yes No	1 (5%) 19 (95%)
Previous systemic therapy	Yes No	20 (100%) 0 (0%)
No of lines of prior chemotherapy	3 (1-3)	20 (100%)
Cycles of weekly paclitaxel	Median (range)	5.5 (4-17)
Best response to weekly paclitaxel	Partial response Stable disease	7 (35%) 13 (65%)
Number of target lesions, n=17	Median (range)	2 (1-5)
Sum of longest diameter (mm), n=17	Median (range)	44 (10-212)
Number of non-target lesions, n=16	Median (range)	2 (1-4)

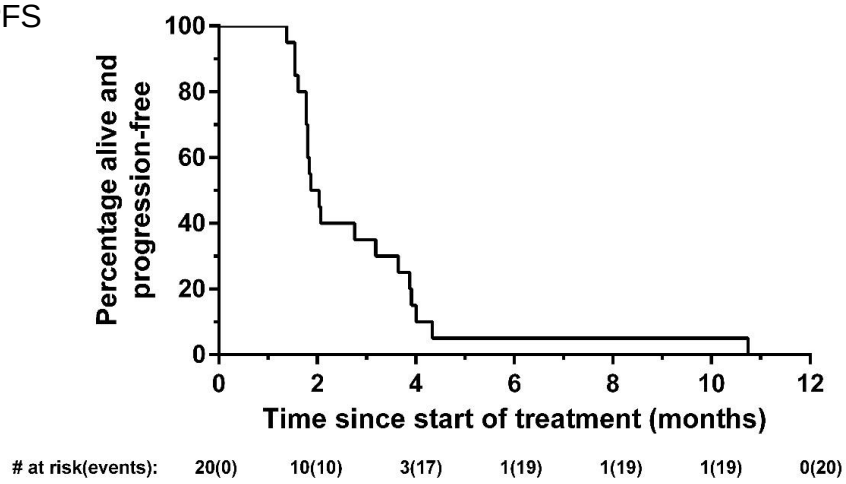
Table 12: Demographic and patient characteristics – all patients enrolled in the PROMPT trial (n=20)
Abbreviations: ECOG – Eastern Cooperative Oncology Group, FIGO – International Federation of Gynaecology and Obstetrics

3.3.2 Efficacy

The median follow up was 16.5 months, with 20 PFS events and 13 OS events. The 6-month PFS rate was 5.0% (95% CI 0.3-20.5). At the first response assessment after C3 pembrolizumab, 13/20 patients had progressive disease, 1/20 had a partial response (PR), 5/20 had stable disease (SD), 1/20 had non-CR, Non-PD. At the time of the second response assessment (after C6) all but 1 patient had progressed. Disease response rate 5.0% (95% CI: 0.1-24.9), median PFS 2.0 months (95% CI 1.8-3.6) and median OS 9.8 months (95% CI 6.2-20.7).

Figure 9: Kaplan Meier curves for PFS and OS

1) PFS



2) OS

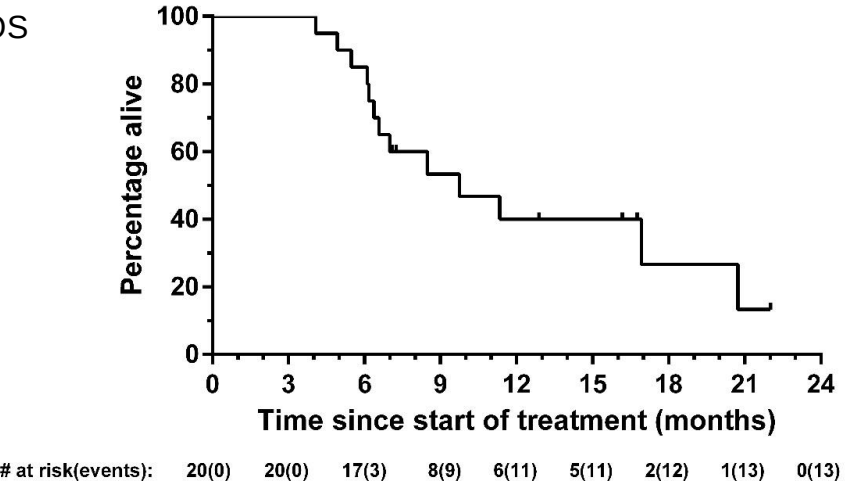


Figure 9: Progression free survival (PFS) and overall survival (OS) for all patients treated with pembrolizumab. Median PFS is 2.0 months and median OS is 9.8 months.

3.3.3 Toxicity

Pembrolizumab was very well tolerated, with very few reported adverse events (AE's). 8 (40%) patients did not experience any treatment related AE's (TRAE). G1 TRAEs were reported in 7 (35%) patients, G2 in 3 (15%), G3 in 2 (10%). There were no reported G4/5 adverse events. As of the data cutoff, there were nine G3 AE's reported in four patients, 4 of which were thought to be related to pembrolizumab. These AE's were managed as per the protocol guidelines, with high dose steroids and supportive agents.

Table 13: Treatment related adverse events in 20 patients treated with pembrolizumab

Adverse event	Any grade (n)	Grade ≥3
Abdominal pain	1	
Nausea	3	
Vomiting	2	
Pain (other)	1	
Gastroesophageal reflux	1	
Pruritis	1	
Anorexia	1	
Constipation	2	
Neuropathy	1	
Diarrhoea	3	1
Dry mouth	1	
Agitation	1	
Hyperthyroidism	1	
Weight loss	1	
Headache	1	
Dry skin	1	
Rash	5	1
Mucositis (oral)	1	
Flatulence	1	
Deranged LFTs	5	2
Fatigue	1	
Muscle cramps	1	
Hypothyroidism	1	

Table 13: Treatment related adverse events (AEs) in 2 patients treated with pembrolizumab

Table 14: G3 Adverse events and their relatedness to pembrolizumab

Patient (ID)	Adverse event	Grade	Relatedness
PRO-10	Nausea	3	Unrelated
	Vomiting	3	Unrelated
	Urticarial rash	3	Related
PRO-12	Raised ALT	3	Related
	Immune-mediated hepatitis	3	Related
	Diarrhoea	3	Related
PRO-13	Chest infection	3	Unrelated
	Pleural effusion	3	Unrelated
PRO-20	Hypertension	3	Unrelated

Table 14: Grade 3 (G3) adverse events (AEs) and their relatedness to pembrolizumab

3.4 Discussion

The key objective for this study, a prolongation in disease control rate was not met. The study highlighted the poor prognosis of patients with PROC. There are multiple ways in which ovarian cancer becomes resistant to chemotherapy, including dysregulation of transport of platinum into and out of ovarian cancer cells, and changes to DNA pathway repair mechanisms (289). Over the years, there have been numerous published phase II trials investigating various different agents in PROC, with only a minority progressing to phase III studies (290), with even fewer reporting positive and practice changing outcomes (78,79). ICPIs, together with chemotherapy or as a single agent, have also not been proven to be effective in OC (232,239,291,292). PROMPT was therefore designed to explore the impact of ICPIs on patients with first platinum-resistant relapse, who responded to weekly paclitaxel to see whether immunotherapy would be effective as maintenance treatment.

There were a number of difficulties with the trial. Most importantly, many patients who were initially thought to be eligible following a response to the first 3 cycles of weekly paclitaxel, started to progress towards the end of treatment (usually 6 cycles) and were therefore no longer eligible. Additionally, there were a small proportion of patients who had such a good response to weekly paclitaxel, making them ineligible for the trial due

to the lack of measurable and biopsiable disease. These factors made it very difficult to recruit and also resulted in a very small number of samples being collected.

However, recruitment improved following the protocol amendment in August 2021, when this requirement became optional, rather than mandatory. Whilst this helped with numbers of patients being treated and provided the clinical data – it was not possible to biopsy all patients, leading to a smaller number of samples available for translational research. Due to the very low 6-month PFS rate of 5.0%, the study stopped early due to futility, having only recruited 20 patients, instead of the planned 28. These results were presented in a poster at the European Society of Gynaecological Oncology (ESGO) 2024 Congress (see appendix).

Translational research remains crucial to understand why most patients with HGSC do not respond to immune checkpoint inhibitors. There were a number of problems with sample collection from the patients recruited to PROMPT. Many patients refused / were too unwell to have bloods and/or a biopsy prior to C4, which was when disease progression was confirmed in most patients.

In early 2020, when it became apparent that recruitment to the study was much slower than anticipated, the objectives of my project were reviewed and changed to exploring the immune microenvironment in ovarian cancer in patients undergoing standard of care treatment. Patients seen in the Gynaecological Medical Oncology clinic at University College London Hospital (UCLH) were recruited and samples collected for translational research at various timepoints throughout treatment. The purpose of this was to explore baseline immune profile in blood +/- tumour of patients with HGSC and to investigate what impact these treatments have on circulating immune cells and within the tumour microenvironment.

The translational analysis performed on these samples will be discussed in the next results chapter, Chapter 4.

Chapter 4: Translational results

Section 1: Assessing the immune landscape of ovarian cancer

4.1 Introduction

To determine the immune landscape of ovarian cancer, patients seen in the gynae-oncology clinic at UCLH with high grade EOC, mainly HGSC, were recruited for this project. Blood samples and tumour were collected at various time points of their treatment. Primary treatment of advanced EOC is a combination of chemotherapy and surgery. All patients with a presumed diagnosis of EOC are discussed at the specialist gynae-oncology multi-disciplinary team meeting (MDT). The extent and distribution of disease will determine whether to proceed with PCS or NACT. Provided patients respond to primary chemotherapy, maintenance treatment, with bevacizumab and/or PARPi, is commonly offered to patients. Decisions regarding maintenance treatment are influenced by the tumour HRD status, although routine testing of this was not yet in place at the time of this project.

Despite improved PFS with maintenance therapies in the first line setting, the majority of patients will relapse and eventually become chemotherapy resistant. There is an urgent need to establish whether alternative systemic treatments are effective in HGSC.

As described in detail in Chapter 1, Introduction, the majority of published clinical trials investigating the use of ICPIs in HGSC have been negative and immunotherapy is not routinely used to treat ovarian cancer. HGSC is marked by a profoundly immunosuppressive TME, and this might partly explain why treatment with immunotherapy to date has been unsuccessful.

The aim of the translational component of this project was to study the immune profile and microenvironment in HGSC and explore whether primary treatments have an impact on circulating immune cells and immune cells found within the TME. To do this, patients were recruited from clinic and samples collected at key timepoints throughout treatment to provide insight into any changes that may occur with standard of care therapy.

The first part of this chapter outlines the patients recruited and their clinical characteristics.

4.2 Patient recruitment and demographics

In total, 37 patients were consented for this project. Blood samples from 36 patients were collected between October 2020 and June 2021 (Table 15). Patients were split into 3 cohorts.

Cohort 1: Patients due to start NACT – a total of 18 patients were consented; at the time of commencing chemotherapy, all patients were planned to proceed with surgery. However, analysis was only performed on 17 patient samples. One patient, TG017, was excluded as it was not possible to collect blood at the completion of chemotherapy and a tissue biopsy sample was unavailable.

Cohort 2: Patients who had undergone PCS – a total of 9 patients were recruited. These patients were planned to start adjuvant chemotherapy +/- maintenance treatment.

Cohort 3: Patients commencing PARPi maintenance – a total of 10 of patients were recruited. These patients had completed and responded to platinum-based chemotherapy (either 1st or 2nd line) and were planned to start PARPi maintenance therapy.

Healthy donors – a total of 19 age matched female healthy donors (HD) were recruited (median age 60yrs; age range 45-77yrs).

Patient characteristics are summarised in table 15.

Table 15: Clinical characteristics of all patients (n=36)

	ALL	NACT	PCS	PARPi
Total number (N)	36	17	9	10
Median age, y (range)	**71 (46-87)**	**75 (58-87)**	**66 (46-79)**	**70 (47-77)**
Histology				
- **HGSC^**	**34**	**17**	**8**	**9**
- **High grade endometrioid**	**2**	**-**	**1**	**1**
Stage at diagnosis				
- **I**	**3**	**-**	**1**	**2**
- **II**	**3**	**-**	**2**	**1**
- **III**	**14**	**6**	**5**	**3**
- **IV**	**16**	**11**	**1**	**4**
Germline BRCA/HR gene mutation				
- **WT**	**27**	**14**	**7**	**6**
- **BRCA1**	**2**	**-**	**-**	**2**
- **BRCA1 VUS**	**1**	**1**	**-**	**-**
- **BRCA2**	**3**	**1**	**1**	**1**
- **RAD51D**	**1**	**-**	**-**	**1**
- **Not tested**	**2**	**1**	**1**	**-**
Timing of surgery				
- **PCS**	**9**	**-**	**9**	**-**
- **ICS**	**7**	**7**	**-**	**-**
- **DCS**	**5**	**5**	**-**	**-**
- **Inoperable**	**5**	**5**	**-**	**-**
Line of maintenance treatment				
- **1st**	**5**	**-**	**-**	**5**
- **2nd**	**5**	**-**	**-**	**5**

Table 15: Tumour characteristics, stage at diagnosis, germline BRCA status and timing of surgery in all patients included, and then split into the 3 cohorts; Neoadjuvant chemotherapy (NACT), primary cytoreductive surgery (PCS) and PARPi.
HGSC - high grade serous ovarian cancer, HR - Homologous recombination gene mutation; BRCA/RAD51D - cancer predisposition genes when altered, WT – wild-type, VUS - variant of unknown significance, PCS - primary cytoreductive surgery, ICS - interval cytoreductive surgery, DCS - delayed cytoreductive surgery.
^ One patient with HGSC with a focus of carcinosarcoma

4.2.1 Cohort 1 (NACT)

17 patients commenced NACT. However, response to treatment varied and not all patients proceeded to surgery. 5 were inoperable, 7 underwent ICS after 3 cycles of chemotherapy and 5 underwent DCS after completion of 6 cycles of chemotherapy. These patients were split into 3 separate cohorts for analysis (figure 7).

Figure 7: NACT cohorts

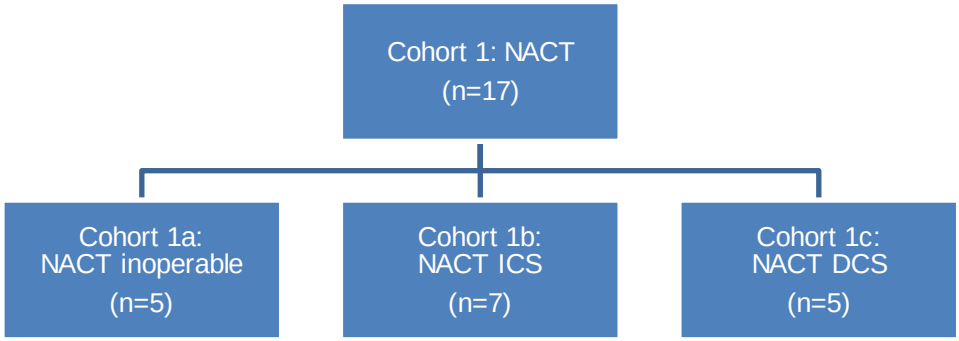


Figure 7: The 3 NACT (neo-adjuvant chemotherapy) cohorts and number of patients in each cohort.

4.2.1.1 Cohort 1a (NACT inoperable)

Five (5/17) patients did not proceed with surgery. By definition, these patients were likely to have a poorer prognosis, either due to their insufficient response to chemotherapy or to their frailty. All patients had high grade serous histology. Clinical characteristics are summarised in table 16.

Table 16: Clinical characteristics of patients in Cohort 1a (n=5)

Patient	Age	Stage	gBRCA	Total CT cycles
TG013	80	IVB	Unknown	6
TG014	79	IIIB	WT	6
TG016	82	IVA	WT	3
TG018	74	IVB	WT	6
TG036	71	IIIC	WT	7

Table 16: Clinical characteristics of Cohort 1a (NACT inoperable)
gBRCA – germline BRCA; WT – wildtype; CT – chemotherapy

4.2.1.2 Cohort 1b (NACT ICS)

Seven (7/17) underwent ICS after 3 cycles of chemotherapy. All patients had high grade serous histology and completed a total of 6 cycles of chemotherapy. Table 17 summarises their clinical characteristics.

Table 17: Clinical characteristics of patients in Cohort 1b (n=7)

Patient	Age	Stage	gBRCA	Surgical outcome	CRS	Maintenance
TG005	78	IIIC	WT	NRD	2	Niraparib
TG008	65	IVB	WT	NRD	2	Niraparib
TG011	75	IIIC	WT	RD	2	Niraparib
TG023	62	IVB	WT	NRD	1	Niraparib
TG025	87	IIIC	WT	RD	U/K	Niraparib
TG035	64	IIIB	WT	NRD	3	Niraparib
TG044	75	IVB	BRCA2	NRD	U/K	Olaparib

Table 17: Clinical characteristics of Cohort 1b (NACT ICS)
gBRCA – germline BRCA; WT – wildtype; CT – chemotherapy; NRD – no residual disease i.e. complete cytoreduction; RD – residual disease; CRS – chemotherapy response score; U/K - unknown

4.2.1.3 Cohort 1c (NACT DCS)

Five (5/17) patients commenced NACT and completed 6 cycles of chemotherapy prior to having surgery. Bloods were taken prior to starting chemotherapy (timepoint 1), at the completion of chemotherapy (timepoint 2) and post operatively (timepoint 3). All patients had high grade serous histology, and all had complete cytoreduction with no residual disease post-operatively. Four (4/5) patients went on to have a further 2 cycles of chemotherapy after surgery, so had another set of bloods following this (timepoint 4). Two patients commenced PARPi maintenance. However, only one of these patients had bloods pre-cycle 4 (timepoint 5). Patient characteristics and treatment are shown in table 18.

Table 18: Clinical characteristics of patients in Cohort 1c (n=5)

Patient	Age	Stage	gBRCA	Total cycles CT	Surgical outcome	CRS	Maintenance therapy
TG006	58	IVB	WT	6	NRD	1	Bevacizumab
TG007	70	IVB	WT	7	NRD	3	Niraparib
TG015	75	IVB	WT	8	NRD	3	Nil
TG032	83	IVA	WT	6	NRD	2	Bevacizumab
TG045	75	IVA	WT	7	U/K	3	Niraparib

Table 18: Clinical characteristics of patients in Cohort 1c (NACT DCS)
gBRCA - germline BRCA; WT – wild-type; CT – chemotherapy; NRD – no residual disease; U/K – unknown; CRS – chemotherapy response score

4.2.2 Cohort 2 (PCS)

Nine (9/36) patients who underwent PCS were recruited. Timepoint 1 bloods were taken following surgery but prior to starting chemotherapy, timepoint 2 following completion of chemotherapy and, if applicable, timepoint 3 bloods taken 3 months after commencing PARPi maintenance therapy. Patient characteristics are summarised in table 19. 1 patient, TG026, declined post-chemotherapy bloods and therefore the PBMC data were excluded from analysis. However, a tissue sample was available.

Table 19: Clinical characteristics of patients in cohort 2 (n=9)

Patient	Age	Stage	Histology	Surgical outcome	gBRCA	Maintenance therapy
TG009	46	IIIB	HGSC	NRD	WT	Niraparib
TG012	59	IIA	HGSC	NRD	WT	Nil
TG022	79	IIIB	HGSC	NRD	WT	Niraparib
TG024	62	IA	High grade endometrioid	NRD	WT	Nil
TG026	60	IIB	HGSC	NRD	BRCA2	Nil
TG027	70	IIIB	HGSC^	NRD	WT	Niraparib
TG030	71	IIIC	HGSC	NRD	WT	Nil
TG037	70	IIIC	HGSC	NRD	WT	Niraparib
TG039	73	IVB	HGSC	RD	WT	Bevacizumab

Table 19: Clinical characteristics of patients in cohort 2 (PCS)
HGSC - high grade serous ovarian carcinoma; ^HGSC with a focus of carcinosarcoma; NRD – no residual disease; RD – residual disease; gBRCA - germline BRCA; WT – wild-type;

4.2.3 Cohort 3 (PARPi)

PARPi are commonly used in the first line maintenance setting, provided there has been a response to platinum-based chemotherapy. The presence of a germline or somatic BRCA mutation or HRD predicts response to PARPi. At the time of this study, germline and somatic BRCA testing was routinely requested, but not HRD.

Ten (10/36) patients were recruited; Five (5/10) patients were given PARPi as first line maintenance and five (5/10) were given PARPi in the second line setting. Timepoint 1 bloods were taken post chemotherapy / pre-PARPi and timepoint 2 bloods taken after 3 months of treatment. Clinical characteristics are summarised in table 20. The PFS for patients on PARPi as second line maintenance was defined from the date of diagnosis to time of first disease progression.

Table 20: Clinical characteristics of patients in cohort 3 (n=10)

Patient	Age	Stage	Histology	gBRCA	PARPi	Line of treatment
TG019	62	IVB	HGSC	BRCA1	Olaparib	1
TG020	66	IIB	HGSC	BRCA2	Olaparib	2
TG021	66	IC	High grade endometrioid	WT	Niraparib	2
TG028	72	IIIC	HGSC	WT	Niraparib	1
TG031	54	IVA	HGSC	BRCA1	Olaparib	1
TG033	47	IIIC	HGSC	RAD51D	Niraparib	1
TG034	77	IIIC	HGSC	WT	Niraparib	2
TG038	52	IC	HGSC	WT	Niraparib	2
TG040	71	IVB	HGSC	WT	Niraparib	2
TG041	68	IVA	HGSC	WT	Niraparib	1

Table 20: Clinical characteristics and outcomes of patients in cohort 3 (PARPi)
HGSC - high grade serous ovarian cancer; gBRCA - germline BRCA; WT – wild-type.

4.3 Blood collection timepoints

Timelines below (figures 8.1-8.3) summarise blood collection timepoints of each cohort.

Figure 8: Blood collection timepoints

Figure 8.1: Cohorts 1b & c (NACT operable)

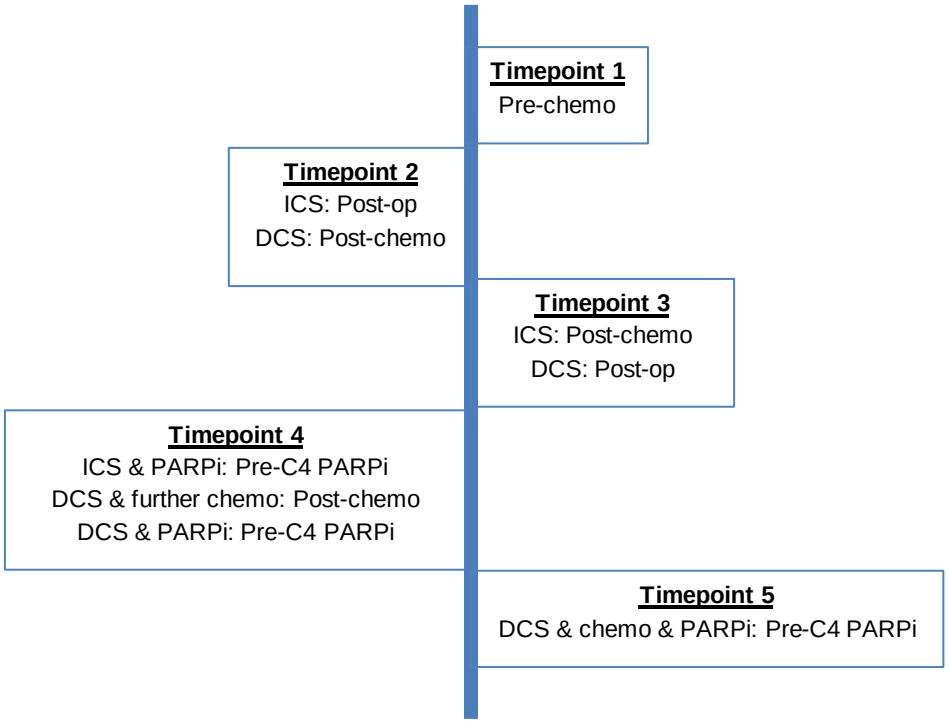


Figure 8.2: Cohort 2 PCS/Cohort 1a NACT inoperable

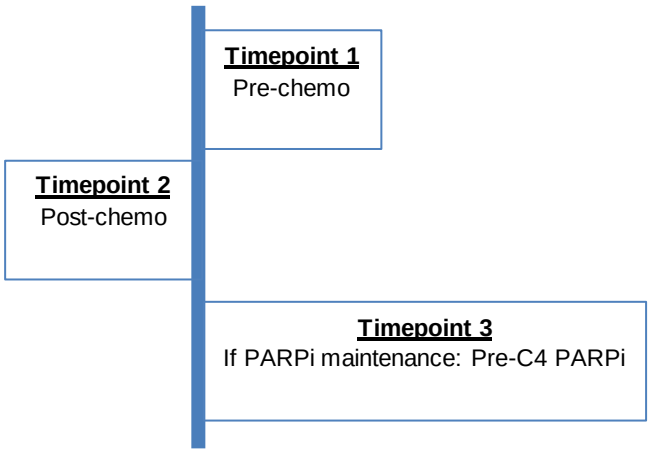
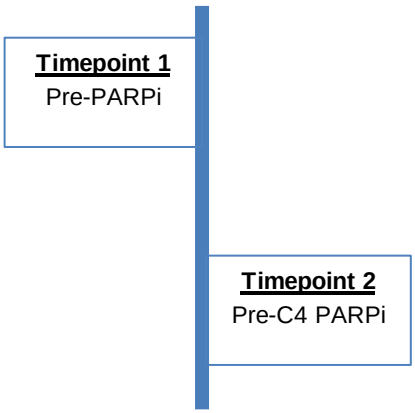


Figure 8.3: Cohort 3: PARPi



Figures 8.1-3: Blood collection at various timepoints for each patient cohort.
Pre-chemo – prior to commencing chemotherapy; post-op – post operatively; post-chemo – following completion of chemotherapy; ICS – interval cytoreductive surgery; DCS – delayed cytoreductive surgery; PARPi – poly-ADP ribose polymerase inhibitor.

4.4 Tumour samples for multiplex IF analysis

Formalin-fixed paraffin-embedded (FFPE) tissue blocks were available for thirty patients. 8 patients had paired samples, 2 patients had only pre-chemotherapy biopsy samples available, and 19 patients had resection specimens only (combination of pre-chemotherapy specimens for those who underwent PCS and post-chemotherapy for those who received NACT).

4.4.1 Samples collected

The following tables (tables 21-25) summarise the bloods and tissue available for analysis in each cohort, together with the status of disease at the time of final blood collection if applicable. Unless otherwise stated, patients were disease free at the final timepoint.

Table 21: Cohort 1a (NACT inoperable) blood & tissue samples

Patient	Bloods Pre-CT (T1)	Bloods Post-CT (T2)	Disease status at T2	Bloods Pre-C4 PARPi (T3)	Disease status at T3	Tissue Biopsy
TG013	✓	✓	PD	N/A	N/A	✗
TG014	✓	✓	NED	✓	Rising Ca125	✓
TG016	✓	✓	PD	N/A	N/A	✗
TG018	✓	✓	PR	N/A	N/A	✗
TG036	✓	✓	SD	✗	N/A	✗

Table 22: Cohort 1b (NACT ICS) blood and tissue samples

Patient	Bloods Pre-CT (T1)	Bloods Post-ICS (T2)	Bloods Post-CT (T3)	Bloods Pre-C4 PARPi (T4)	Tissue Biopsy	Tissue Resection
TG005	✓	✓	✓	✓	✗	✓
TG008	✓	✓	✓	✓	✗	✓
TG011	✓	✓	✓	✓	✗	✓
TG023	✓	✓	✓	✓	✓	✓
TG025	✓	✓	✓	✓	✓	✗
TG035	✓	✓	✓	✓	✓	✓
TG044	✓	✓	✓	✗	✓	✓

Table 23: Cohort 1c (NACT DCS) blood and tissue samples

Patient	Bloods Pre-CT (T1)	Bloods Post- CT (T2)	Bloods Post-DCS (T3)	Bloods After post-op CT (T4)	Bloods Pre-C4 PARPi (T5)	Tissue Biopsy	Tissue Resection
TG006	✓	✓	✓	✓	N/A	✗	✓
TG007	✓	✓	✓	✓	✓	✗	✗
TG015	✓	✓	✓	✓	N/A	✗	✓
TG032	✓	✓	✓	N/A	N/A	✗	✓
TG045	✓	✓	✓	✓	✗	✓	✓

Table 24: Cohort 2 (PCS) blood and tissue samples

Patient	Bloods Pre-CT (T1)	Bloods Post-CT (T2)	Bloods Pre-C4 PARPi (T3)	Tissue Resection
TG009	✓	✓	✓	✓
TG012	✓	✓	N/A	✓
TG022	✓	✓	✗	✓
TG024	✓	✓	N/A	✗
TG026	✓	✗	N/A	✓
TG027	✓	✓	✗	✗
TG030	✓	✓	N/A	✓
TG037	✓	✓	✓	✓
TG039	✓	✓	N/A	✓

Table 25: Cohort 3 (PARPi) blood and tissue samples

Patient	Bloods Pre-PARPi (T1)	Disease status at T1	Bloods Pre-C4 (T2)	Disease status at T2	Tissue Biopsy	Tissue Resection
TG019	✓	NED	✓	NED	✓	✓
TG020	✓	NED	✓	NED	✓	✓
TG021	✓	SD	✓	SD	✗	✓
TG028	✓	NED	✓	SD	✓	✓
TG031	✓	NED	✓	NED	✓	✓
TG033	✓	NED	✓	NED	✗	✗
TG034	✓	SD	✓	SD	✗	✓
TG038	✓	PR	✓	Rising Ca125	✗	✓
TG040	✓	SD	✓	SD	✗	✓
TG041	✓	NED	✓	Rising Ca125	✗	✓

Tables 21-25: Tables summarising all bloods and tumour specimens collected for all patient cohorts

PD – progressive disease; NED – no evidence of disease; SD – stable disease; PR – partial response; N/A – not applicable.

4.5 Survival

Data cut off was 24th March 2024. At this time, 2/36 (6%) patients were lost to follow up. 11/36 (31%) patients were alive and disease free. 23/36 (64%) patients had progressed; 9/23 were still alive and 14/23 had died. PFS is defined as the time in months between the date of diagnosis and date of radiological progression or death. For the 5 patients who were recruited in cohort 3 prior to commencing second line PARPi maintenance, PFS is defined from date of diagnosis to date of first relapse. OS is time in months between date of diagnosis and date of death from any cause. Median PFS was 26.5 months. Outcomes and survival curves are below (table 26 and figure 9). The two patients lost to follow up have been excluded from the analysis. TG040 had a progression date, but was then lost to follow-up, so I have censored OS as being alive at data cut off. Median OS is unreliable due to the long PFS of TG038.

Table 26: Survival data for all patients

Patient	Cohort	PFS (mths)	OS (mths)
TG013	1a	5.5	11.6
TG014	1a	15.2	29.9
TG016	1a	2.7	9.4
TG018	1a	5.4	5.4
TG036	1a	12.4	14.8
TG005	1b	-	-
TG008	1b	27.8	-
TG011	1b	-	-
TG023	1b	11.0	27.8
TG025	1b	11.3	28.2
TG035	1b	-	-
TG044	1b	Lost to f/up	Lost to f/up
TG006	1c	8.3	16.4
TG007	1c	17.5	-
TG015	1c	27.0	28.2
TG032	1c	30.0	-
TG045	1c	12.1	30.1

Patient	Cohort	PFS (mths)	OS (mths)
TG009	2	26.0	-
TG012	2	-	-
TG022	2	-	-
TG024	2	-	-
TG026	2	20.4	-
TG027	2	Lost to f/up	Lost to f/up
TG030	2	15.6	33.8
TG037	2	-	-
TG039	2	20.7	-
TG019	3	44.0	-
TG020	3	45.1	-
TG021	3	21.6	-
TG028	3	24.2	-
TG031	3	-	-
TG033	3	-	-
TG034	3	42.4	-
TG038	3	123.4	148.9
TG040	3	94.0	Lost to f/up
TG041	3	12.6	35.5

Table 26: Survival data for all patients recruited to the study
Includes all patients recruited to project.
PFS – progression free survival in months; OS – overall survival in months; f/up – follow-up

Figure 9: Kaplan Meier survival curves

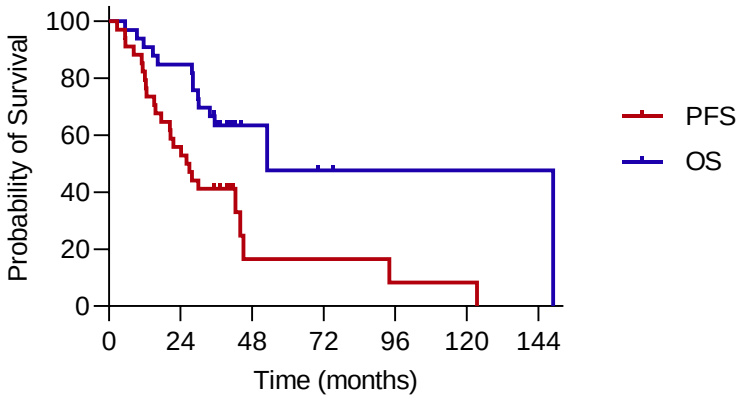


Figure 9: Kaplan Meier (KM) survival curves (n=34)
Excluding the 2 patients who were lost to follow up and progression free/overall survival unknown
Red – progression free survival (PFS); Blue – overall survival (OS)

4.6 Summary

The first section of this chapter describes all the patients recruited for this project and their clinic details and outcomes. Section 2 will focus on results from the translational aspect of this project.

Section 2: Immune cells in ovarian cancer

4.7 Introduction

To mount an effective immune response against cancer, antigens need to be available. They are presented to and activate T-cells, which then enter the tumour. Upon antigen exposure, naïve T-cells are activated and differentiate into short-lived effector cells. A small proportion of these activated cells will become memory T-cells, and therefore play a key role in the immune response upon re-exposure of the specific disease related antigen. Central memory T-cells (Tcm) cells express high levels of chemokine receptor 7 (CCR7), a homing receptor, resulting in effective homing to lymph nodes. They do not have significant cytotoxic activity, rather they proliferate promptly after antigen re-exposure. Effector memory T-cells (Tem) do not express CCR7 and typically infiltrate non-lymphoid and/or inflamed tissues. TEMRA cells are a subset of effector memory T-cells that re-express CD45RA, often seen in situations with continuous/constant stimulation. They are terminally differentiated cells and express the senescent markers KLRG1 and/or CD57 and display cytotoxic and pro-inflammatory properties (293).

One mechanism of resistance to this immune response is the expression of PD-L1 on tumour cells, which interact with PD-1 on T-cells, resulting in the inhibition of T-cell cytotoxic functions (294). Additionally, continuous antigen stimulation occurs in cancer, causing activated T-cells to become exhausted. This is defined by a reduction in effector function and expression of a combination of inhibitory receptors, such as PD-1, TIM-3, LAG-3, CTLA-4 and TIGIT (293). By preventing PD-1 from binding with its ligand, PD-L1, exhausted T-cells are reinvigorated and can therefore restore anti-tumour immune responses (295) . PD-1 blockade with ICPIs has proven to be effective in a number of solid tumours; however, to date, they do not appear to have a role in the treatment of HGSC.

Tumour biopsies are the best samples to provide an understanding of the TME. However, due to tumour heterogeneity, a sample taken from a specific site of disease may differ from disease elsewhere in that patient. It would be virtually impossible to biopsy multiple sites of disease at varying timepoints throughout a patient's treatment and an alternative way of tracking immune responses is required.

Bloods tests are routinely required throughout treatment and during follow up for cancer and therefore provide an opportunity to collect additional minimally invasive samples for translational research. PBMCs are made up of circulating immune cells, including lymphocytes and myeloid cells, such as monocytes. They are isolated from fresh blood, making them more easily accessible than tumour tissue. It remains unclear whether circulating immune cells mirror the immune cells within the tumour microenvironment of solid tumours. If this were the case, they could potentially provide insight into the effect of systemic therapy on the immune microenvironment.

The presence of TILs in the ovarian cancer (OC) immune microenvironment is a strong prognostic marker in OC. Increased number of CD3+ and CD8+ cytotoxic lymphocytes are associated with better outcomes. The location of these immune cells within the TME is also important, with increased survival seen when these cells are found within the neoplastic epithelium (intraepithelial TILs), rather than in the stroma (stromal TILs) (296).

TILs are recruited into the tumour, with the aim of mounting an anti-tumour immune response. There are multiple mechanisms in which this anti-tumour response can be disrupted. The PD-1 inhibitor pathway is very prominent in promoting tumour immune escape. PD-1 is expressed on TILs and interacts with its ligand, PD-L1, expressed on tumour cells, macrophages and other cells within in TME. The binding of these two receptors inhibits T-cell proliferation and production of inflammatory cytokines, thereby preventing immune attack of cancer cells. Other immunosuppressive cells found within the TME and also contribute to immune evasion include Tregs and TAMs (297).

ICPIs, specifically anti PD-1/ PD-L1 antibodies, have dramatically changed the treatment landscape of numerous advanced stage solid tumours (298), with durable responses seen in some patients. However, despite these promising results, there are many patients who do not respond and there is an urgent need to identity predictive biomarkers. PD-L1 expression is thought to influence 'tumour immune escape' and could be a potential biomarker for response to immunotherapy. Whilst PD-1 positive TILs and PD-L1 positive OC cells are found within the ovarian cancer TME (299), they are only weakly predictive of predictive of response to ICPIs (292).

4.8 Aims

Two 20 colour flow cytometry panels were designed and optimised to explore the circulating T-cells and myeloid cells in patients with high grade EOC to determine the following:

- Identify the different subsets of T-cells and myeloid cells in PBMCs of patients with ovarian cancer at baseline and compare these to those seen in PBMCs of healthy donors
- Explore whether surgery and/or systemic therapy (with chemotherapy and/or PARPi) affects the proportions and function of these immune cells.
- Correlate these findings with clinical outcomes.

The hypotheses were as follows:

- Chemotherapy will reduce the total number of T-cells but may not affect the proportion of CD4 / CD8 cells.
- Ovarian cancer patients will have an increased number of circulating T-regs compared to healthy donors.
- Higher numbers of circulating Tregs would be seen in patients with a higher burden of disease e.g. those patients requiring NACT.
- Response to treatment would cause a reduction in the number of circulating Tregs.
- Higher Tregs would be associated with a poorer prognosis.

4.9: Flow cytometry analysis of PBMCs

This section will describe the T-cell analysis of isolated PBMCs using multi-parametric flow cytometry.

4.9.1 T-cell analysis

T cell subsets were gated based upon CD3+ cells within the lymphocyte gate (FSC-A vs SSC-A) after excluding doublets and non-viable cells. CD3+ T-cells were then further divided into CD8+ T-cells, Treg (CD4+ FoxP3+ CD25+) cells and CD4+ effector (Teff) (CD4+ FoxP3- CD25-) cells. CD8+ and CD4+Teff cells were then further divided into naïve (CD45RA+ CCR7+), central memory (CD45RA- CCR7+), effector memory (CD45RA- CCR7-) and TEMRA (CD45RA+ CCR7-) (figure 10).

Figure 10: Gating strategy for T-cells

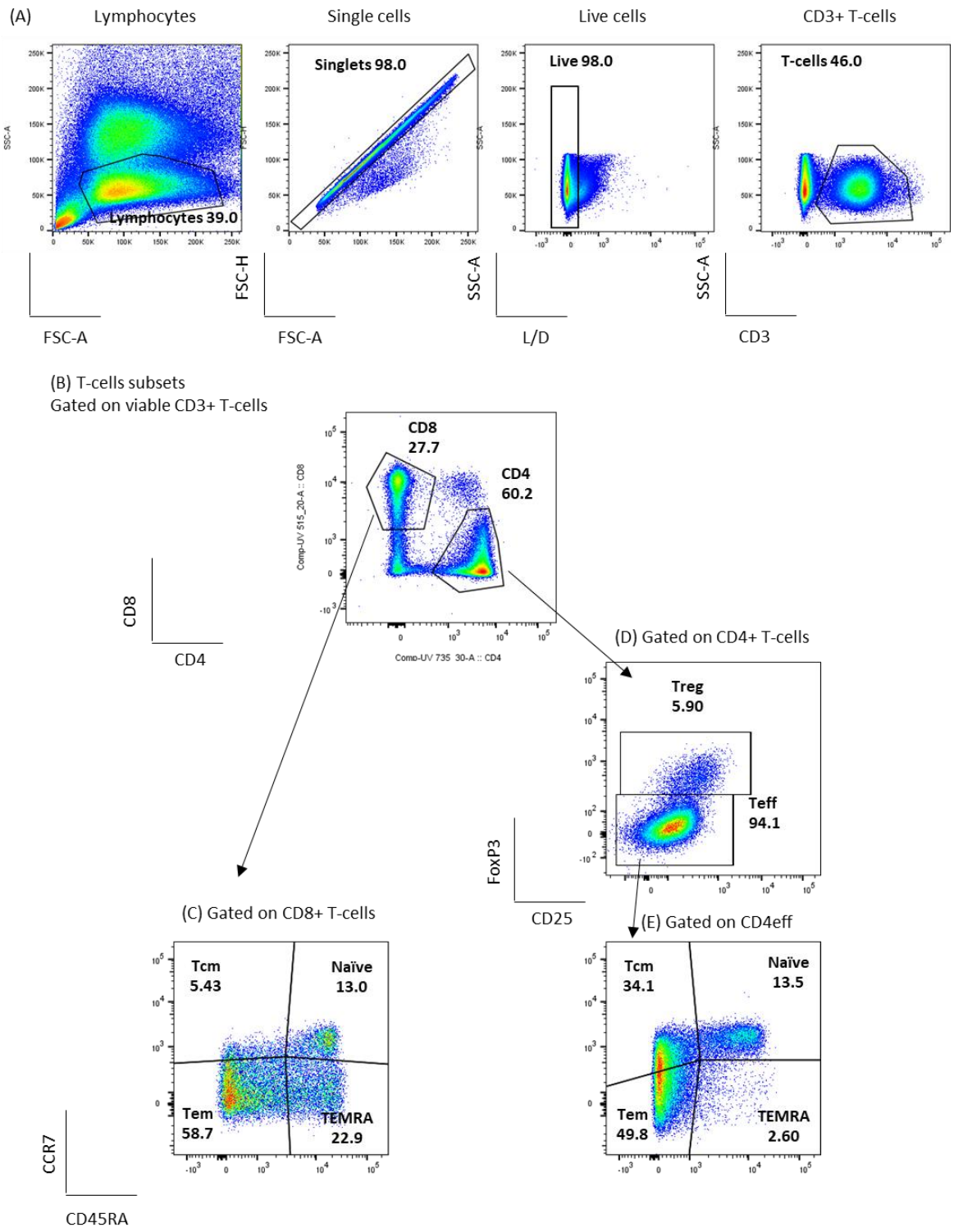


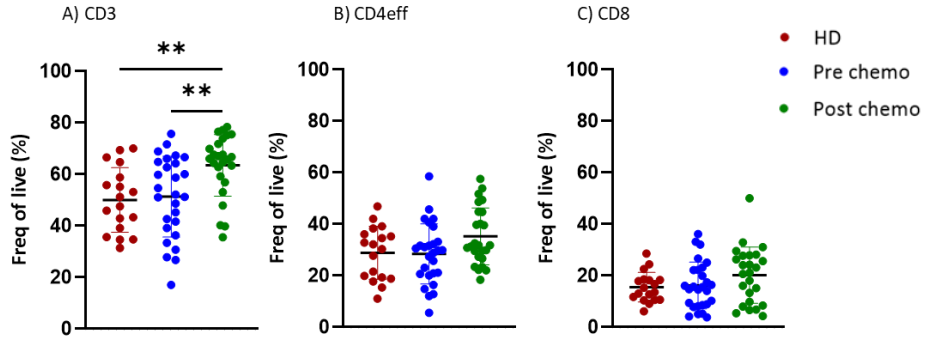
Figure 10: T-cell gating strategy

Multi-parametric flow cytometry was used to identify the T-cell subsets in PBMC samples of all patients and healthy donors (HD). Dot plots and representative gating strategies are illustrated from a representative HD (TG047). (A) Viable (L/D), singlet CD3+ cells were gated for within the lymphocyte gate. (B) CD8+ and CD4+ T-cells were identified from within the population of CD3+ cells. (C) Naïve, central memory (cm), effector memory (em) and TEMRA cells were identified from within the CD8+ populations. (D) Regulatory T-cells (Treg) (CD4+ FoxP3+ CD25+) and (E) effector CD4+ cells (CD4 Teff) (CD4+ FoxP3- CD25-) were identified from the CD4+ population and then further memory phenotypes were identified from within the CD4 Teff gate.

Across cohorts 1 and 2, there were a total of 26 pre- and post-chemotherapy samples. The figures below show the difference in total numbers of T-cells and the impact of chemotherapy on these (figure 11.1). Across all 3 cohorts, there were 20 patients with bloods taken prior to starting PARPi and 3 months into treatment. Figure 11.2 illustrates the proportions of T-cells before and after PARPi. Both sets of data were compared to HD blood. In healthy individuals, T-cells make up approximately 45-70% of total PBMCs(300), of which 5-25% are CD8+ T-cells and CD4+ T-cells up to 60% total PBMCs. Chemotherapy and PARPi can both cause pancytopenia, but this tends to be short lived and blood counts usually return to normal after completion of treatment.

Figure 11: Proportion of T-cells in HD (n=19) vs diseased bloods

11.1) T-cells Pre- and post-chemotherapy (n=26)



11.2) T-cells Pre- and post-PARPi (n=20)

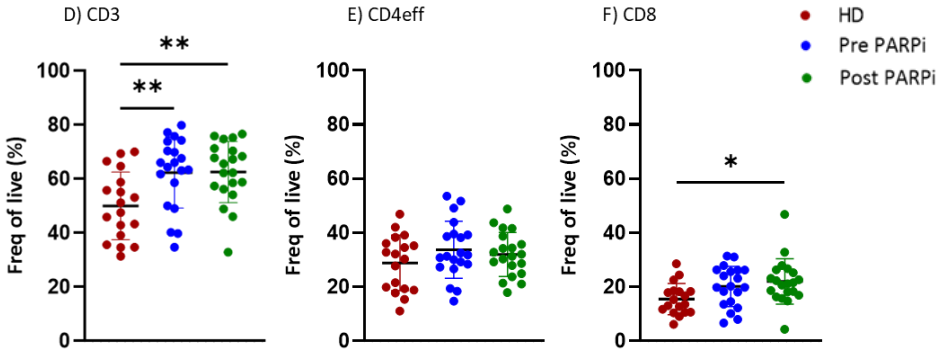


Figure 11: Proportion of T-cells of all live cells

A-C) Pre- and post-chemotherapy; D-F) Pre- and post-PARPi

A&D) Total number of CD3+ T-cells as proportion of all live cells. B&E) Total number of CD4eff (CD3+CD4+FoxP3-CD25-) cells as a proportion of live cells. C&F) Total number of CD8+ (CD3+CD8+) T-cells as a proportion of live cells.

* denotes statistical significance - * - p ≤0.05; ** p ≤0.01 (ordinary one-way ANOVA with multiple comparisons).

Figure 11.1A illustrates that HD have similar numbers of circulating CD3+ T-cells compared to treatment naïve patients with ovarian cancer. A statistically significant increase in the proportion of CD3+ cells (T-lymphocytes) is seen following systemic therapy. However, there does not appear to be a change in the total proportion of CD4eff and CD8 T-cells post-chemotherapy (figures 11.1B and 11.1C).

4.9.2 T-cell phenotypes

The following analyses explored the proportion of different phenotypes of CD4eff and CD8 T-cells (figure 12); naïve, central memory (CM), effector memory (EM) and terminally differentiated (TEMRA) cells. Although the proportion of CD4eff and CD8 T-cells does not appear to change significantly with disease or systemic therapy, this next section was planned to establish whether T-cell phenotypes differed between HD and patients and if the proportions of these changed with systemic therapy. CD45RA and CCR7 were used to identify the number of naïve, central memory (CM), effector memory (EM) and effector memory cells that re-express CD45RA (TEMRA) cells. The proportion of these cells in healthy individuals is highly variable, and will be affected by age, baseline characteristics and prior antigen exposure. On average, one would expect to see 40% naïve CD8+ T-cells, 20-25% EM, 20-25% TEMRA and a small number of CM cells. There should be an inversely proportional relationship between naïve and TEMRA cells e.g. as naïve cells reduce, TEMRA cells should increase (301).

Figure 12.3L and 12.4P show that patients with ovarian cancer have a higher proportion of circulating CD8+ TEMRA cells. Figure 13 illustrates, that as expected, CD8+ TEMRA cells express CD57 and granzyme B (GzmB), a marker of cytotoxicity. The proportion of CD57+ and GzmB+ CD8+ TEMRA cells are significantly increased in ovarian cancer patients when compared to HD, however there is no change following treatment with chemotherapy. The median fluorescence intensity (MFI) is unchanged in HD and patients, although there is a statistically significant increase in the MFI of GzmB in cancer patients, which is likely to represent the increase in cytotoxic activity of these cells in patients with advanced cancer.

Figure 12: T-cell phenotypes in HD (n=19) vs diseased bloods

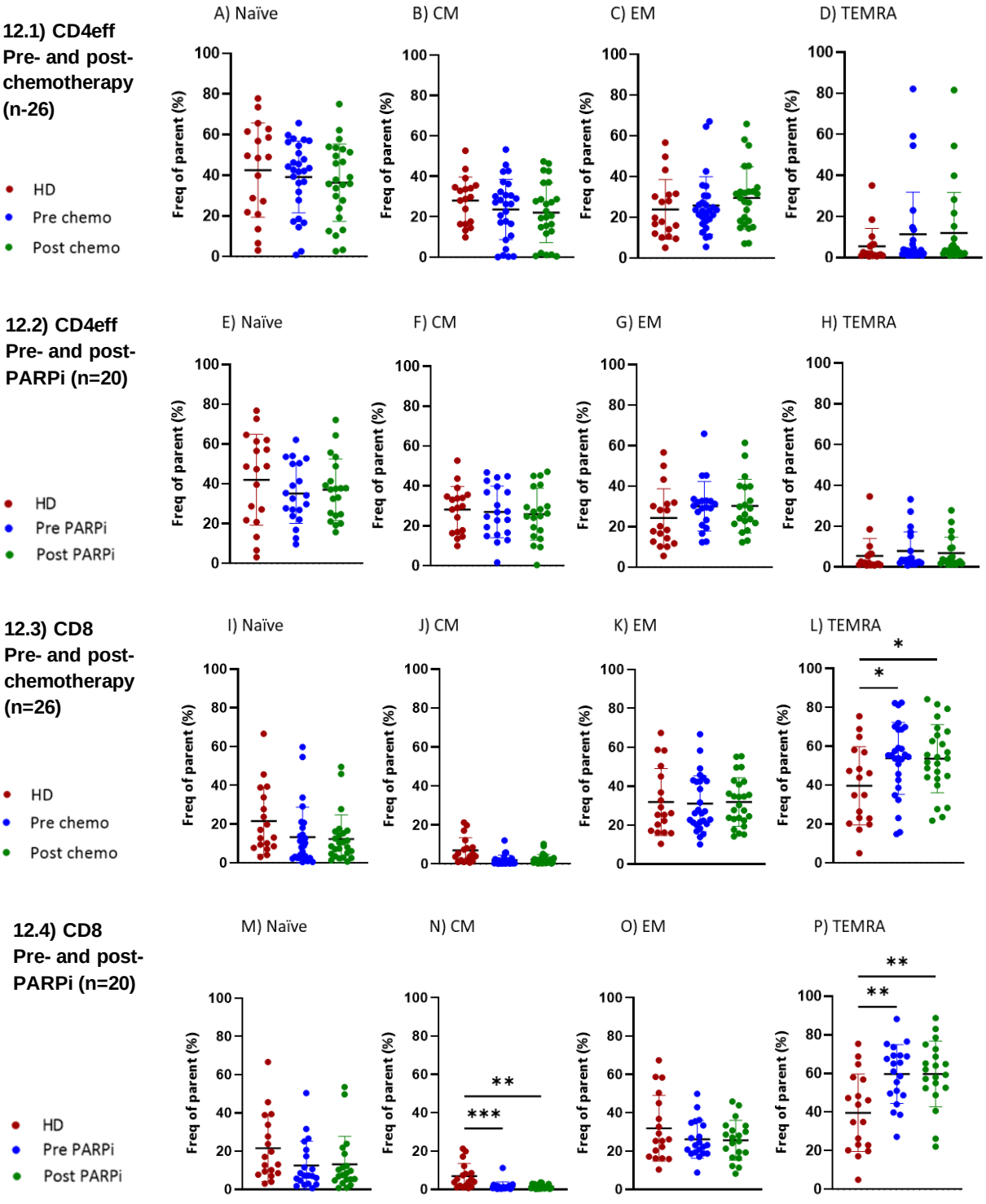


Figure 12: T-cell phenotypes

Proportions of A,E,I,M) CD4+ naïve B,F,J,N) CD4+ central memory (CM) C,G,K,O) CD4+ effector memory (EM) and D,H,L,P) CD4+ TEMRA cells. Figures 4.1 & 4.2 illustrate the CD4eff cells and 4.3 & 4.4 the CD8 T-cells. * denotes statistical significance - * - p≤0.05; *; ** - p ≤0.01; ***- p ≤0.001 (using ordinary one-way ANOVA with multiple comparisons and Kruskal-Wallis test in those that did not pass normality test).

Figure 13: CD57 and GzmB expression of CD8+ TEMRA in HD (n=19) vs diseased bloods

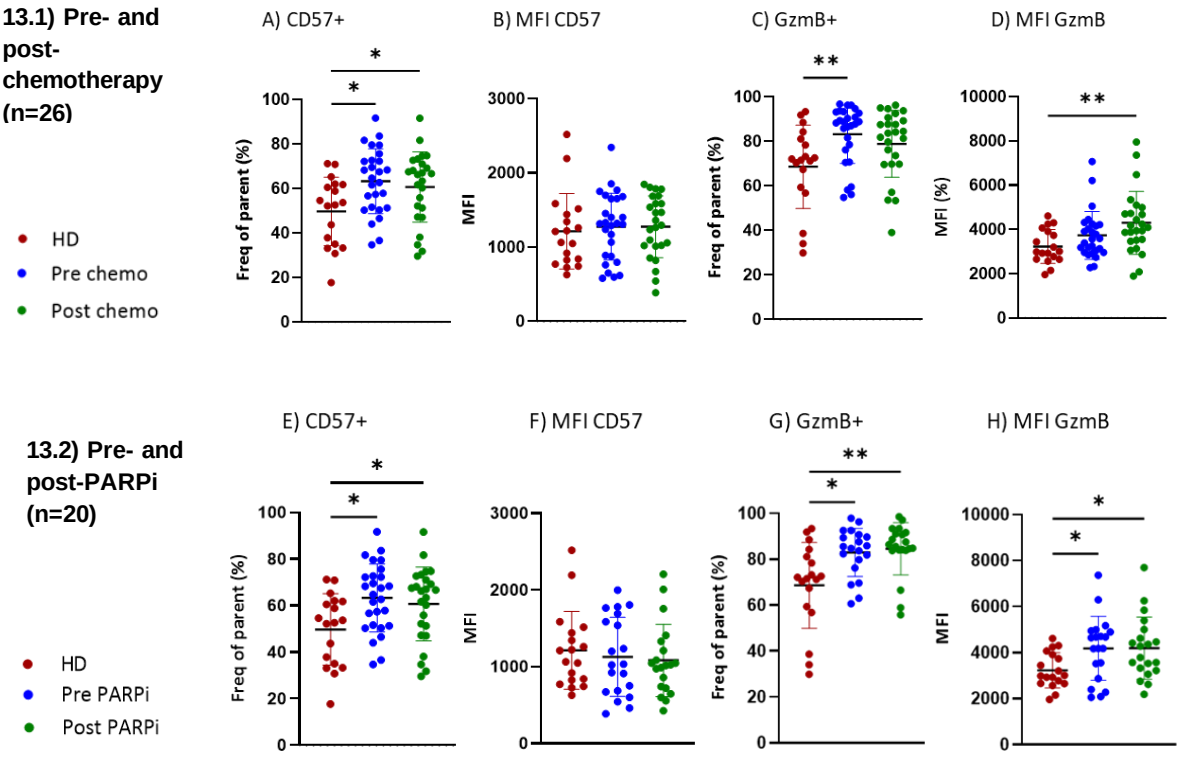


Figure 13: CD8+ TEMRA cells CD57 and GzmB expression

7.1) Pre-& post-chemotherapy; A&C) Proportion of CD57+ and GzmB+ CD8 TEMRA cells, B&D) Median fluorescent intensity (MFI) of CD57+ and GzmB+ CD8 TEMRA cells

7.2) Pre- & post-PARPi; E&G) Proportion of CD57+ and GzmB+ CD8 TEMRA cells, F&H) Median fluorescent intensity (MFI) of CD57+ and GzmB+ CD8 TEMRA cells

* denotes statistical significance - * - p≤0.05; ** - p ≤0.01 (using ordinary one-way ANOVA with multiple comparisons and Kruskal-Wallis test in those that did not pass normality test).

4.9.3 Regulatory T-cells

Tregs are defined as CD4+CD25+FoxP3+ immune cells and in a healthy individual comprise approximately 3-5% of total CD4+ T-cells. Their dominant function is to prevent autoimmunity. In cancer, they play a critical role in suppressing T effector function, thereby preventing anti-tumour immunity (302,303). Their ability to do this is also influenced by the CD8+/Treg ratio and prognosis appears to be better with a higher CD8+/Treg ratio (304). They can also suppress the function of NK cells, monocyte/macrophages and APCs (305). It has been reported that patients with ovarian cancer have a higher number of circulating Tregs (83). The hypothesis was that, when compared to HD, patients with ovarian cancer would have an increased number of circulating Tregs, with a reduction of Tregs if treatment reduced the burden of disease.

Figure 14 illustrates an increased number of Tregs in patients with ovarian cancer when compared to HD, in keeping with published literature. However, there is no significant change in the proportion to Tregs following treatment with chemotherapy, despite most patients responding to treatment. Included in this figure are representative dot plots from the flow cytometry analysis of the HD and patient data.

Figure 14: Circulating Tregs in HD (n=19) vs diseased bloods

14.1: Pre-and post-chemotherapy (n=26) **14.2: Pre- and post- PARPi (n=20)**

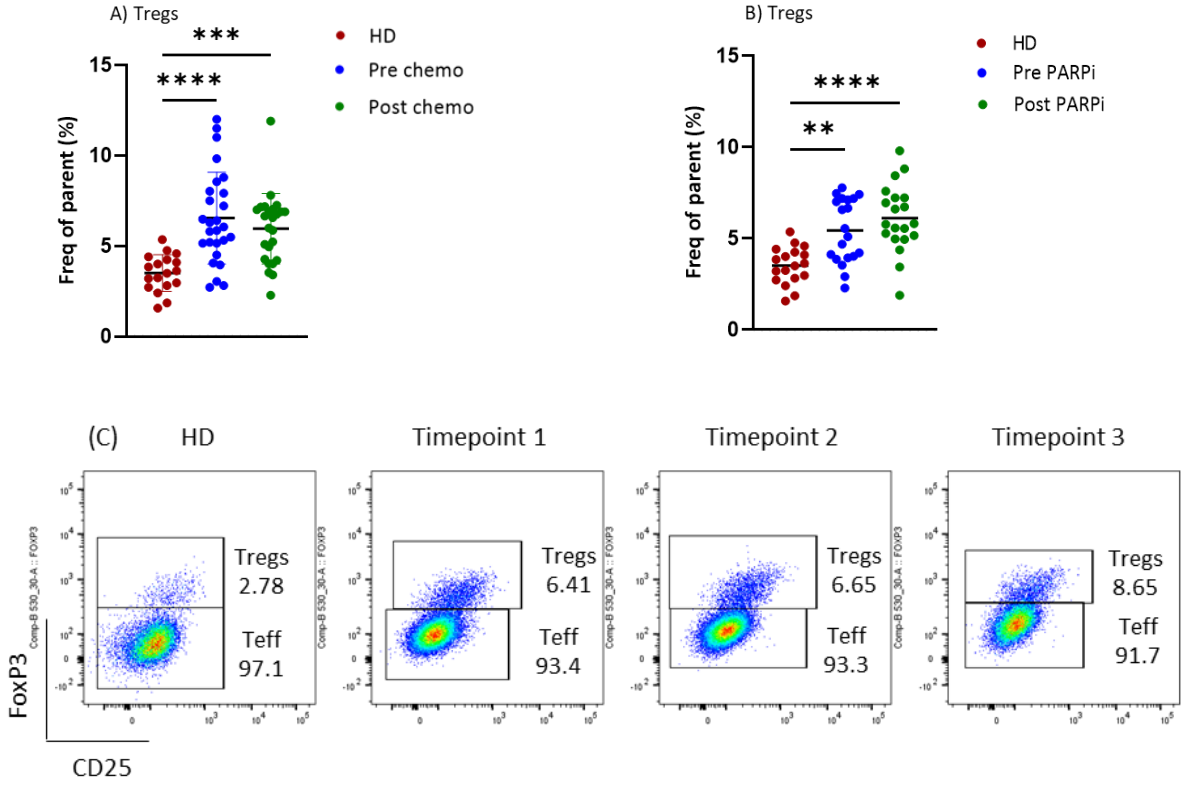


Figure 14: Proportion of Tregs HD vs diseased patient bloods
Graphs representing the proportions of Tregs as a proportion of CD4+ cells)
(A) Pre- and post-chemotherapy
(B) Pre- and post-PARPi
(C) Representative dot plots illustrating the different proportions of Tregs in a representation HD (TG048) and that of a patient (TG009) at timepoint 1 (pre-chemo), timepoint 2 (post-chemo) and timepoint 3 (post-C4 PARPi)
* denotes statistical significance - ** - p ≤0.01; *** - p ≤0.001; ****- p ≤0.0001 (using ordinary one-way ANOVA with multiple comparisons and Kruskal-Wallis test in those that did not pass normality test).

Although Figure 14 does not show any significant change in Tregs following treatment of ovarian cancer, figure 15 shows a trend towards a reduction of Tregs following chemotherapy. Although not statistically significant (adjusted p value >0.9999 using Kruskal-Wallis non-parametric test), there appears to be a trend towards fewer circulating Tregs following chemotherapy. This is not seen in the pre- and post-PARPi cohort. One explanation for this could be chemotherapy is cytotoxic and given with intent to kill cancer cells, whereas PARPi are given as maintenance treatment, having already responded to chemotherapy and patients may not have any visible disease.

Figure 15: Changes in Tregs in patients treated with systemic therapy

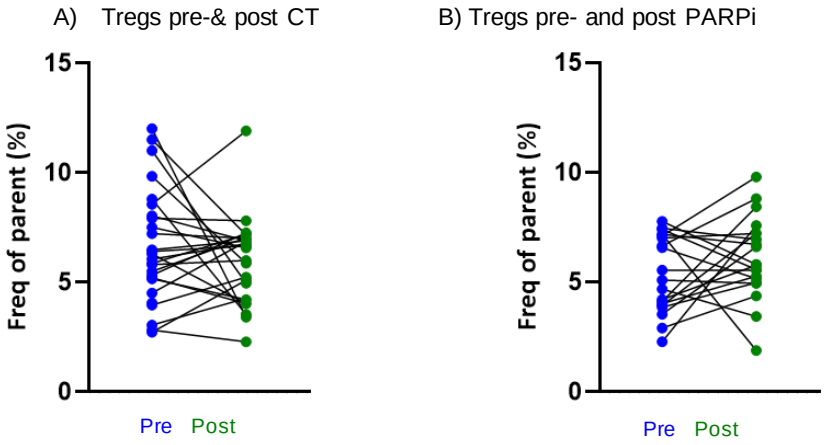


Figure 15: Differences in Tregs pre- and post-systemic therapy
A) Pre- (blue) and post- (green) chemotherapy; B) Pre- (blue) and post-(green) PARPi

The fold change (FC) of Tregs at completion of treatment was calculated and correlated with all the myeloid subsets. No correlation between change in Tregs and myeloid cells was found, and these data have not been included.

As Tregs are immunosuppressive, the next section explores whether Tregs in ovarian cancer patients express more inhibitory receptors than those from HD and whether systemic therapy alters this expression in any way. When compared to HD, there appears to be an increase in expression of PD-1 and TIM-3 (figure 16). The change in PD1 expression is not statistically significant, and the figures suggest there may be 2 cohorts of patients within this population; one with low PD-1 expression and the other with high PD-1 expression. The increase in number of TIM-3+ Tregs was statistically significant, although there is also a suggestion of 2 separate cohorts of patients in the PARPi group. Representative dot plots from the flow cytometry analysis are included (figures 16E & F).

Figure 16: PD-1 and TIM-3 expression on Tregs in HD (n=19) and diseased blood

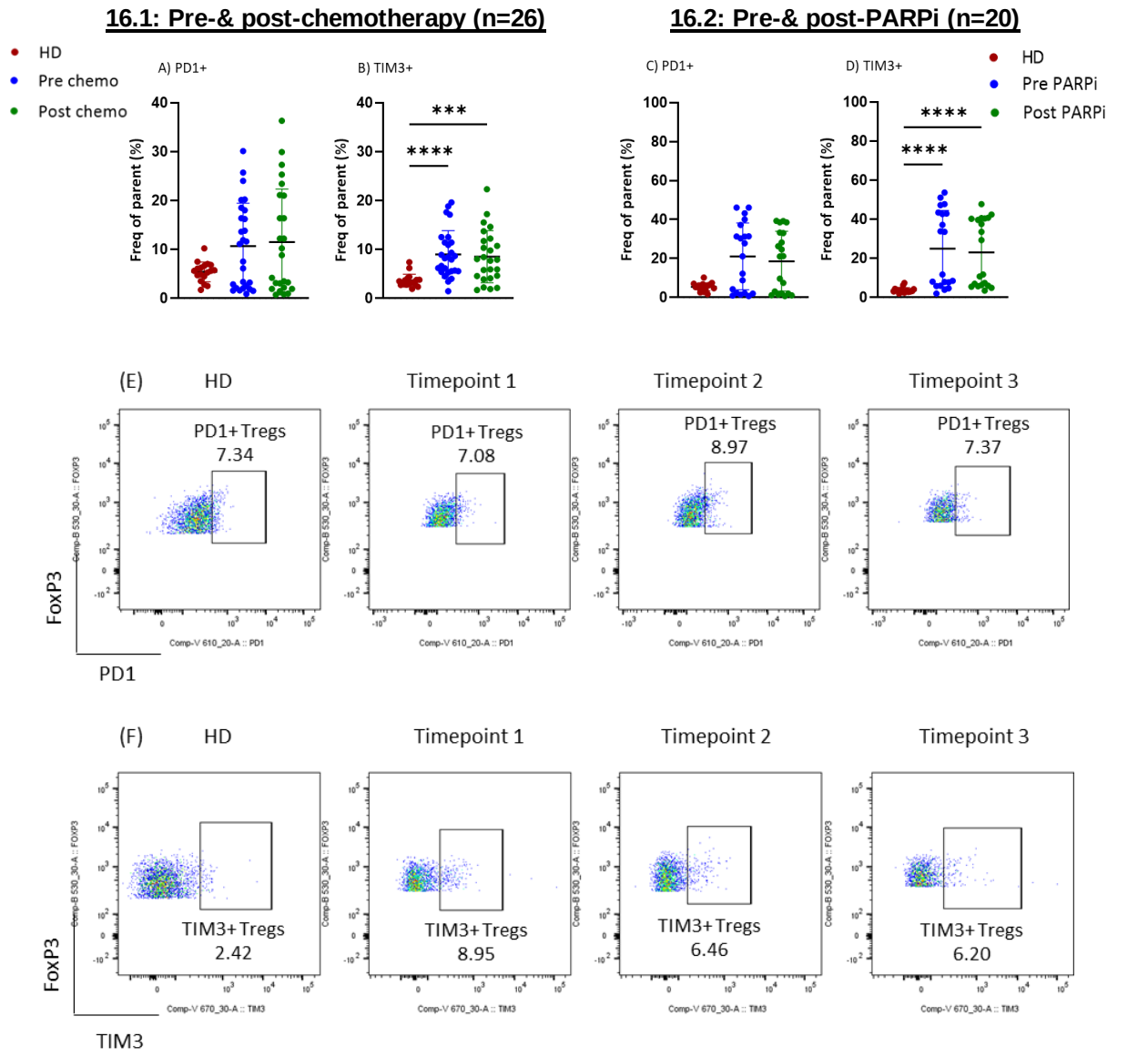


Figure 16: Proportion of PD-1 & TIM-3+ Tregs

A&B) Pre- and post-chemotherapy; C&D) Pre and post-PARPi

Graphs representing the proportions of:

A&C) PD-1 and B&D) TIM-3 expression in Tregs

Representative dot plots of E) PD-1+ and F) TIM-3+ Tregs in HD (TG047) and TG009 at timepoint 1 (pre-chemo), timepoint 2 (post-chemo) and timepoint 3 (post-PARPi)

* denotes statistically significant differences - *** - $p \leq 0.001$; ****- $p \leq 0.0001$ (using ordinary one-way ANOVA with multiple comparisons and Kruskal-Wallis test in those that did not pass normality test).

It has been reported that a high CD8/Treg ratio in tumour infiltrating lymphocytes (TILs) is associated with improved response in patients treated with NACT for breast and bladder cancers (306,307). This has also been reported in HGSC (308). However, there are no data looking at the CD8+/Treg ratio in PBMCs and its impact on response to treatment / prognosis.

The CD8/Treg ratio pre- and post- systemic therapy was calculated and correlated with clinical outcomes (figure 17).

Figure 17: CD8/Treg ratio pre- and post-systemic therapy

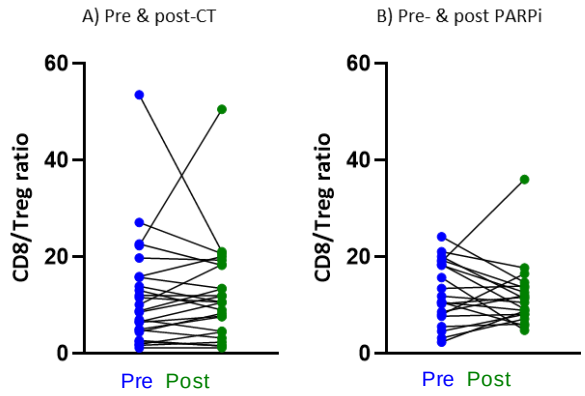


Figure 17: CD8/Treg ratio in PBMCs
A) Pre- (blue) and post- (green) chemotherapy (n=26)
B) Pre- and post- PARPi (n=20)

As shown in figure 17, there is no significant change in the CD8/Treg ratio following treatment with systemic therapy. There are 2 outliers in the chemotherapy arm – TG023 who appears to have a large reduction in the CD8/Treg ratio from 53.5 to 21.1 and TG036, whose CD8/Treg ratio increases from 22.3 to 50.5. In the PARPi group, TG033 seems to have an increased CD8/Treg ratio. Their clinical histories are summarised below in Table 13.

Table 27: Patient details of those with change in CD8/Treg ratio

Patient	CD8/Treg ratio	Clinical history
TG023	Decreased	Stage IVb HGS PPC Platinum sensitive relapse (PFS 11 months) No response to further lines of chemotherapy OS 27.8 months
TG036	Increased	Stage IIIC HGSC Partial response to first line chemotherapy, but disease too extensive at attempted DCS Platinum sensitive relapse (PFS 12.4 months). Pt died before starting second line chemotherapy. OS 14.8 months
TG033	Increased	Stage IIIC HGSC; gRAD51D mutation March 2024: Remains on maintenance niraparib with no evidence of disease

Table 27: Patient details of those with change in CD8/Treg ratio
PPC – primary peritoneal carcinoma; PFS – progression free survival (months); HGSC – high grade serous ovarian cancer; DCS – delayed cytoreductive surgery; gRAD51D – germline mutation in RAD51D gene.

These results imply that CD8/Treg ratio in bloods is unaffected by systemic therapy and does not appear to have any predictive or prognostic significance.

4.10 Myeloid cell analysis

The second flow cytometry panel was a myeloid panel, designed to explore whether myeloid cell subsets differed between HD and ovarian cancer patients and whether systemic therapy had any impact on circulating myeloid cells. Figure 18 illustrates the gating strategy used for this panel.

Figure 18: Myeloid gating strategy

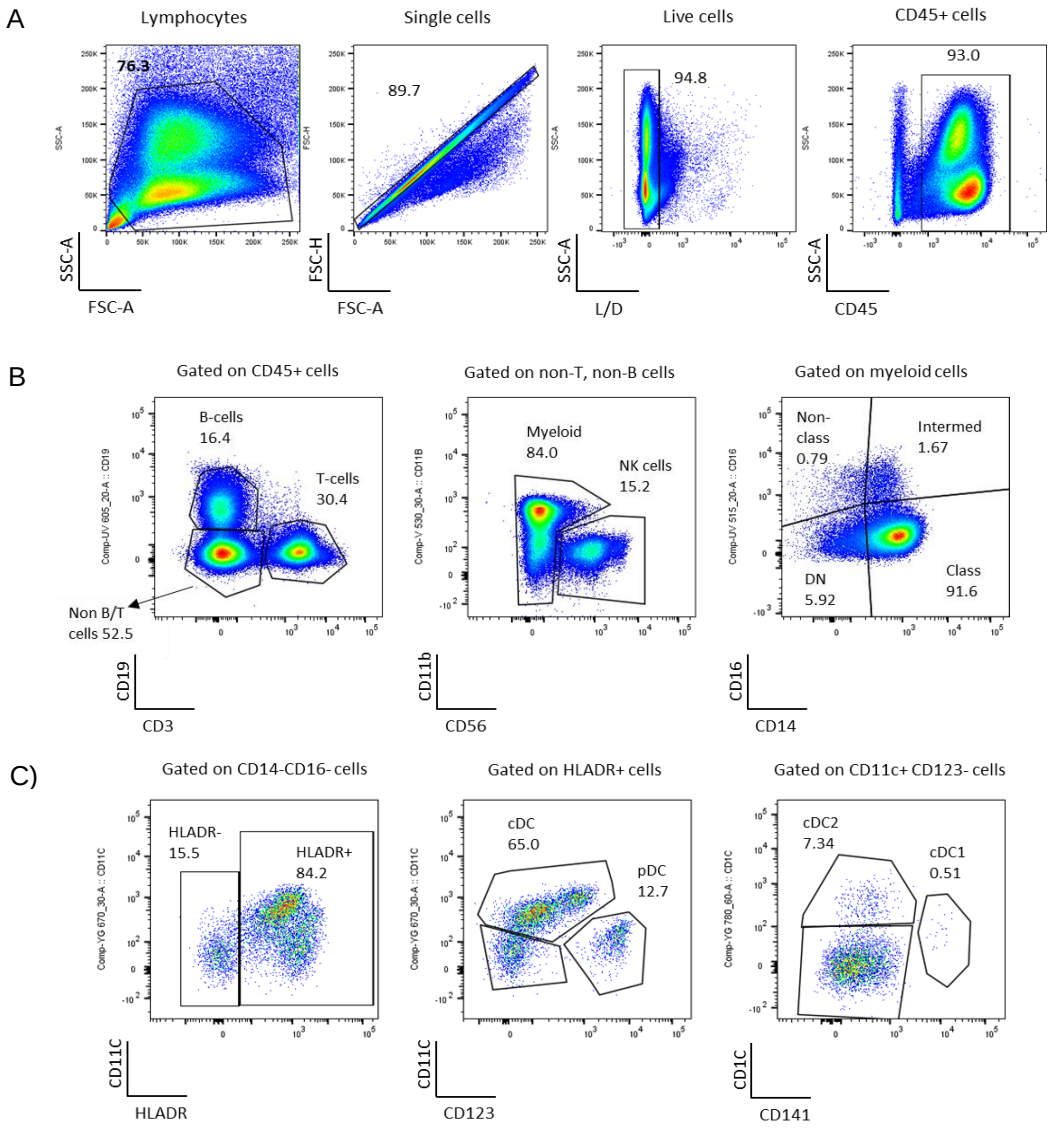


Figure 18: Flow cytometry gating strategy for myeloid cells
Multi-parametric flow cytometry was used to identify the myeloid in PBMC samples of all patients and healthy donors (HD). Dot plots and representative gating strategies are illustrated from a representative HD (TG041). (A) Viable (L/D), singlet CD45+ cells were gated for within the lymphocyte gate. (B) T-cells (CD3+), B-cells (CD19+) and Non-B/T cells (CD45+ CD19- CD3-) were identified from the CD45+ population. NK (CD56+) and myeloid cells (CD11b+/-, CD56-) were then gated for within the non-T/B cell population. Monocytes were then gated for within the myeloid population and gated into classical (CD14+ CD16-), non-classical (CD14- CD16+), intermediate (CD14+ CD16+) and double negative. (C) Dendritic cells (DCs) were gated on the double negative population (CD14- CD16-). Conventional DCs (cDC) (CD11c+) and plasmacytoid DCs (pDCs) (CD123+); cDCs were then further gated into cDC1 (CD141+ CD1c-) and cDC2 (CD141- CD1c+) populations.

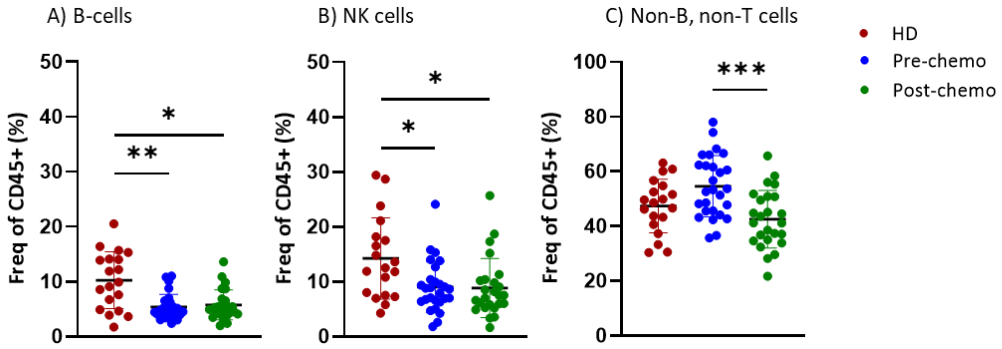
4.10.1 Myeloid cells

The full myeloid panel is shown in the methods and materials chapter. It included a B-cell, T-cell and NK-cell marker, and myeloid cells were assumed to be part of the non-B, non-T cell group. This analysis was performed on cryopreserved PBMCs, rather than whole blood, therefore the panel did not include a neutrophil marker.

The proportion of B- and NK-cells within the overall CD45+ population was identified. As shown in figure 11, there are fewer total B- and NK-cells in patients with OC, compared to HD. However, there is no significant change in these cells following systemic therapy. Figure 19.1 C) shows a significant reduction in total non-B, non-T cells (presumed to be myeloid cells) following treatment with chemotherapy. This panel did not include functional markers for B- and NK-cells, precluding any further analysis on these cells.

Figure 19: Non-T cells pre- and post- systemic therapy compared to HD

19.1) Pre- & Post-chemotherapy



19.2 D-F) Pre- & Post-PARPi

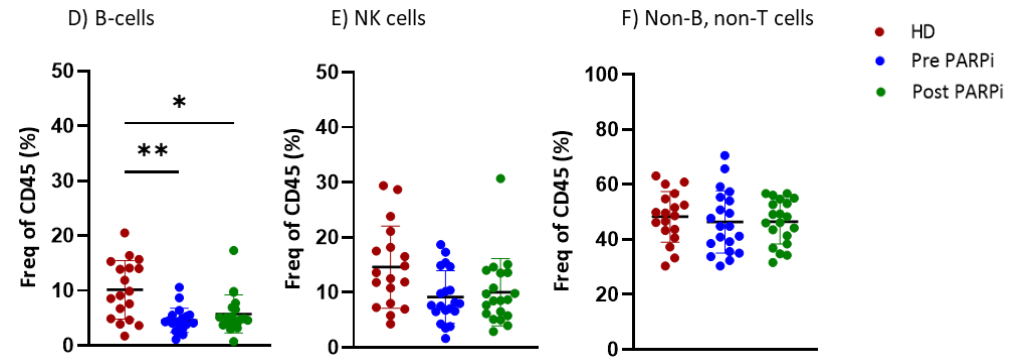


Figure 19: Proportion of non-B/non-T cells, B-cells and NK-cells in patients vs HD
A&D) B-cells (CD45+CD19+CD3-) cells in HD vs ovarian cancer patients; B&E) NK cells (CD45+CD19-CD3-CD11b-CD56+); C&F) Non-B/non-T cells (CD45+CD19-CD3-)
* denotes statistical significance - * - p≤0.05; ** - p ≤0.01; *** - p ≤0.001 (using ordinary one-way ANOVA with multiple comparisons and Kruskal-Wallis test in those that did not pass normality test).

Figure 20 : Dot plots for B cells, NK cells and myeloid cells

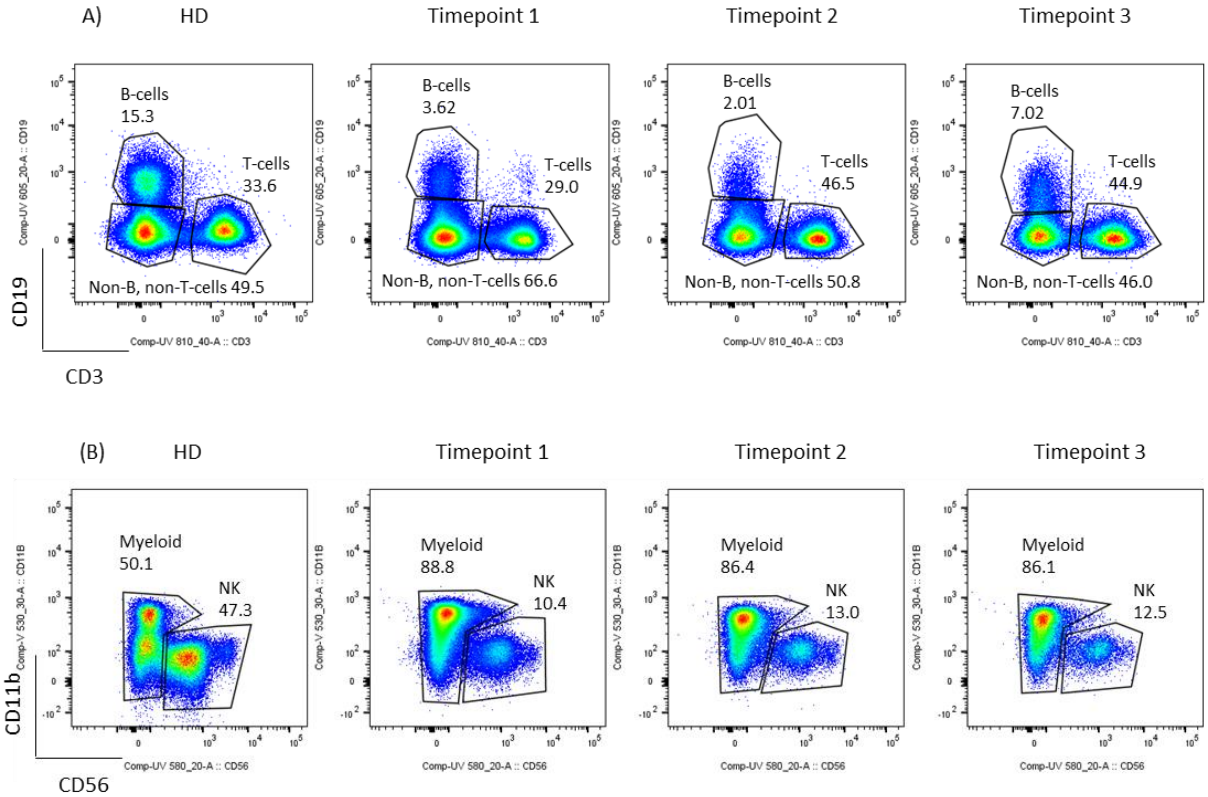


Figure 20: Representative dot plots for B-cell, NK cells, non-B, non-T cells and myeloid cells
HD (TG048) and patient sample TG009 (timepoint 1 – pre-chemotherapy, timepoint 2 – post-chemotherapy and timepoint 3 – pre-C4 PARPi
A) Gated on CD45+ cells; B-cells (CD19+ CD3-), non-B, non-T-cells (CD19- CD3-) and T-cells (CD3+ CD19-) in HD vs ovarian cancer patients;
B) Gated on non-B, non-T cells; NK cells (CD11b-CD56+) and myeloid cells (CD11b+ CD56-).

4.10.2 Monocytes

Monocytes are a major component of the innate immune system. They are mononuclear phagocytes that circulate in peripheral blood and play an important role in cancer development and progression. It is thought that monocytes are derived from common monocyte progenitors found in bone marrow and umbilical cord blood. They all express HLADR (MHC class II (MHCII) receptors), CD11b and CD86. There are 3 established monocyte subsets, identified by CD14 and CD16 expression; classical (clas) (CD14+CD16-), non-classical (non-clas) (CD14-/lo, CD16+) and intermediate (int) (CD14+CD16+). The progenitors develop into classical monocytes within the circulation, with subsequent differentiation into non-classical monocytes. The half-life of circulating classical monocytes is less than 24hrs, whereas it is approximately 7

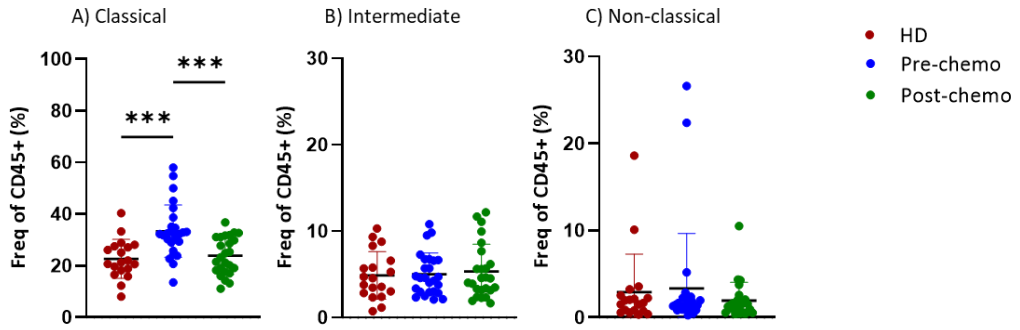
days for non-classical monocytes. Intermediate monocytes may represent a transition state (309).

In the event of disease/inflammation, classical monocytes are rapidly recruited and induce an immune response by secreting cytokines and anti-microbial factors. Monocyte recruitment occurs at all stages of tumour development and progression and display both pro-tumoural and anti-tumoural characteristics (310).

One hypothesis was that diseased bloods would have increased numbers of classical monocytes compared to HD bloods, with and a reduction in number of circulating classical monocytes seen with a response to treatment. The frequency of monocyte subsets of total circulating CD45+ cells are shown in figure 21, with representative dot plots below (figure 21).

Figure 21: Monocyte subsets pre- and post-systemic therapy

21.1 Pre- & Post-chemotherapy



21.2 Pre- & Post PARPi

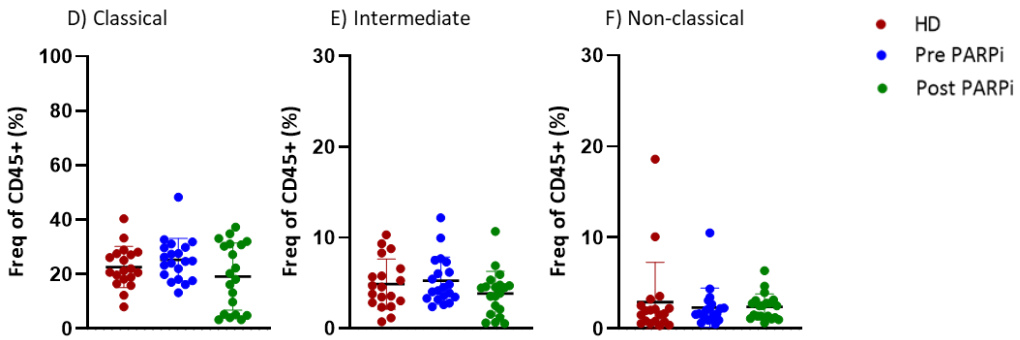


Figure 21: Proportion of monocytes in patients vs HD pre-& post-systemic therapy
A&D) Classical monocytes B&E) Intermediate monocytes C&F) Non-classical monocytes
*** - p ≤0.001 (using ordinary one-way ANOVA with multiple comparisons and Kruskal-Wallis test in those that did not pass normality test).

Figure 22: Representative dot plots for monocytes

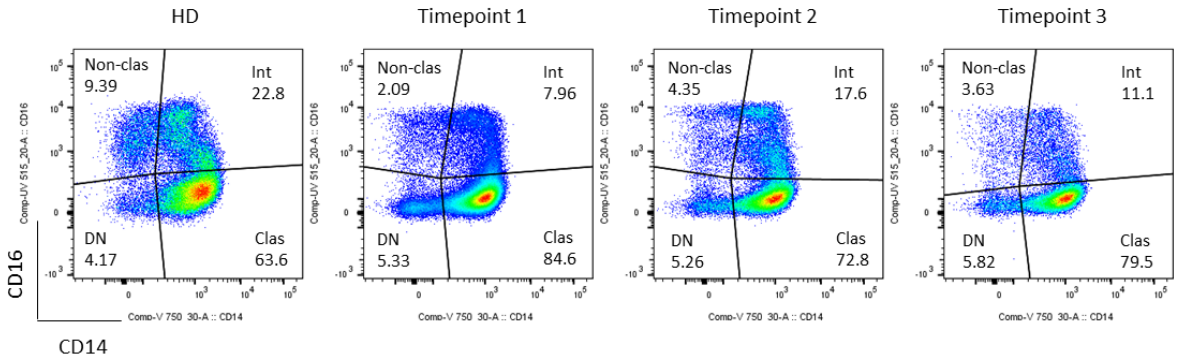


Figure 22: Representative dot plots for monocytes
HD (TG048) and patient sample TG009 (timepoint 1 – pre-chemotherapy, timepoint 2 – post-chemotherapy and timepoint 3 – pre-C4 PARPi
Cells gated on myeloid cells (CD11b+CD56-) cells
Non-classical (non-clas) CD16+CD14-, intermediate (int) CD16+CD14+, classical (clas) CD14+CD16- and double negative (DN) CD14-CD16-.

As shown in figure 21.1(A), classical monocytes are significantly higher in diseased patients when compared to HD, with a statistically significant reduction following chemotherapy. Whilst the PARPi data do not show any statistically significant changes, there also appears to be a trend towards fewer circulating classical monocytes following treatment. The number of intermediate and non-classical monocytes appear similar in HD and diseased bloods, with no significant change following systemic therapy.

The changes in classical monocytes were analysed further, using a paired, non-parametric t-test, assuming that these data were not normally distributed (figure 23). As demonstrated above, this confirms the statistically significant reduction in classical monocytes following treatment with chemotherapy and shows a similar trend in post-PARPi blood samples.

Figure 23: Classical monocytes pre- and post-systemic therapy

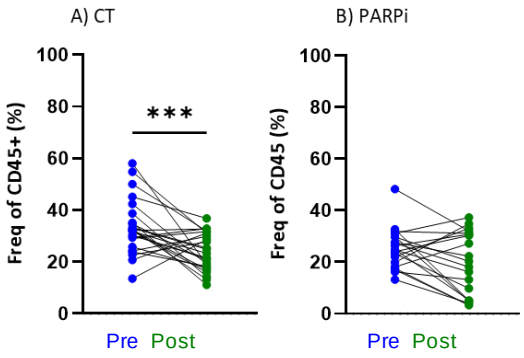


Figure 23:Changes in classical monocytes following treatment for ovarian cancer
A) Shows changes before and after chemotherapy (CT)
B) Shows changes at the commencement of PARPi (following completion of chemotherapy) and after 3 months of treatment.
*** denotes statistical significance ($p \leq 0.05$) (using Wilcoxon signed rank test and ordinary one way ANOVA)

Blue – pre-treatment
Green – post-treatment

4.10.3 Tregs and classical monocytes and relationship to progression

At the time of writing, there was >2yr follow up data on some patients. Data cut off for this analysis was 1st June 2023. The most significant finding is an increased number of Tregs in diseased blood when compared to HD. Additionally, the myeloid dataset illustrates an increased number of circulating classical monocytes, which appear to reduce following treatment, more significantly after completion of chemotherapy, although this trend was seen after treatment with three cycles of maintenance PARPi.

The next analyses explored the relationship between the number of Tregs and classical monocytes at the final timepoint and time to progression. The hypothesis was that patients who relapsed earlier would have a higher number of circulating Tregs compared to those who have not yet progressed. Classical monocytes can have both pro-tumoural and anti-tumoural functions. Therefore, an increase at the time of completion of chemotherapy could represent pro-tumoural activity, resulting in a poorer prognosis. This shown in figures 24-28 below and tables 28-32 summarise the clinical history.

Figure 24: Cohort 1a - NACT inoperable (n=5)

24.1 Tregs **24.2 Classical monocytes**

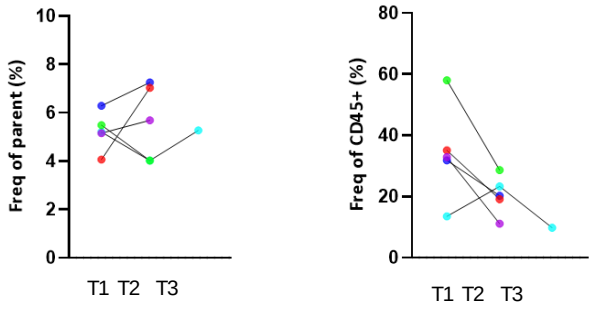


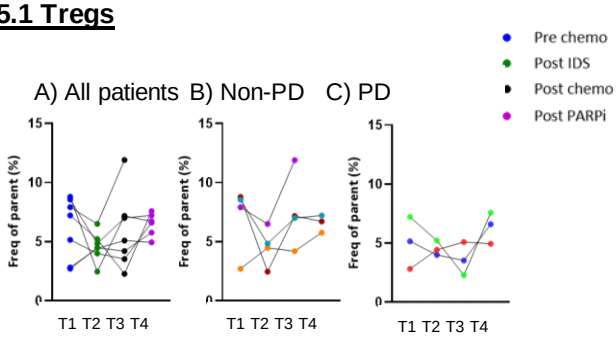
Table 28: Cohort 1a – NACT inoperable patients with disease progression

Patient	Platinum free interval	Tregs	Clas monocytes	PFS (mths)	OS (mths)
TG013	Platinum refractory	Increased	Decreased	5.5	11.6
TG014	Platinum sensitive (6-12 months)	Increased	Decreased	15.2	29.9
TG016	Platinum refractory	Decreased	Decreased	2.7	9.4
TG018	No progression	Increased	Decreased	5.4	5.4
TG036	Platinum sensitive (6-12 months)	Increased	Decreased	12.4	14.8

Figure 24 and Table 28: Cohort 1a – NACT inoperable
Changes in Tregs and classical monocytes at timepoints 1-3 (T1, T2, T3).
All patients had progressed at the time of analysis.
Tregs – regulatory T-cells; Clas – classical monocytes, PFS – progression free survival (months); OS – overall survival (months).

Figure 25: Cohort 1b - NACT ICS patients (n=7)

25.1 Tregs



25.2 Classical monocytes

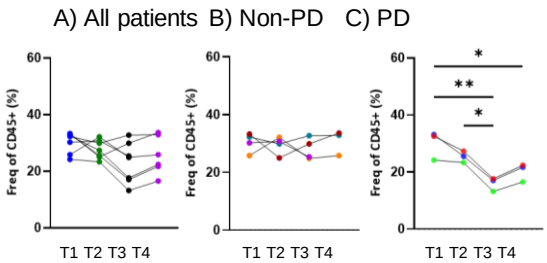


Table 29: Cohort 1b - NACT ICS patients with disease progression

Patient	Platinum free interval	Tregs	Clas monocytes	PFS (mths)	OS (mths)
TG008	Platinum sensitive (>12 months)	Increased	Decreased	27.8	N/A
TG023	Platinum sensitive (6-12months)	Stable	Decreased	11.0	27.8
TG025	Platinum sensitive (6-12months)	Increased	Decreased	11.3	28.2

Figure 25 and Table 29: Cohort 1b – NACT ICS
Changes in Tregs and classical monocytes at timepoints 1- 4 (T1, T2, T3, T4).
3/7 patients had progressed at the time of analysis.
Tregs – regulatory T-cells; Clas – classical monocytes, PFS – progression free survival (months); OS – overall survival (months).

Figure 26: Cohort 1c - NACT DCS patients (n=5)

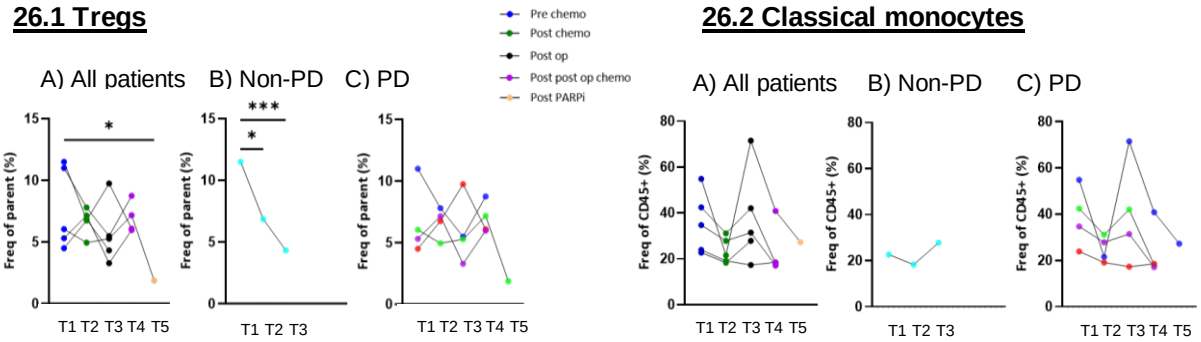


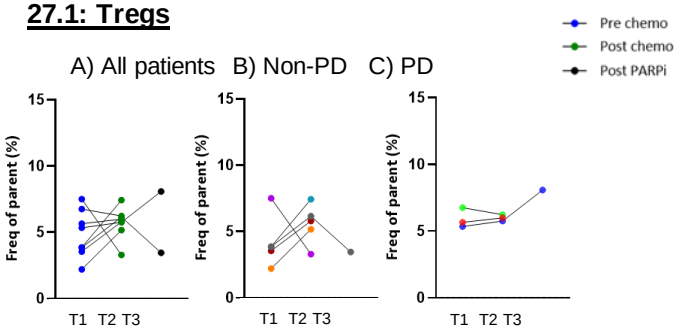
Table 30: Cohort 1c - NACT DCS patients with disease progression

Patient	Platinum free interval	Tregs	Clas monocytes	PFS (mths)	OS (mths)
TG006	Platinum sensitive (6-12months)	Increased	Decreased	8.3	16.4
TG007	Platinum sensitive (>12 months)	Decreased	Decreased	17.1	N/A
TG015	Platinum sensitive (>12 months)	Decreased	Decreased	27.0	28.2
TG045	Platinum sensitive (6-12 months)	Increased	Increased	12.1	30.1

Figure 26 and Table 30: Cohort 1c – NACT DCS
Changes in Tregs and classical monocytes at timepoints 1- 5 (T1, T2, T3, T4, T5).
4/5 patients had progressed at the time of analysis.
Tregs – regulatory T-cells; Clas – classical monocytes, PFS – progression free survival (months); OS – overall survival (months).

Figure 27: Cohort 2 - PCS patients (n=8)

27.1: Tregs



27.2: Classical monocytes

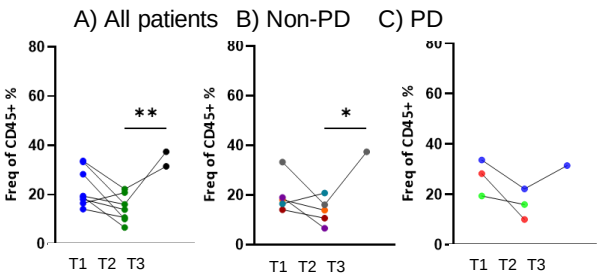


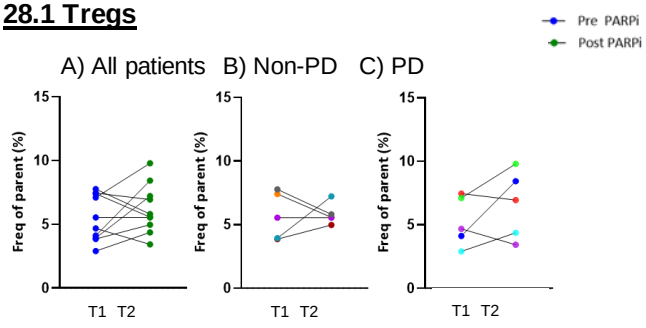
Table 31: Cohort 2 - PCS patients with disease progression

Patient	Platinum free interval	Tregs	Clas monocytes	PFS (mths)
TG009	Platinum sensitive (>12 months)	Increased	Increased	26
TG030	Platinum sensitive (>12 months)	Decreased	Decreased	15.6
TG039	Platinum sensitive (>12 months)	Increased	Decreased	20.7

Figure 27 and Table 31: Cohort 2 – PCS
Changes in Tregs and classical monocytes at timepoints 1-3 (T1, T2, T3)
3/8 patients had progressed at the time of analysis.
Tregs – regulatory T-cells; Clas – classical monocytes, PFS – progression free survival (months); OS – overall survival (months).

Figure 28: Cohort 3 - PARPi patients (n=10)

28.1 Tregs



28.2 Classical monocytes

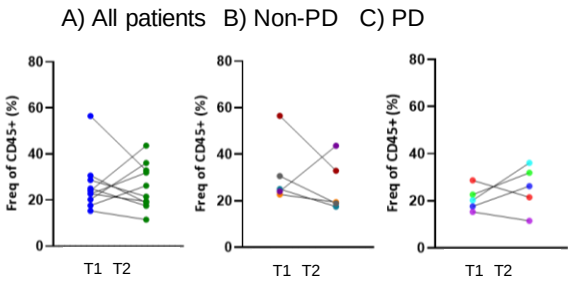


Table 32: Cohort 3 -PARPi patients with disease progression (n=5)

Patient	Line of maintenance	Tregs	Clas monocytes	PFS (mths)	OS (mths)
TG028	1	Increased	Increased	24.2	-
TG034	2	Decreased	Decreased	42.4	53
TG038	2	Increased	Increased	123.4	148.9
TG040	2	Decreased	Decreased	94	Lost to f/up
TG041	2	Increased	Increased	12.6	35.5

Figure 28 and Table 32: Cohort 3 – PARPi
Changes in Tregs and classical monocytes at timepoints 1-2 (T1, T2)
5/10 patients had progressed at the time of analysis.
Tregs – regulatory T-cells; Clas – classical monocytes, PFS – progression free survival (months); OS – overall survival (months).

In relapsed ovarian cancer, the platinum free interval (PFI) is a prognostic indicator and is often used to determine the next line of treatment. If PFI is >6 months, patients are considered to have a high likelihood of response to platinum-based chemotherapy. Patients with platinum refractory/resistant disease have a poorer prognosis, and response rates to chemotherapy (both platinum and non-platinum based) regimens are poor.

Based on the data above, there does not appear to be any clear relationship between the numbers of Tregs / classical monocytes and platinum free interval.

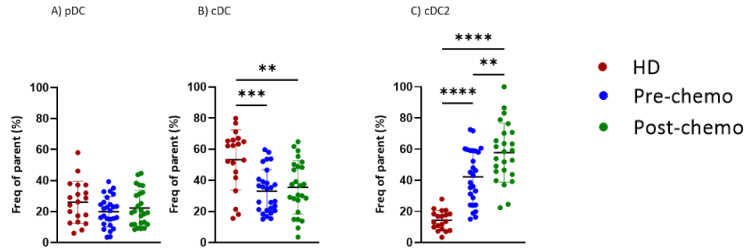
4.10.4 Dendritic cells

DCs are APCs that play an integral role in T-cell activation by transporting tumour-antigens to the local drainage lymph nodes. There are 2 main types of DCs; plasmacytoid DCs (pDCs) and conventional DCs (cDCs). cDCs are further subdivided into cDC1 (CD141+) and cDC2 (CD1c+). All DCs represented below were gated on HLADR+ CD14- CD16- non-B, non-T cells. cDCs are CD11c+ and pDCs are CD123+. cDC1s account for a very small proportion of circulating myeloid cells in human blood (<0.01% of haematopoietic cells) (311).

Unfortunately, CD11c staining did not work well on the 10 PARPi patients or the 2 patients who underwent PCS and went on to have PARPi maintenance therapy. These samples were excluded from the DC analysis. Ten patients in Cohort 1 – NACT went onto maintenance PARPi. NACT IDS – TG004, 8, 11, 23, 25, 35, NACT DDS – TG007, NACT inoperable – TG014, PDS – TG009, TG037. The total number of plasmacytoid and conventional DCs are shown as a percentage of their parent cells, as well as conventional DC2s (figure 29).

Figure 29: Dendritic cells in HD (n=19) vs diseased blood

29.1 Pre- & Post-chemotherapy (n=26)



29.2 Pre- & Post-PARPi (n=10)

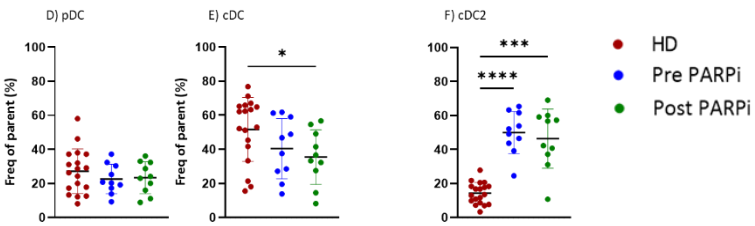


Figure 29. Dendritic cells in HD vs diseased bloods pre- and post-systemic therapy
pDC – plasmacytoid dendritic cells, cDC – conventional dendritic cells, cDC2 – conventional dendritic cells type 2
Figures are shown as a frequency of parent.
* denotes statistical significance - * - p≤0.05; ** - p ≤0.01; *** - p ≤0.001; **** - p≤0.0001 (using ordinary one-way ANOVA with multiple comparisons and Kruskal-Wallis test in those that did not pass normality test).

There appears to be a non-statistically significant difference between the HD and diseased patients, with a trend showing a smaller number of cDCs, but increased number of cDC2. These levels do not change significantly following treatment. The pre- and post-PARP data is difficult to interpret due to the small numbers of patients. Representative dot plots are illustrated in figure 30.

Figure 30: Representative dot plots of dendritic cells

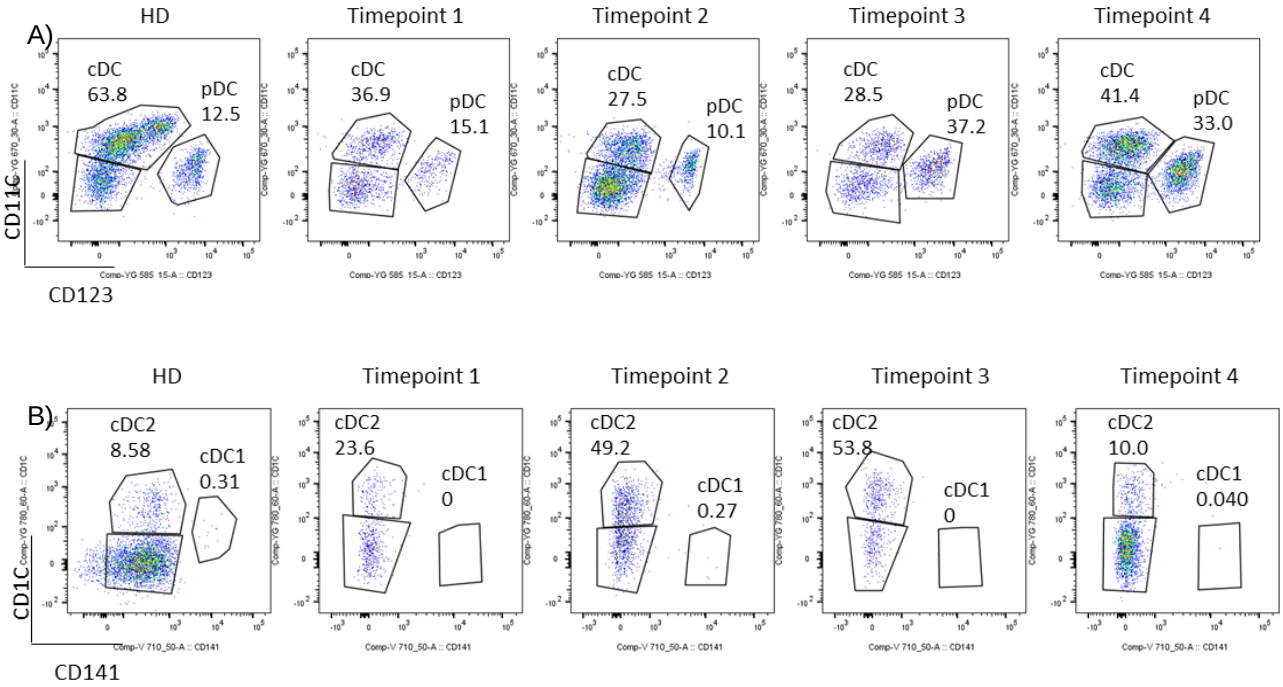


Figure 30: Representative dot plots of dendritic cells
HD TG047 and diseased bloods belong to TG035 Timepoint 1 pre-chemo, timepoint 2 post-ICS, timepoint 3 post-chemo and timepoint 4 – post PARPi
A) Dot plots illustrating cDC (lin-DR+CD11c+) and pDC (lin-DR+CD141+) subtypes (gated on CD45+lin-HLADR+)
B) Dot plots illustrating cDC1 (CD141+) and cDC2 (CD1c+) (gated on CD11C+ cells)

4.11 Discussion

In this chapter, I have analysed peripheral blood mononuclear cells in a cohort of patients with advanced high grade epithelial ovarian cancer before, during and after first line therapy, and compared them to samples from a cohort of age-matched healthy donors.

The cohort had a median age at diagnosis of 71 years, which is slightly older than the UK average (63 years). However, 28/36 (77%) had stage III or IV disease at the time of diagnosis and 5/36 (13.9%) had pathogenic germline mutations in BRCA1 or BRCA2. Thus, the cohort was largely representative of patients with advanced high grade epithelial ovarian cancer.

The most pertinent finding is the increased number of circulating Tregs in patients with ovarian cancer compared to female, age matched HD, which is consistent with published literature (312,313). The significance of this remains unclear and further analysis will be required to gain a deeper understanding of their role in ovarian cancer. Tregs comprise a small proportion of total circulating immune cells. In healthy individuals, they play a pivotal role in preventing autoimmunity and are an important component of the immune response (314). In ovarian cancer, circulating Tregs and Tregs within the cancer TME are reported to be associated with a poor prognosis (315,316). It is also assumed that circulating Tregs are recruited into the omentum via chemokine receptors. Peritoneal/omental disease is commonly seen in advanced ovarian cancer, and the accumulation of Tregs within the omentum is another predictor of poor outcomes (317). Circulating Tregs in other solid tumours such as NSCLC and breast cancer have also been shown to be prognostic. In early-stage NSCLC, increased levels of circulating and tumour-infiltrating Tregs are associated with a higher risk of recurrence and a worse prognosis (318). Similarly in breast cancer, increased levels of circulating FOXP3+ Tregs in breast cancer resulted in poor pathological responses after NACT (319).

The initial hypothesis for this dataset was that the proportion of circulating Tregs would be higher in patients compared to HD, with increased numbers of Tregs in patients with a larger burden of disease, which should reduce following treatment. However, despite response to chemotherapy and / or surgery, the proportion of circulating Tregs

remained the same. This is best illustrated in the NACT ICS and DCS cohorts, where levels of Tregs did not change following effective chemotherapy and surgery. Published reports in other solid tumours, such as breast and gastric cancer have shown a reduction in the number of peripheral Tregs following surgery and systemic anti-cancer therapy in other solid tumours (320–322). The significance of the lack of change in Tregs following treatment in this project is unclear and needs to be confirmed with a larger sample size but could reflect the timing of sampling – it is unclear how rapidly Treg counts might reduce following treatment. Additionally, the size of this cohort precluded meaningful analysis of the prognostic implication of Treg numbers.

Tumour infiltrating Tregs express immune checkpoints, but their expression on circulating Tregs in diseased blood is less well defined (323). PD-1 prevents autoimmunity by inhibiting the immune system from killing cells expressing self-antigens, which may be the mechanism by which malignant cells avoid the immune response. PD-L1 on normal tissues prevents autoimmune damage from Tregs expressing PD-1. Similarly, tumours that express PD-L1 manage to evade the immune system (324). The glycoprotein TIM-3 is another inhibitory checkpoint, which is also expressed on a small proportion of circulating Tregs, with a significantly higher proportion of TIM-3+ Tregs cells seen within the tumour microenvironment (325). TIM-3+ Tregs are immunosuppressive cells and are thought to play a role in tumour progression. Here, circulating Tregs in ovarian cancer express higher levels of PD-1 and TIM-3 compared to HD blood. These could represent dysfunctional / exhausted Tregs but could also be a marker of Treg activation (326). The expression of multiple inhibitory checkpoints could also indicate increased immunosuppressive activity. PD-1 expression did not change with treatment. However, there appears to be a trend towards depletion of TIM-3 expression following systemic therapy. This could symbolise less immunosuppression perhaps correlating with response to treatment. Despite the increased PD-1 expression on Tregs demonstrated in this cohort of patients, PD-L1 expression was not seen on dendritic (antigen presenting) cells. It is not possible to ascertain whether the expression of these inhibitory checkpoints has any clinical significance here. However, in melanoma, a reduction in circulating PD-1+ Tregs predicts better outcomes following treatment with ICPIs (327). Dynamic changes in circulating Tregs in NSCLC have been reported, with a significant decrease

noted in patients who responded to ICPIs and an increase in those who progressed on treatment (328). Higher levels of baseline circulating Tregs have also been reported in melanoma patients. They reduce in number following treatment with ipilimumab and appear to be associated with improved OS (329,330).

Further work is required to identify the relevance of circulating Tregs in ovarian cancer, whether they are a useful and important biomarker with regards to prognosis and / or predicting response to treatment, including ICPIs. Additionally, analysis of chemokine expression on these circulating Tregs, alongside identification of chemokine receptors on omental Tregs could also be of relevance. If indeed circulating Tregs are recruited to the omentum, resulting in inhibition of anti-tumour immunity, this could be a potential therapeutic target (317).

Reduced levels of circulating CD3+CD8+ T-cells in ovarian cancer patients have also been reported (331), although this was not seen here. A high CD8/Treg ratio within the tumour microenvironment is associated with improved response in patients treated with NACT for breast and bladder cancers (306,307) and this has also been reported in HGSC (308). However, there are no published data on the CD8+/Treg ratio in PBMCs and its impact on response to treatment and/or prognosis. In this patient cohort, systemic therapy did not impact the CD8/Treg ratio in the circulation, and it is not possible to deduce from this analysis whether the circulating CD8/Treg ratio has any prognostic significance.

B cells play a key role in immune function by producing antibodies. The data above illustrate reduced numbers of circulating B-cells in ovarian cancer patients compared to HD, with no change in B-cell levels following treatment. This is in keeping with published literature, which report lower numbers of B-cells in ovarian cancer, particularly in advanced stages, platinum resistant disease and clear cell histology (332). The flow panel in this project did not include any functional markers for B-cells and therefore precluded further exploration of circulating B-cells. Currently, their role and significance in ovarian cancer immunity is not well understood.

NK cells are cytotoxic cells that produce inflammatory cytokines and chemokines that inhibit tumour growth. They express receptors that recognise stress-induced proteins on cancer cells and are able to kill these cells in the absence of prior sensitisation

(333,334). NK cells within the TME are associated with a better prognosis in a number of solid tumours, including ovarian cancer (335). However, the role of circulating NK cells is unknown. The data above demonstrates fewer circulating NK cells in diseased blood compared to HD. As with Tregs and B-cells, systemic therapy does not impact the proportion of these. However, this flow panel was not designed to allow further exploration into NK cell activity, nor the range of activating and inhibitory receptors expressed on circulating NK cells, which may influence prognosis.

Dendritic cells (DCs) are potent antigen-presenting cells (APCs) that play a vital role in the immune response. To induce an effective anti-tumour response, three signals between DCs are T-cells need to occur; antigen presentation via MHC-peptide complex (CD4+ T-cells via MHC-II and CD8+ T-cells via MHC-1), activation of co-stimulatory molecules from the DC to the TC and immune-stimulatory cytokines within the immune microenvironment.

Circulating DCs in PBMCs are generally very low in number, and this is represented in the dot plots above, which show very few events. These naturally circulating DCs comprise two subsets; myeloid/conventional DCs (cDCs) and plasmacytoid DCs (pDCs). cDCs are the more important and are pivotal for anti-tumour immunity. They are found in the marginal zone of lymph nodes and express MHC II and CD11c. They stimulate naïve T-cells and generate T-cell differentiation into Th1 (type 1 helper T-cells), which induce cell-mediated immunity (336). They are further subdivided into 2 subsets; cDC2s (CD1c+) and cDC1s (CD141+). cDC2s are the most common type of cDCs in blood and are the more potent stimulators of naïve T-cells (337). The data above illustrate that although the total number of DCs is lower in diseased blood when compared to HDs, there is a higher proportion of cDC2s. Further exploration into their function is needed to determine whether this has prognostic implications.

Circulating cDC1s are difficult to isolate from PBMCs, as they only make up a very small proportion of circulating leukocytes (338). However, they are very efficient in priming cytotoxic T-cells due to their higher cross-presentation ability when compared to other DCs and are associated with a better prognosis in cancer (337). As shown in the dot plots above (figure 30), there were very few cDC1s and further analysis of these is not possible.

In pancreatic cancer, circulating cDCs are lower in patients compared to HD. Treatment with surgery and systemic therapy improves cDC function, thought to be related to reduced immunosuppressive cytokine levels (339). The total number of cDCs appears to have prognostic significance; with improved survival seen in patients with higher numbers of cDCs (336).

pDCs are shaped like plasma cells and play an important role in response to viruses. However, when present in the cancer TME, they tend to be tolerogenic and are associated with a poor prognosis (337,338).

Whilst the number of pDCs in this data set does not appear to differ between HD and diseased bloods, there appears to be a reduction in the total number of cDCs in diseased blood, with an increased number of cDC2s. These data are only able to quantify the numerical change in DCs in diseased blood when compared to HD and do not provide any insight into the impact on prognosis in these patients. Further flow cytometric analysis could include phenotypic characterisation of DCs and expression of inhibitory and co-stimulatory molecules.

4.12 Conclusion

In summary, the flow cytometry data above provides some insight into the circulating immune landscape of patients with advanced EOC. Tregs are a heterogeneous group of cells and further exploration into their role in HGSC is warranted, and if thought to be prognostic, could be a potential target for treatment. However, caution is required when targeting Tregs as this could result in autoimmune toxicities (340), as are seen with inhibition of CTLA-4.

Additionally, future work should include exploration of circulating myeloid derived suppressor cells (MDSCs). MDSCs are immature myeloid cells that are increased in ovarian cancer and are thought to be associated with tumour development and growth (341). They are identified by their lack of HLADR expression and are positive for CD11b and CD33. There are 2 distinct subtypes of MDSCs – Polymorphonuclear (PMN) MDSCs, which express CD15 and are negative for CD14, and monocytic (m-) MDSCs, which express CD14 and are negative for CD15. These cells were not identified in the samples used in this analysis and therefore the data are not shown. This may reflect the use of cryopreserved PBMCs in these analyses, which will significantly reduce the number of MDSCs. Fresh samples should be used in order to accurately quantify these cells (342), as increased numbers of circulating MDSCs have been reported in ovarian cancer and are predictive of prognosis (343–346).

The multiplex immunofluorescence (mIF) analysis of the tumour samples collected for this project will be shown in the next section of this chapter.

Section 3: Multiplex immunofluorescence analysis of tumour

4.13 Aims

1) To investigate the location and density of subpopulation of TILs within the TME and their association with clinical outcomes.
2) In the paired (pre- and post-chemotherapy) samples to determine whether chemotherapy causes significant changes within the TME.

The hypotheses were as follows:

- Low expression of PD-1 and PD-L1 on both tumour and immune cells may explain lack of response to ICPIs in HGSC.
- Evidence of an increased immunosuppressive microenvironment in patients with a poorer prognosis.
- Presence of PD-1 positive Tregs contributing to immunosuppression.
- A reduction in number of tumour cells and increase in immune cells in paired samples pre- and post-NACT.

All the mIF staining was performed by Ayse Akarca at University College London (UCL) and spatial analysis was performed in collaboration with Dr Hanyun Zhang at the Institute of Cancer Research (ICR), London.

4.14 Sample selection

Of the 36 patients recruited for this project, tissue was available for 29. Two patients only had biopsy samples, 19 had resection specimens only and there were 8 paired samples. Prior to analysis, all stained slides underwent a quality control (QC) check. 24 samples from 19 patients passed QC and images from the three panels were aligned. One of these patients (TG044) was excluded from the analysis as no follow up data were available. 4 patients had paired samples; 3 patients, TG019, TG023, TG028 had diagnostic biopsies and a specimen from interval surgery; 1 patient, TG020 had a diagnostic biopsy and repeat biopsy taken at the time of relapse. TG038 had one biopsy sample available at relapse, 10 years after the original diagnosis. Where possible, the diagnostic / pre-chemotherapy sample was used for analysis. Comparisons were made between original samples from patients who were disease free (n=5) and those who had progressed (n=10) at the time of data cut off (24thMarch 2024). Patient characteristics and specimens used are shown in tables 33 and 34. The 3 mIF panels are outlined in table 35.

Table 33: Disease free patients

Patient ID	Stage	Cohort	Specimen	Maintenance Rx	Time from diagnosis (mths)
TG005	IIIC	1b	ICS	Niraparib	41.1
TG011	IIIC	1b	ICS	Niraparib	39.7
TG012	IIA	2	PCS	Nil	40.3
TG022	IIIB	2	PCS	Niraparib	37.8
TG037	IIIC	2	PCS	Niraparib	37.4

Table 34: Patients with disease progression

Pt ID	Stage	Cohort	Specimen	Maintenance Rx	PFS (mths)	OS (mths)
TG006	IVB	1c	DCS	Bevacizumab	8.3	16.4
TG009	IIIB	2	PCS	Niraparib	26.0	-
TG014	IIIB	1a	Biopsy	Niraparib	15.2	29.9
TG019	IVb	3	Biopsy	Olaparib 1st line	44.0	-
TG020	IIB	3	PCS	Olaparib 2nd line	45.1	-
TG021	IC	3	PCS	Niraparib 2nd line	21.6	-
TG023	IVB	1b	Biopsy	Niraparib	11.0	27.8
TG028	IIIC	1b	Biopsy	Niraparib	24.2	-
TG038	IC	3	Biopsy (relapse)	Niraparib 2nd line	123.4	148.9
TG041	IVA	1c	DCS	Niraparib	12.6	35.5

Tables 33 & 34: Summary of patients whose samples were included in analysis
Table 33: Disease free patients; Table 34: Patients with disease progression
Rx – treatment; Cohort 1a – NACT inoperable; cohort 1b – NACT ICS; cohort 1c – NACT DCS; cohort 2 – PCS; cohort 3 – PARPi; PFS – progression free survival (months); OS – overall survival (months).

Table 35: Multiplex IF panels

	Panel Description	Markers
Panel 1	T-cell/myeloid	CD4, CD8, FoxP3, CD14, CD15, CD11b
Panel 2	Dysfunctional T-cell	CD4, CD8, TCF1, TIM3, GzmB, PD1
Panel 3	Macrophage	CD68, CD163, CD86, MHC-II, PD-L1, PAX8

Table 35: Multiple IF panels designed for this project
Panel 1 – T-cell/myeloid markers; Panel 2 – Dysfunction T-cell markers; Panel 3 – Macrophage markers

4.15 Slide images

Below are representative H&E samples (figure 31) from patient TG019, with a paired biopsy and ICS sample and representative stained composite images from patient TG023 (figure 32).

Figure 31: Representative H&E slides

A) Biopsy **B) ICS specimen**

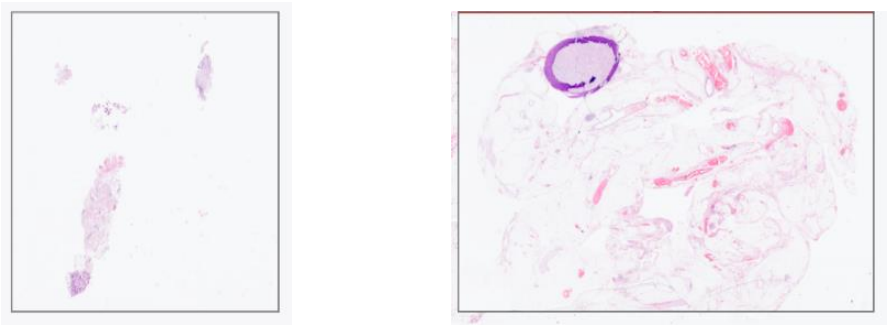
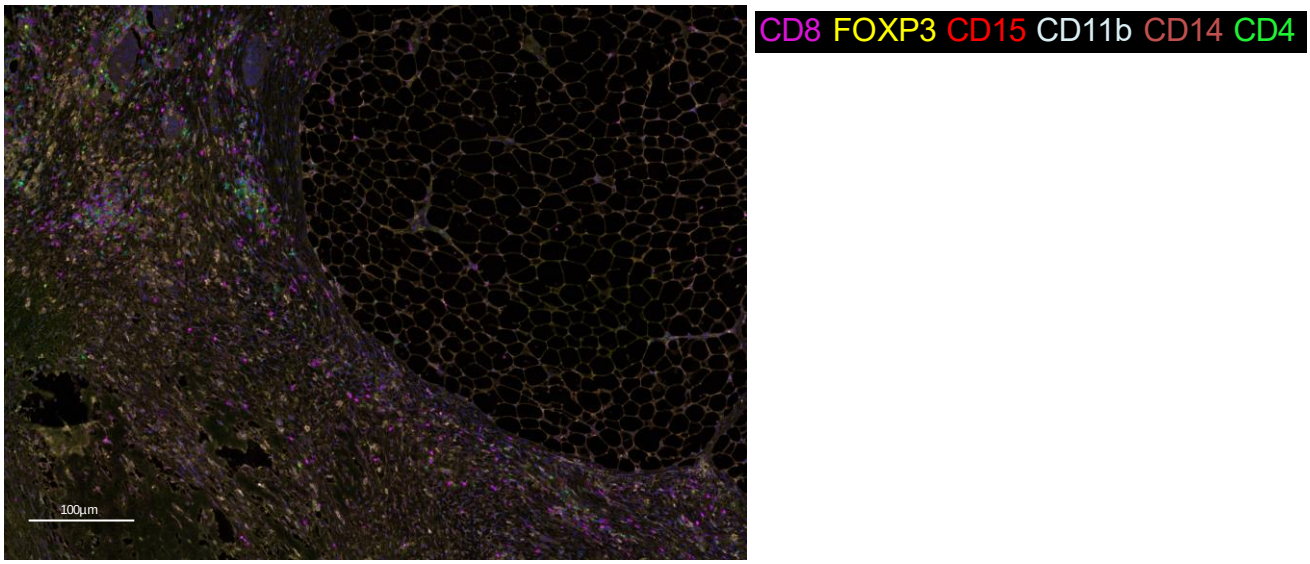


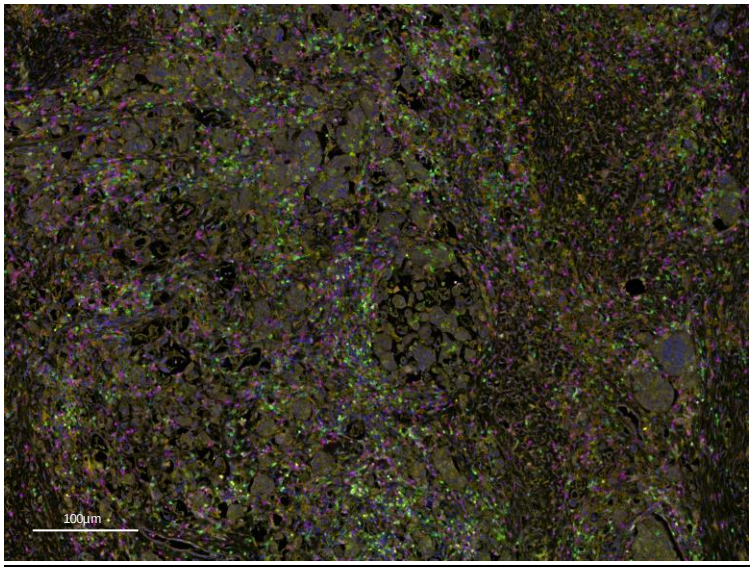
Figure 31: H&E slides of A) biopsy and B) ICS specimen from patient TG019 with region of interest circled (top left) identifying residual tumour cells

Figure 32: Representative stained composite images of each mIF panel

A) Panel 1 - T-myeloid

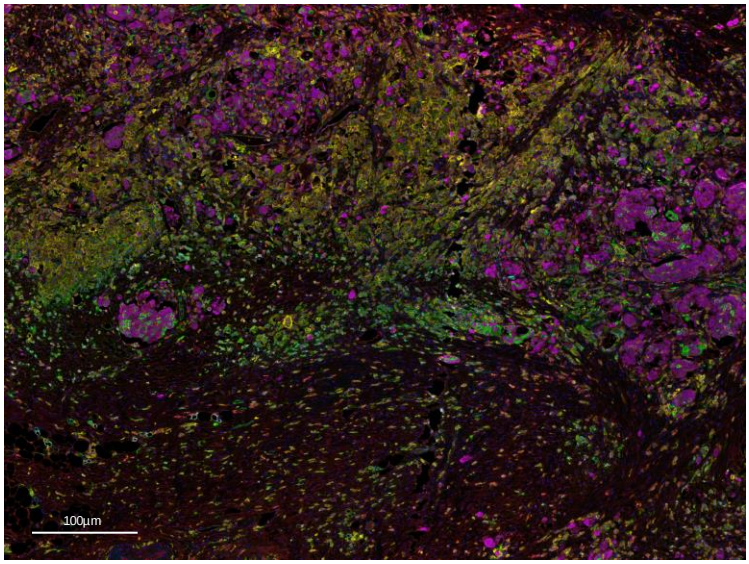


B) Panel 2 - Dysfunctional



CD8 CD4 Gzmb TCF1 PD1 TIM3

C) Panel 3 - Macrophage



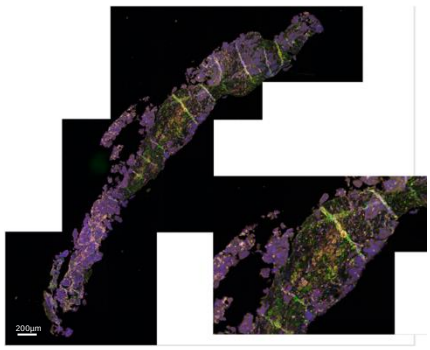
PAX8 CD68 PDL1 CD163 CD86 MHCII

Figure 32: Examples of staining for each panel identifying colours for each marker.
A) T-myeloid panel; B) dysfunctional panel; C) macrophage panel

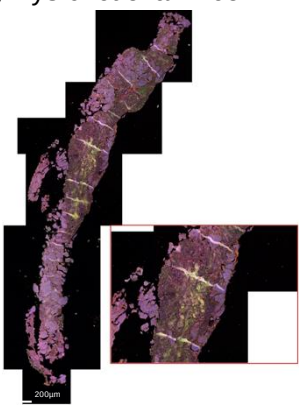
Figure 33: Stained whole slide image of biopsy and resection specimen for patient TG023

33.1 Biopsy

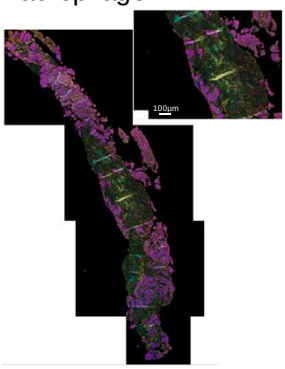
A) T-myeloid



B) Dysfunctional T-cell

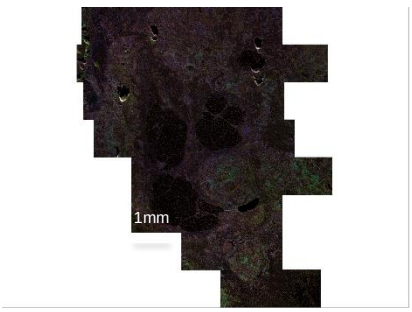


C) Macrophage

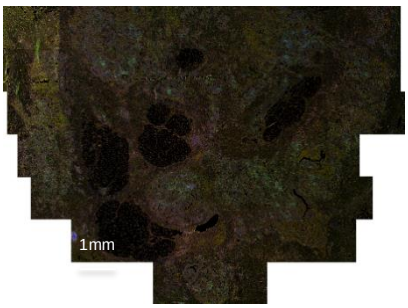


33.2 ICS specimen

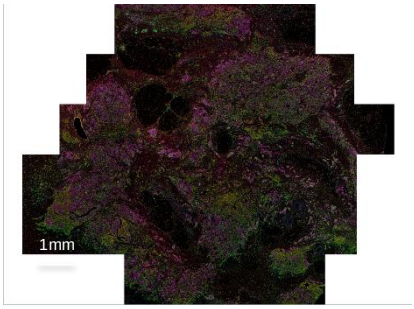
D) T-myeloid

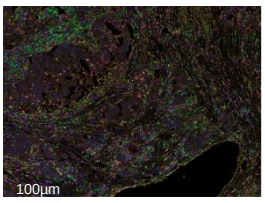


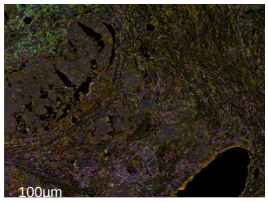
E) Dysfunctional T-cell



F) Macrophage







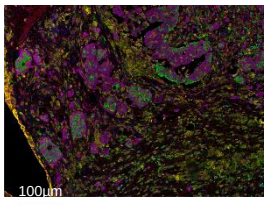


Figure 33: Examples of staining for each panel of paired samples for patient TG023.

33.1: Biopsy specimen stained with 3 panel. Whole slide image (WSI), with zoomed in image of a specific region.
33.2: Resection specimen after ICS with WSI above and zoomed in image of a specific region below.

4.16 Cell abundance, density and distance to tumour cells

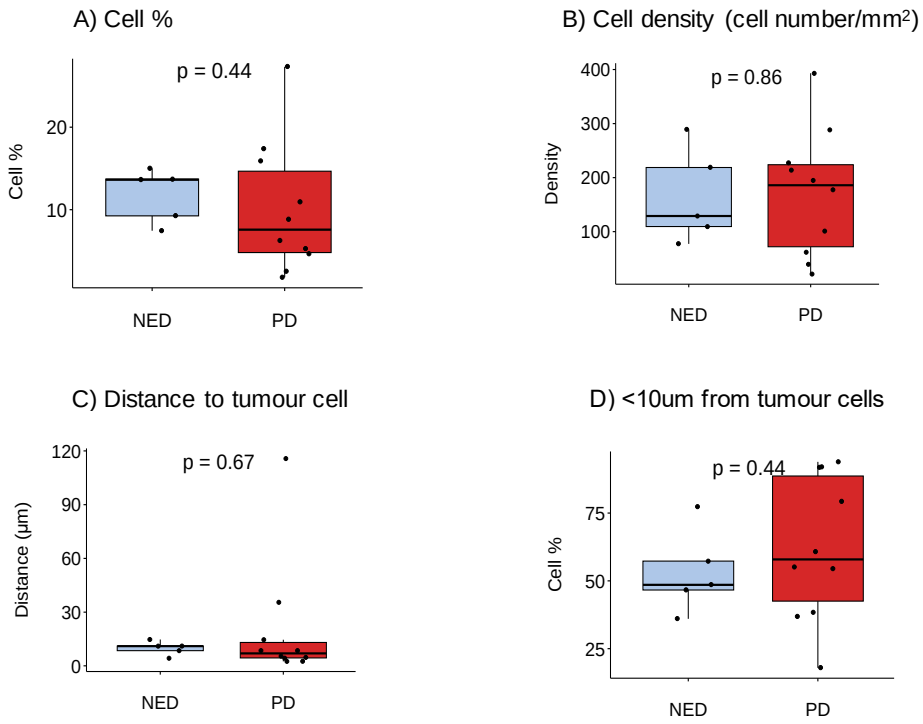
The mIF analysis for this project was performed to assess the ovarian cancer immune microenvironment at diagnosis. The ultimate aim was to explore the number and distribution of immune cells and whether they have any prognostic implications. This analysis was further split into reviewing T-cells and expression of dysfunctional markers, macrophages and myeloid cells. Cell abundance, density and distance to tumour cells were also explored.

4.16.1 T cell analysis

CD8 TILs are associated with a good prognosis (101). CD4 T-cells are T-helper cells that support CD8 T-cell cytotoxicity (347) within the TME. The distribution of immune cells and their proximity to tumour cells may also be important in helping to establish whether these cells are non-functional suppressed inflammatory cells or functionally active (348). Distance from tumour cells was also measured, with box plots illustrating those that are further away, and those that are ≤10μm from tumour cells (figure 34 C, D, G, H).

Figure 34: CD4 and CD8 T-cells number, density and distance to tumour cells

34.1) CD4+ T-cell



34.2) CD8+ T- cells

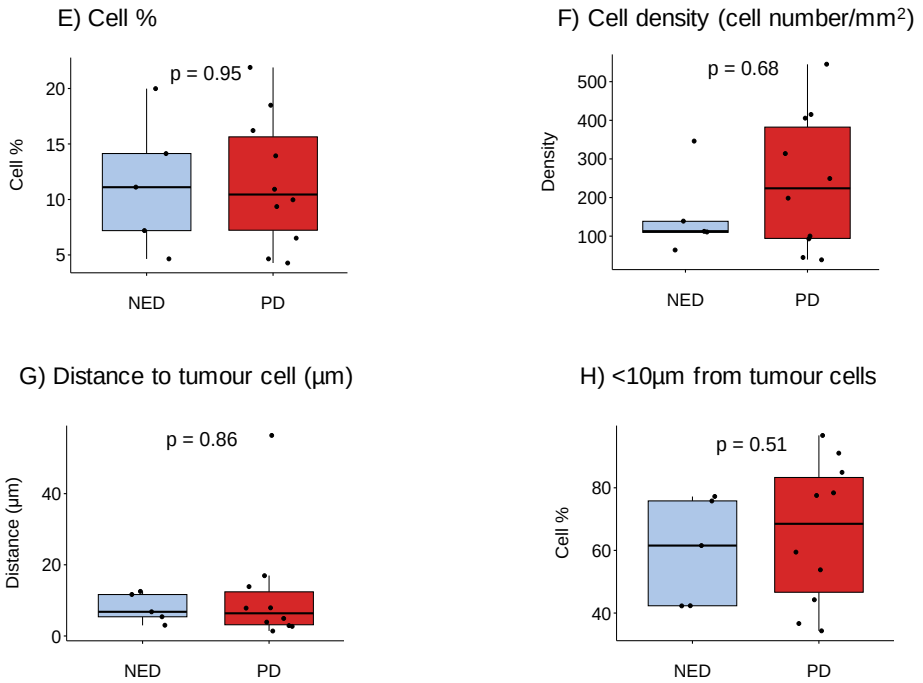


Figure 34: 34.1) CD4 T-cells; 34.2) CD8+ T-cells within the TME
A&E) Proportion of all classified cells across the 3 panels (%); B&F) Cell density (cell counts/mm2); C&G) Distance to tumour cells (µm); D&H) Proportion of cells <10µm from tumour cells
NED (blue) – no evidence of disease; PD (red) – disease progression

Figure 34 illustrates that the absolute number CD4+ and CD8+ T-cells within the TME are not different between the two patient groups. Naive T-cells can differentiate into different phenotypes depending on the cytokine environment within the TME (349). There was a trend, but not-statistically significant increase in density of CD4+ TCF1+ (naïve) T-cells, is seen in patients with a poor prognosis compared to those who are disease free (figure 35 A&B). Distance to tumour cells has a broader range in the progressed group, with a non-statistically significant reduction in the number of cells < 10µm from tumour cells (figures 35C & D).

Figure 35: CD4+TCF+ (naïve) T-cells

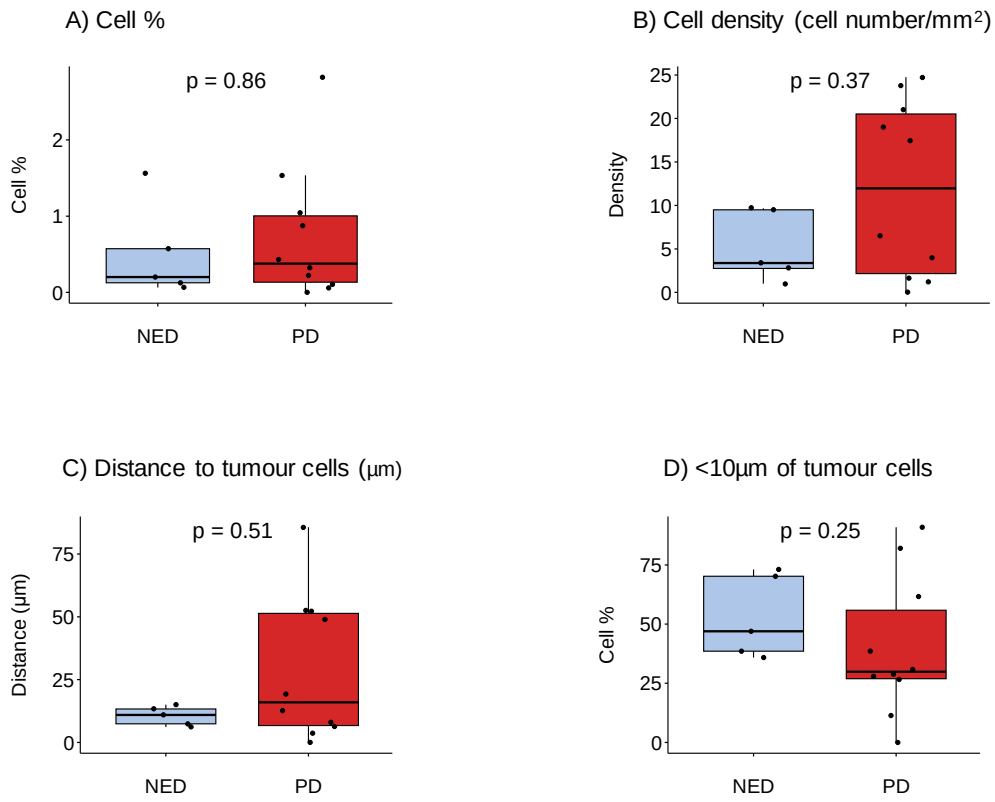


Figure 35: Naïve T-cells within the TME
A) Proportion of cells (%); B) Cell density (cell counts/mm2); C) Distance to tumour cells (µm) D) Cells <10µm from tumour cells
NED (blue) – no evidence of disease; PD (red) – disease progression

Tregs play an important role in immunosuppression in cancer. Data presented earlier have already demonstrated that circulating Tregs are increased in patients with ovarian cancer when compared to HD. Within the tumour samples, fewer Tregs were identified than CD8+ cells, with no difference in total number and density seen between samples from patients who had and had not progressed (figure 36).

Figure 36: Tregs

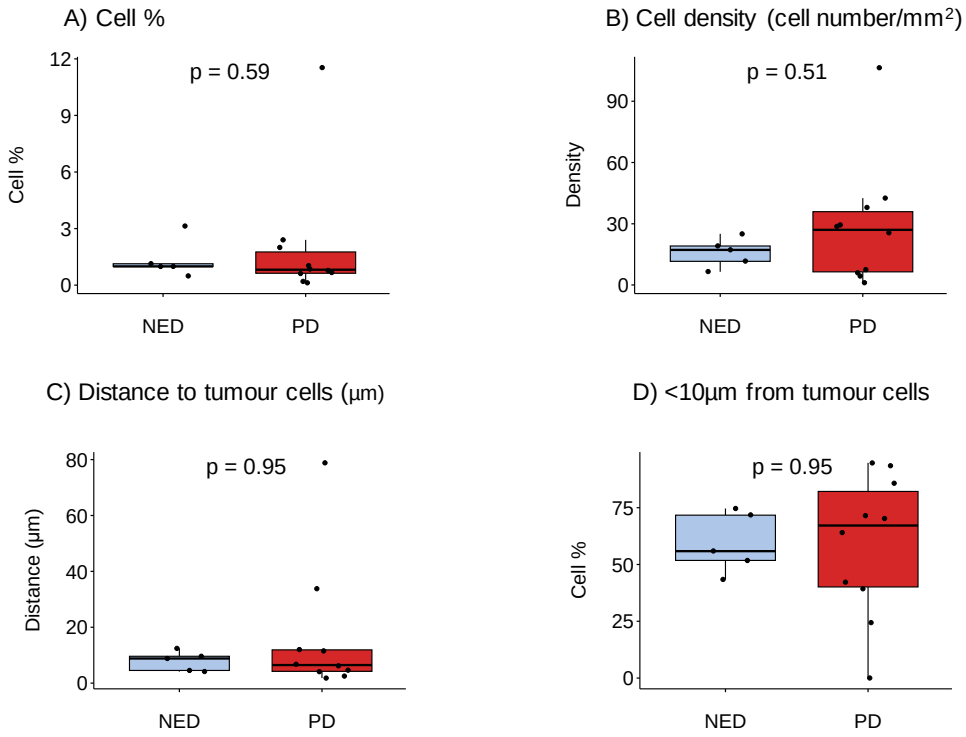
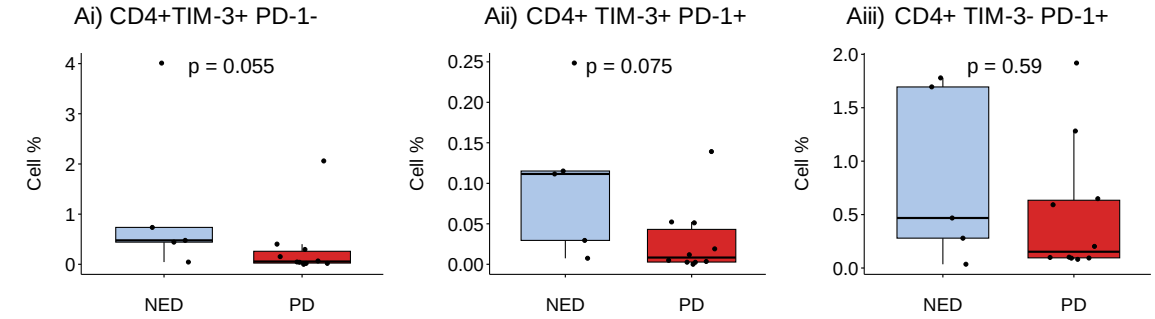


Figure 36: Tregs
A) Proportion of cells (%); B) Cell density (cell counts/mm2); C) Distance to tumour cells (µm);
D) Proportion of cells <10µm from tumour cells
NED (blue) – no evidence of disease; PD (red) – disease progression

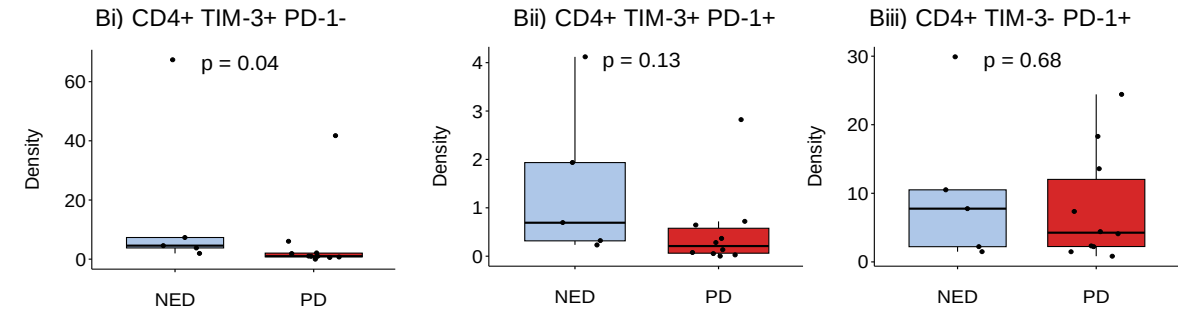
Panel 2 was designed to explore whether there was any change in dysfunctional T-cell numbers between the two groups. There is a non-statistically significant reduction in the percentage of exhausted/dysfunction CD4+ cells. These cells either express TIM3 and/or PD1. Change in density was less marked between the two groups. There was no difference in distance from tumour cells in either group (figure 37).

Figure 37: Numbers, density and distance to tumour cells of dysfunctional CD4+ T-cells

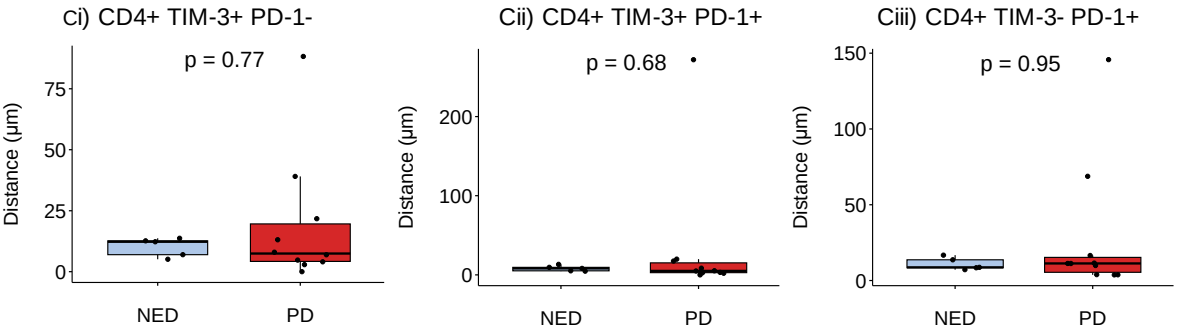
A) Total cell percentage (%)



B) Cell density (cell number/mm^2)



C) Distance to tumour cells (µm)



D) <10µm from tumour cells

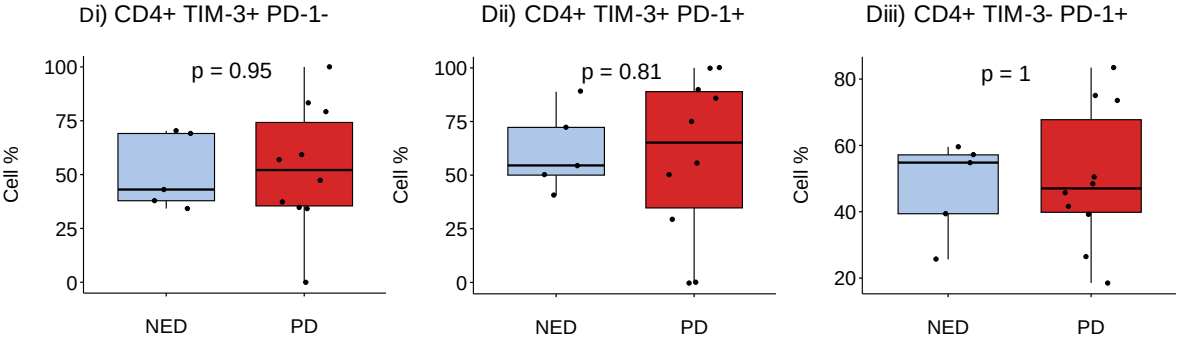


Figure 37: Dysfunctional CD4+ T-cells

Dysfunctional CD4+, defined by TIM-3 expression, PD-1 expression of co-expression of both

A) Proportion of cells (%); B) Cell density (cell counts/mm2); C) Distance to tumour cells (µm) D) Proportion of cells <10µm from tumour cells

NED (blue) – no evidence of disease; PD (red) – disease progression

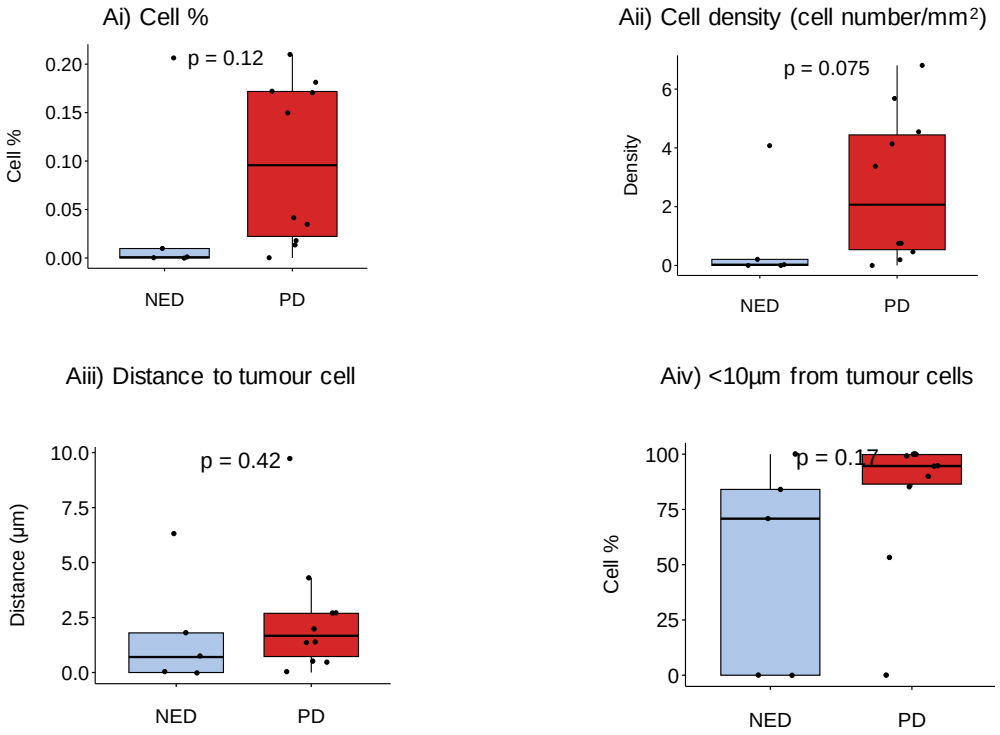
4.16.2 Tumour associated myeloid cells

Tumour associated myeloid cells include tumour associated macrophages (TAMs) and myeloid derived suppressor cells (MDSCs) and make up the largest proportion of the immune cells within the HGSC TME. They can be immunosuppressive and often associated with a poor prognosis (350).

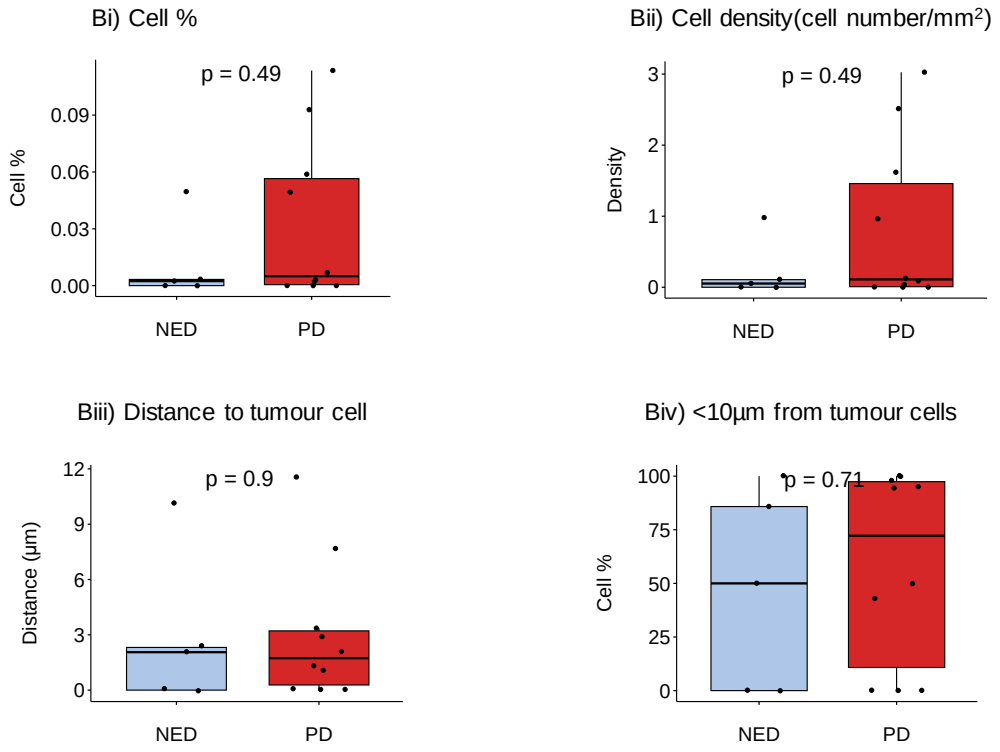
For this analysis, I have defined a macrophage as MHCII+ CD68+. 'M2-like' macrophages are defined by CD163 expression (114) and CD86 is expressed by 'M1-like' macrophages (351). There was an increase in number and density of macrophages in the poor prognostic group when compared to those who are disease free (figure 38), with an increase in the number of MHCII+ CD68+ CD163+ cells <10µm to the tumour (figure 38Aiv).

Figure 38: Tumour associated macrophages

A) MHCII+ CD68+ CD163+ CD86- cells



B) MHCII+ CD68+ CD86+ CD163+ cells



C) MHCII+ CD68+ CD86+ CD163- cells

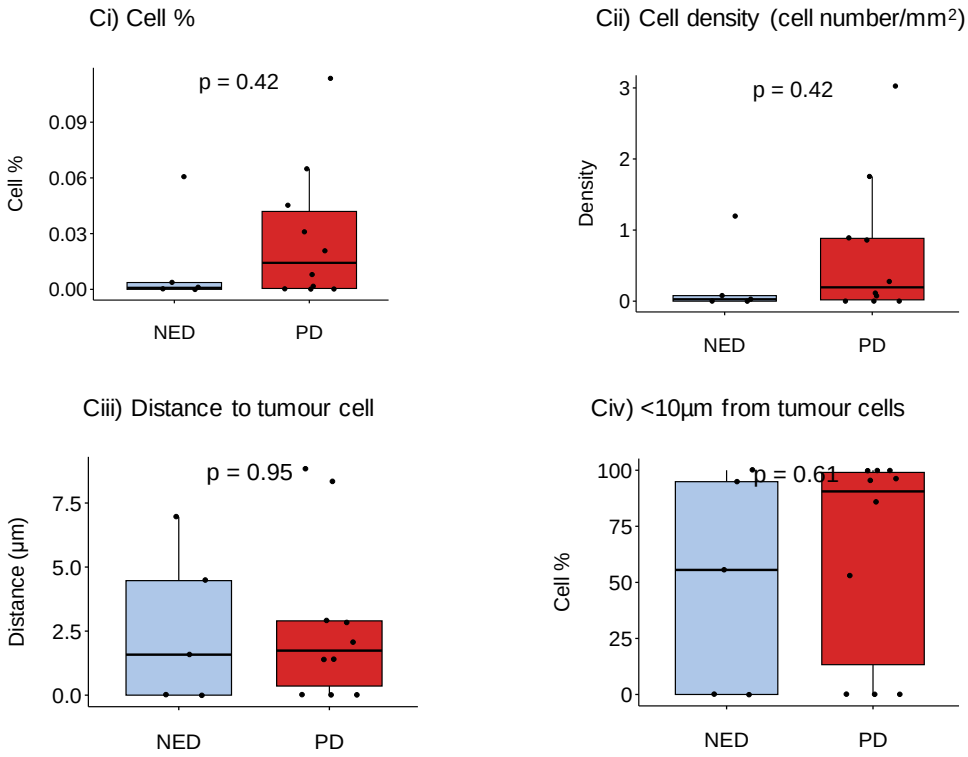


Figure 38: Tumour associated macrophages
A) MHCII+ CD68+ CD163+ CD86-cells; B) MHCII+ CD68+ CD86+ CD163+ cells;
C) MHCII+ CD68+ CD86+CD163- cells i) Cell number (%), ii) Cell density (cell number/mm², iii) Distance to tumour cell (µm), iv) Proportion of cells <10µm from tumour cells.
NED (blue) – no evidence of disease; PD (red) – disease progression

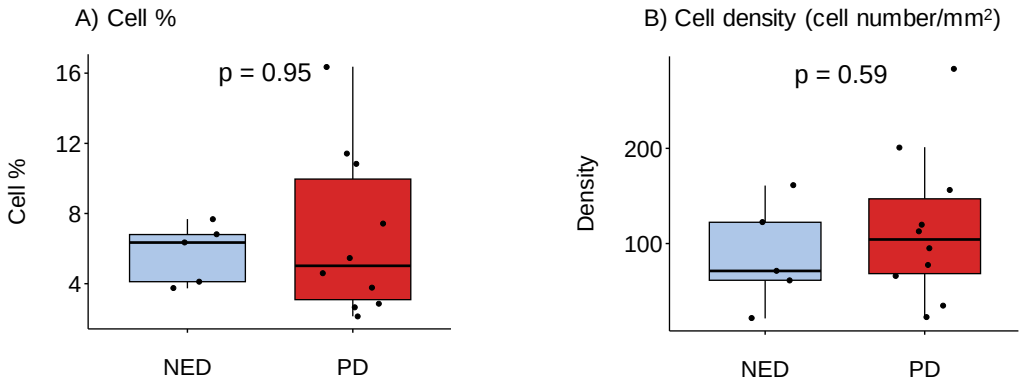
The next marker of interest was PD-L1, although there is no clear consensus whether the expression of PD-L1 on tumour or immune cells is predictive of response to ICPIs (352). Here, very few immune and tumour cells expressed PD-L1, and these data are not shown.

4.16.3 Myeloid derived suppressor cells

Myeloid derived suppressor cells (MDSCs) are divided into 2 main subtypes; monocytic (M-MDSCs) and polymorphnuclear (PMN-MDSCs). M-MDSCs are defined by CD11b+ CD14+ HLA-DR-/lo CD15- and PMN-MDSCs by CD11b+ CD14- CD15+(350). There is no significant difference in the numbers or density of M-MDSCs (figure 39.1). There was a numerical but non-significant reduction in density of PMN-MDSCs (figure 39.2D). Distance to tumour cells was the same between the 2 groups (data not shown).

Figure 39: MDSCs

39.1 CD11b+ CD14+ CD15- (M-MDSCs)



39.2 CD11b+ CD14- CD15+ (PMN-MDSCs)

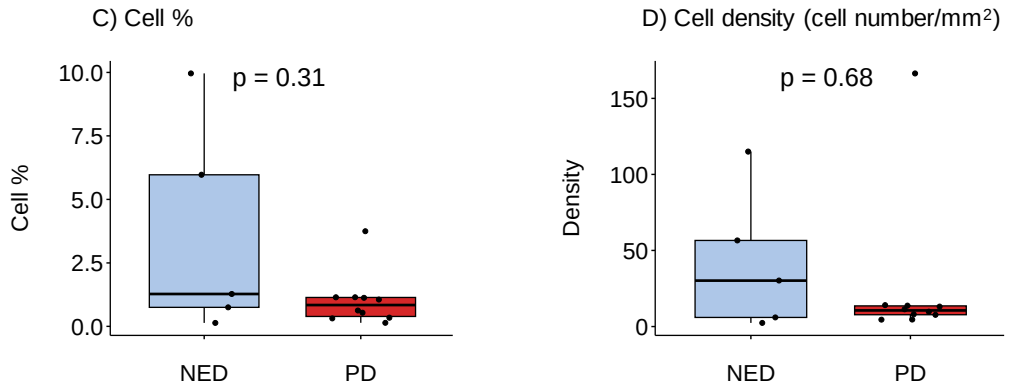


Figure 39: Number and density of MDSCs with the TME
39.1 M-MDSCs A) Number (%), B) Density (cell count/mm^2)
39.2 P-MDSCs C) Number (%), D) Density (cell count/mm^2)
NED (blue) – no evidence of disease; PD (red) – disease progression

4.17 Paired samples

4 patients (TG019, TG023, TG028 and TG044) had matching biopsy and resection samples that passed QC. Their clinical histories are summarised below (table 35). All patients had NACT, with interval cytoreduction to NRD. 3/4 patients had progressed at the data cut off whilst the final patient was lost to follow up due to referral for ongoing management to another hospital. All patients had a biochemical response (defined as >50% reduction in Ca125) to chemotherapy. However, the Ca125 fell to within the normal range in 1 patient (TG044). Unfortunately, this patient was lost to follow up, with no PFS data available. The chemotherapy response score (CRS) on the omentum (range 1-3, with 1 being no or minimal response and 3 defined as complete or near-complete response to chemotherapy) is used to evaluate pathologic response to NACT and correlates with PFS (353).

Table 36: Patient characteristics with paired samples available for analysis

Patient	Stage	Germline genetics	Baseline Ca125	Ca125 prior to ICS	CRS	PFS (mths)	OS (mths)
TG019	IVB	BRCA1	1303	79	2	44.0	-
TG023	IVB	WT	4728	199	1	11.0	27.8
TG028	IIIC	WT	3075	55	2	24.2	-
TG044	IVB	BRCA2	374	18	U/K	U/K	U/K

Table 36: Paired samples available for analysis
BRCA – breast and ovarian cancer susceptibility genes; CRS – chemotherapy response score; PFS – progression free survival (months); OS – overall survival (months); U/K – unknown; ICS – interval cytoreductive surgery; Ca125 – tumour marker elevated in ovarian cancer (ref range 0-35 kIU/L) and useful in determining biochemical response to treatment if elevated at diagnosis.

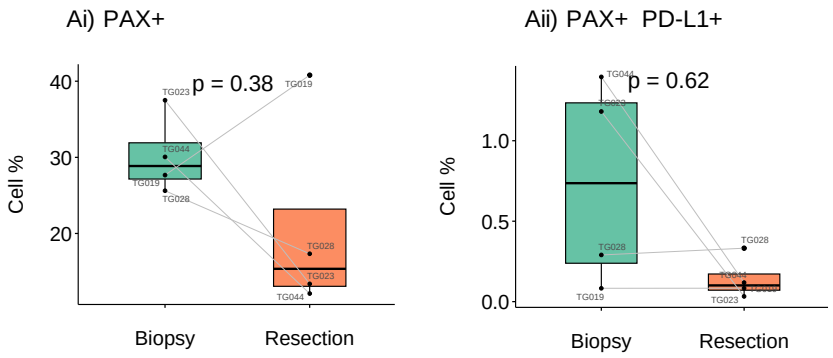
Analysis of paired samples provides the opportunity to assess changes in the TME at diagnosis and following treatment with chemotherapy. As shown, TG023 had a low CRS (1), with a PFS of 11 months and OS of 27.8 months.

4.17.1 Tumour cells

As expected, the number and density of tumour cells, identified as PAX8+ cells, reduced in number and density following chemotherapy in 3 patients, but were increased in TG019. PD-L1+ tumour cells also showed a non-statistically significant reduction in number and density following chemotherapy (figure 40).

Figure 40: Tumour cells

A) Cell %



B) Cell density (cell counts/mm²)

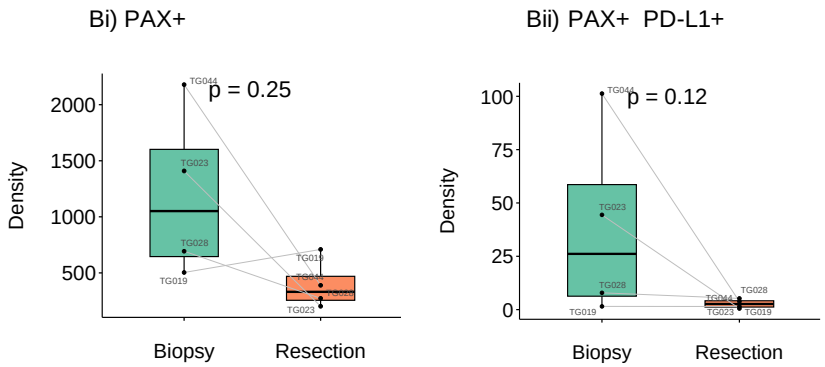


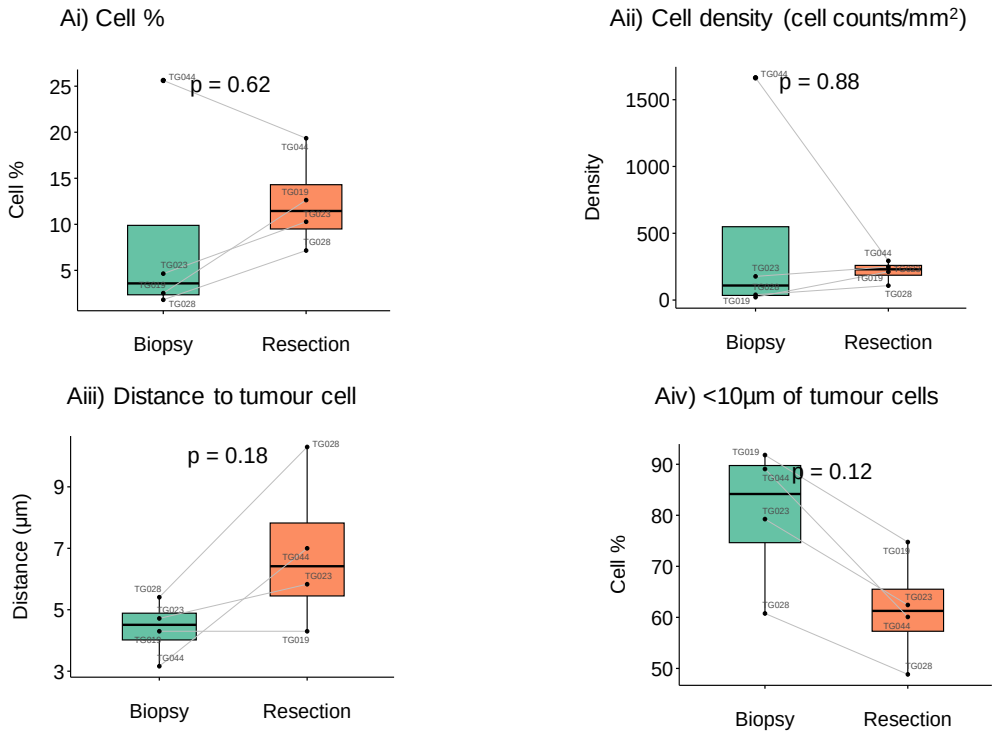
Figure 40: Number and abundance of tumour cells pre- and post-chemotherapy
A) Total cell percentage and B) Density of cells i) PAX+ cells and ii) PAX8+ PD-L1+ cells
Green – original biopsy specimen; orange – resection specimen

4.17.2 T-cell analysis

Following chemotherapy, there was minimal change in the number and density of CD4+ and CD8+ T-cells. Distance to tumour cells is also shown. There was a trend towards immune cells being further from the tumour cells, with fewer cells in close proximity, which is in keeping with a reduced number of tumour cells overall (figure 41).

Figure 41: T-cells

A) CD4+ T-cells



B) CD8+ T-cells

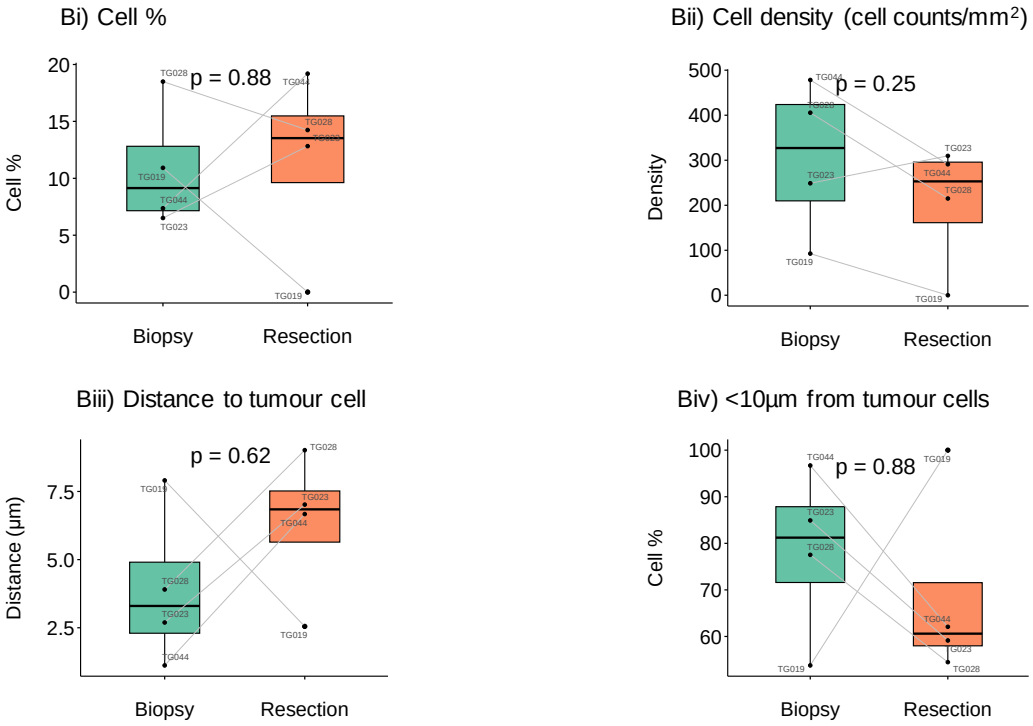


Figure 41: CD4+ and CD8+ TILS - Number, abundance, density and distance to tumour cells pre- and post-chemotherapy

A) CD4+ T-cells; B) CD8+ T-cells

i) Total cell percentage and ii) Density of cells iii) Distance to tumour cells (µm) iv) Proportion of cells <10 µm from tumour cells

Green – original biopsy specimen; orange – resection specimen

The following analysis demonstrated a trend towards an increase in number and density of cytotoxic CD8+ T-cells post-chemotherapy, with an increased distance between these cells and tumour cells in the resection specimens (figure 42).

Figure 42: CD8+ GzmB+ T-cells

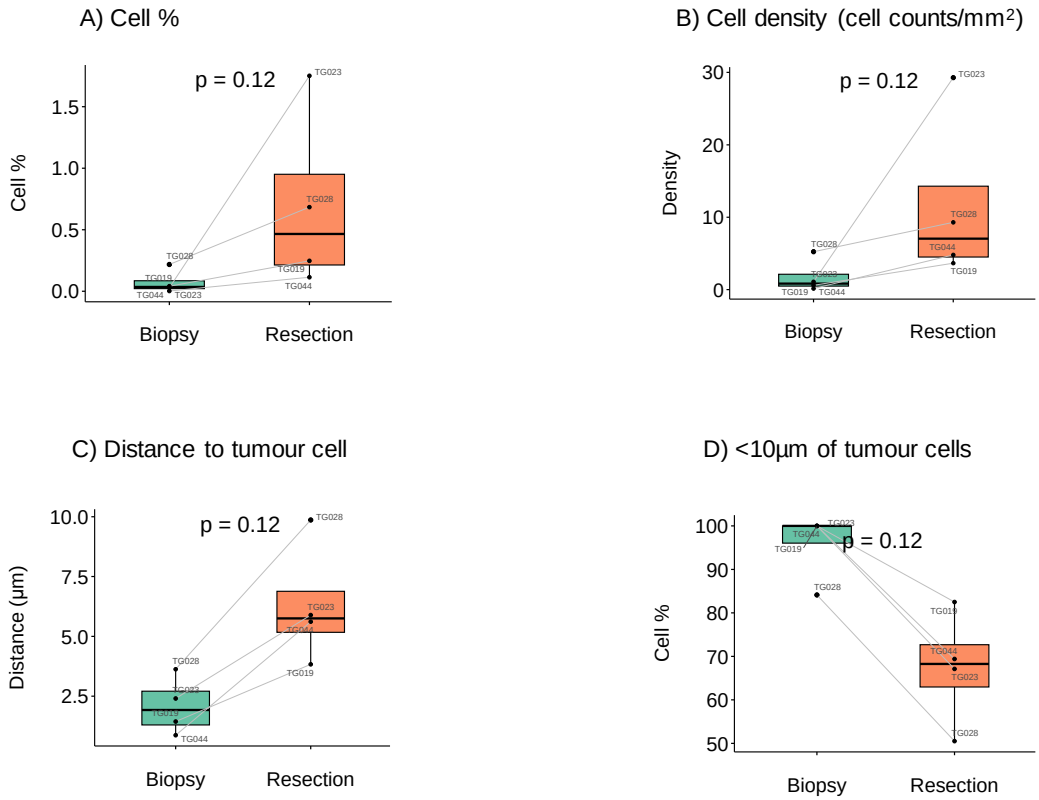


Figure 42: CD8+ GzmB+ TILS - Number, abundance, density and distance to tumour cells pre- and post-chemotherapy
A) Total cell percentage; B) Density of cells; C) Distance to tumour cells (µm); D) Proportion of cells <10 µm from tumour cells
Green – original biopsy specimen; orange – resection specimen

4.17.3 Tregs

Here, Tregs were relatively unchanged within the TME. There appeared to be an increase in Tregs in TG044, whilst the rest of the patients had similar numbers in both specimens (figure 43 A-B). The distance between the tumour cells and Tregs appears to fall in patient TG019 and increase in the other 3 patients (figure 43C).

Figure 43: Treg abundance, density and distance to tumour cells

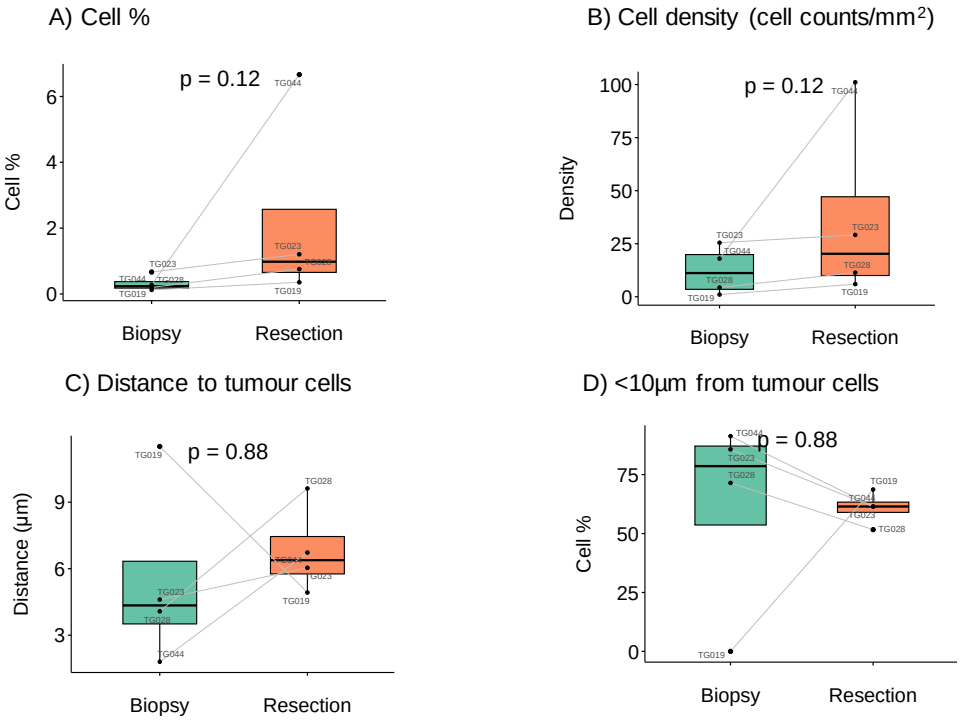


Figure 43: Tregs - Number, abundance, density and distance to tumour cells pre- and post-chemotherapy

A) Total cell percentage; B) Density of cells (cell counts/mm^2); C) Distance to tumour cells (µm); D) Proportion of cells <10 µm from tumour cells.

Green – original biopsy specimen; orange – resection specimen

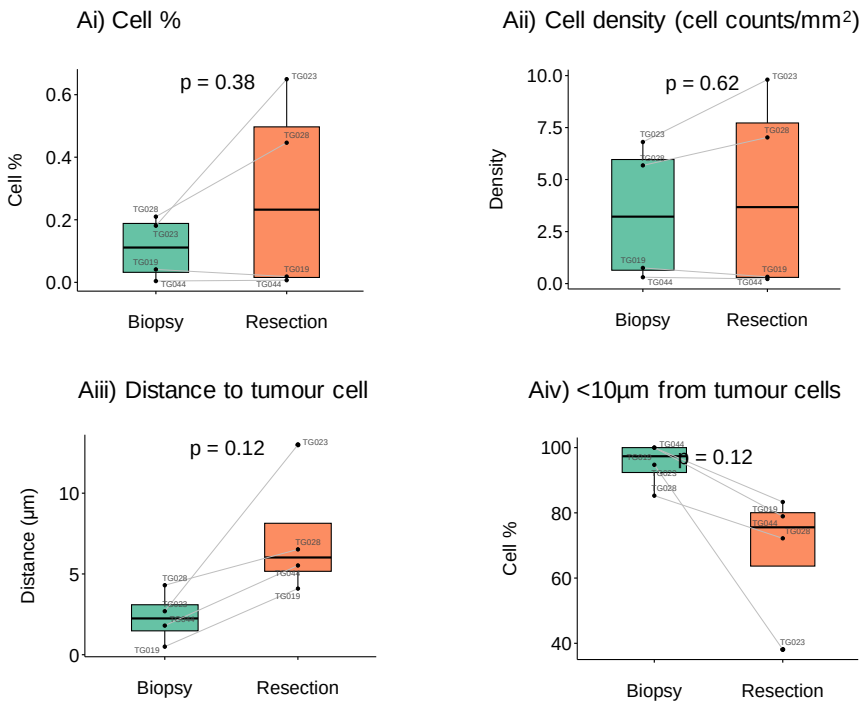
There was no significant change in dysfunctional / exhausted CD4+ TILs following NACT. These data are not shown.

4.17.4 Myeloid analysis

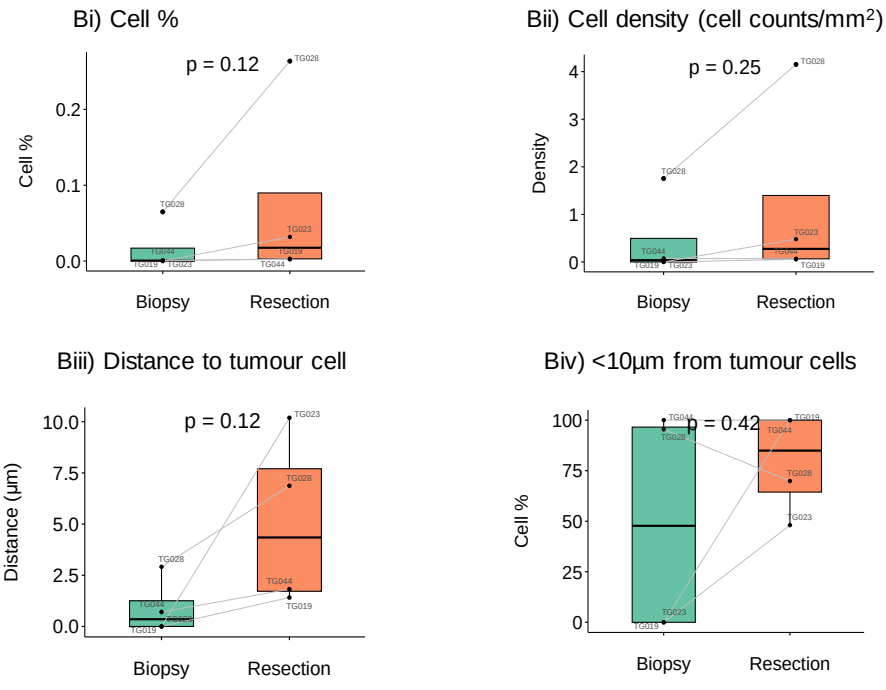
Macrophages, defined as cells with expression of MHCII and CD68, and then subdivided into CD163+, CD86+ and cells that co-express CD163 and CD86, are relatively unchanged following NACT in the 4 paired samples available (figure 44).

Figure 44: Changes in macrophages following NACT

A) MHCII+ CD68+ CD163+ CD86-



B) MHCII+ CD68+ CD86+ CD163-



C) MHCII+ CD68+ CD86+ CD163+

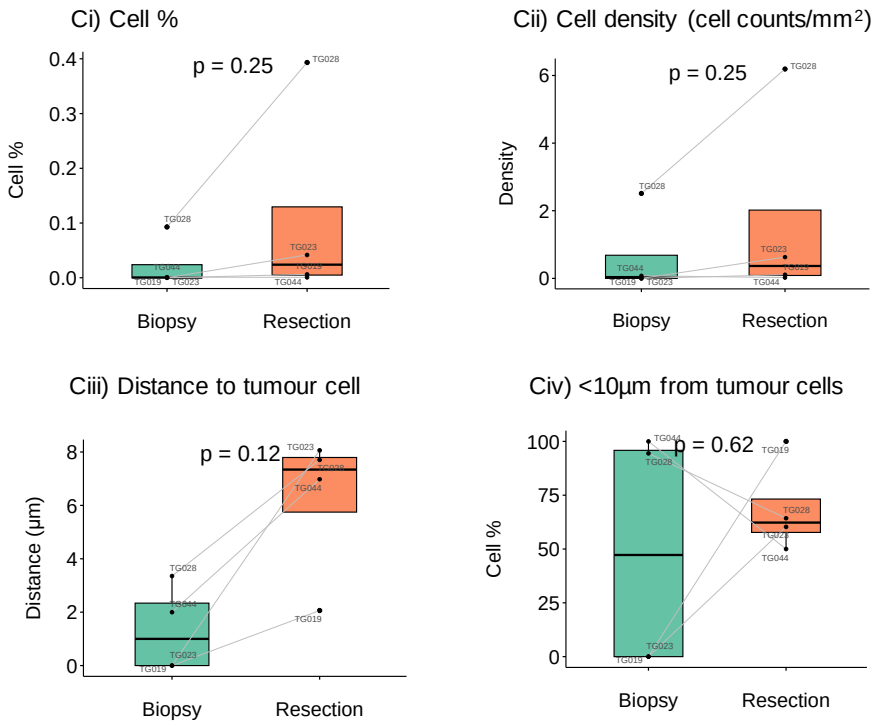


Figure 44: Macrophages - Number, abundance, density and distance to tumour cells pre- and post-chemotherapy
A) MHCII+ CD68+ CD163+ CD86-; B) MHCII+ CD86+ CD68+ CD163-;
C) MHCII+ CD68+ CD86+ CD163+
i) Total cell percentage; ii) Density of cells; iii) Distance to tumour cells (µm); iv) Proportion of cells <10 µm from tumour cells
Green – original biopsy specimen; orange – resection specimen

There was no significant change in number and density of MDSCs post-NACT in the 4 paired samples (figure 45). Distance to tumour cells is not shown.

Figure 45: MDSCs

Figure 45.1: CD11b+ CD14+ CD15- (M-MDSCs)

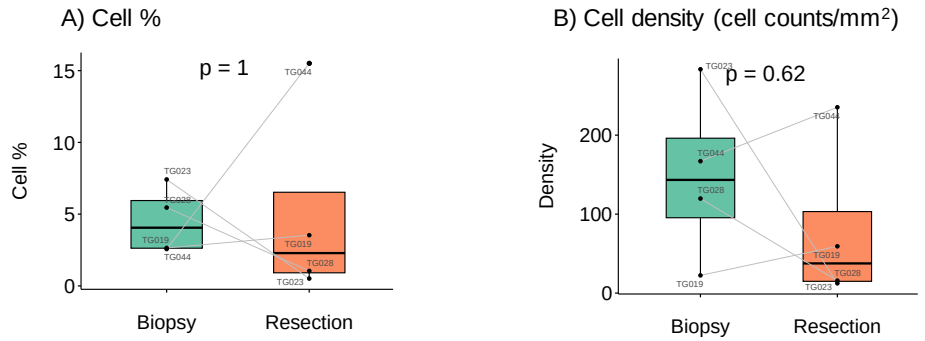


Figure 45.2: CD11b+ CD14- CD15+ (PMN-MDSCs)

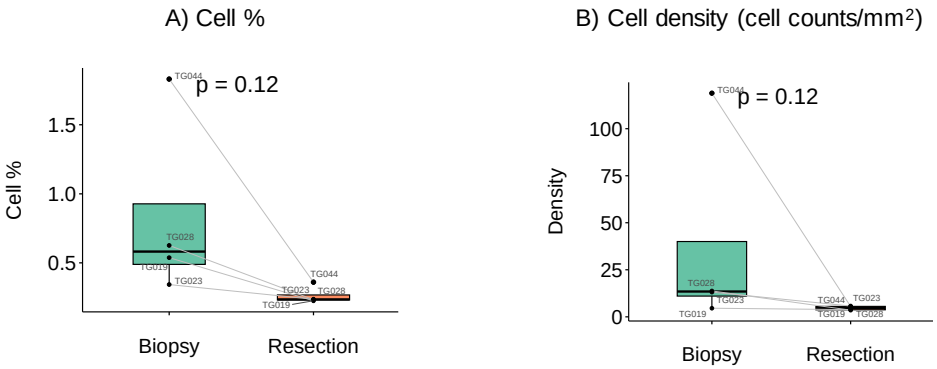


Figure 45: MDSCs – Number and density pre- and post-chemotherapy
45.1: Monocytic (M)-MDSCs (CD11b+ CD14+); 45.1 Polymorphonuclear (PMN)-MDSCs (CD11b+ CD15+)
A) Cell number (%) B) Cell density (cell counts/mm²)
Green – original biopsy specimen; orange – resection specimen

4.18 Discussion

Despite numerous clinical trials exploring the use of immunotherapy in ovarian cancer, the ovarian immune microenvironment is still poorly understood. In order to explore the tumour microenvironment for this project, FFPE blocks were chosen. This was due to the fact that they are readily available, and no additional interventional procedures were required.

In an attempt to explore whether any changes have prognostic implications, analysis was performed by looking at patients who were disease free compared to those who had progressed at time of data cutoff. The first patient was recruited in October 2020 and the final patient in June 2021. Data cutoff for the mIF analysis was 24th March 2024, providing almost 3 years for clinical follow up. In view of the small number of samples available for analysis after QC checks, it was not possible to break this analysis down further e.g. disease-free vs alive with recurrent disease vs progressed and dead.

This dataset shows that there is no significant change in numbers of CD4+ and CD8+ TILs, regardless of prognosis. This is most likely due to the small number of samples, as it is well documented that CD8+ TILs within the ovarian TME are associated with a good prognosis (101). Interpretation of the paired analysis needs to be done with caution due to the very small numbers (n=4). However, there were also no significant difference in CD4+ and CD8+ TILs following NACT. There are reports that NACT in ovarian cancer increases the density of CD8+ T-cells, CD20+ B-cells and CD68+ PD-L1+ macrophages in ovarian cancer and higher infiltrates of these cells are associated with improved survival (128).

Tregs are immunosuppressive cells, and these data show them to be increased in the circulation in patients with ovarian cancer when compared to HD. However, in the mIF analysis of tumour, very few Tregs were seen, with no statistically significant difference between those who are disease free and those who have progressed.

The main changes identified in this patient cohort is the difference between CD4+ TIM-3+ +/- PD-1+ T-cells, which appear to be reduced in patients who had progressed compared to those who remain disease free. Some of these cells could represent Tregs, however, FoxP3 was only used in panel 1 and therefore co-expression of these

markers was not easily identified. Although the slides used for staining were sequential, the aligned images were not high resolution, making it more difficult to determine which of these cells co-expressed FoxP3. There are very few CD8+ TIM3+ T-cells identified in these samples, with no significant change in abundance or number in patients with NED compared to those with PD (data not shown).

Similar to PD-1, TIM-3 is an inhibitory immune checkpoint and contributes to T-cell exhaustion (354). TIM-3 upregulation is commonly seen on immune cells in solid tumours, including ovarian cancer. High rates of CD8+ TIM-3+ PD-1+ TILS are prognostic and found in the TME of advanced disease and are associated with a high risk of relapse (355,356). TIM-3 is often co-expressed with PD-1, causing a reduction in T-cell proliferation and cytokine production. CD8+ TIM-3+ PD-1+ cells are immunosuppressive, resulting in tumour immune escape (357) High levels of CD4+ TIM-3+ T-cells have been reported within the TME of solid tumours and, due to co-expression of FoxP3, are thought to represent Tregs within the TME (358). Whilst not definitive, findings in this analysis suggest that a proportion of these CD4+ TIM-3+ cells are Tregs, although as demonstrated above, CD4+ FoxP3+ cells (Tregs) were similar in both groups of patients. As they are immunosuppressive cells, it is difficult to explain why they are reduced at the time of diagnosis in the patients with progressive disease, as they tend to be associated with a poorer prognosis. In this study, increased levels of PD-1 and TIM-3 expression were seen on circulating Tregs when compared to HD.

The therapeutic target for cancer immunotherapies focuses on CD8+ cytotoxic lymphocytes. However, whilst not clearly defined, the role of CD4+ T-cells is important in maintaining anti-tumour immunity (347). Here, there seem to be fewer naïve CD4+ T-cells (CD4+ TCF1+) in close proximity to tumour cells in the poor prognostic group. Cytokines within the TME will determine what these naïve CD4+ T-cells can differentiate into (359). As the samples collected for this project were biopsies taken at diagnosis, this is a snapshot of the TME at the time of diagnosis in one anatomical location. These cells could differentiate into various subpopulations of CD4+ T-cells, all with different functions, some of which could be immunosuppressive and contributing to worse outcomes.

Another pertinent finding in this dataset is the increase in macrophages in patients with progressive disease. These cells express MHCII, CD163 and CD68, with another group of cells co-expressing MHCII, CD86, CD163 and CD68.

Tumour associated macrophages (TAMs) are the most common immune cells in the HGSC TME and are a vital component of the TME. Circulating tumour-secreting factors recruit monocytes from the peripheral circulation into the TME, which are then transformed (360,361). Tissue-resident macrophages seeded during embryogenesis are also found within the cancer TME (362). Macrophages are a heterogenous group of cells and different stimuli trigger their development into differing phenotypes and have both pro-tumoural and anti-tumoural activity(360). They can be broadly defined as 2 distinct phenotypes; M1-like macrophages, are pro-inflammatory, with anti-tumour properties and M2-like macrophages express anti-inflammatory cytokines e.g. IL10 and TGF-b and promote tumour growth. M1 macrophages are identified by the expression of CD86 and CD80, whereas M2 macrophage markers include CD163 and CD206 (363). However, macrophages are more complex than this and it is important to remember that the definition and function of TAMs goes far beyond the M1 and M2 phenotype (364). CD68 is expressed on a variety of myeloid cells (365). The macrophages identified in the TME of these patients express CD68 and CD163 and are increased in patients with PD compared to those who are disease free. Whilst it is difficult to know definitively the exact phenotype of the macrophages seen in these samples, although they could be in keeping with an M2-like phenotype. Overexpression of CD68 and increased densities of TAMs have been reported to be prognostic and associated with worse clinical outcomes (365). Interestingly, the flow cytometry analysis illustrates a reduction in circulating monocytes, particularly in patients with a poorer prognosis e.g. patients in Cohort 1a, NACT inoperable. One explanation for this could be that these monocytes are recruited to the TME – resulting in a more immunosuppressive microenvironment and a poorer prognosis. Unfortunately, the biopsy samples for the patients in this particular cohort were unavailable or did not pass the QC check and therefore analysis of their biopsy samples was not possible. Additionally, where possible, future work should include biopsies at progression to enable paired analyses and identification of changes following treatment.

MDSCs are immature progenitors to myeloid cells and are activated by a variety of factors expressed in the ovarian cancer TME. They also produce a large number of pro-inflammatory mediators and increased numbers of MDSCs in ovarian cancer are associated with advanced, high-grade disease (366). The data above do not show a significant change in MDSCs, regardless of outcomes.

4.19 Conclusion

I have demonstrated that there are definite changes within the immune microenvironment of ovarian cancer, with reduced numbers of dysfunctional/exhausted CD4+ T-cells and increased numbers of macrophages in patients with a poorer prognosis. How these changes impact response to immunotherapy remains unclear.

The most successful immunotherapy agents in solid tumours are the anti-PD1 and PD-L1 antibodies, which target T-cells. They have changed the treatment paradigm and survival of numerous solid tumours. However, despite multiple clinical trials investigating the use of these agents in advanced EOC, there is currently no role for single agent ICPIs in the standard treatment of EOC. PD-L1 expression has been identified as a predictive marker of response to ICPIs in some tumours, for example NSCLC and cervical carcinoma, but it is not routinely tested for in all tumour groups (367,368). Increased PD-L1 expression has been reported in ovarian cancer cells, and its expression on tumour cells influences progression and development of peritoneal disease (369,370). This may be associated with worse outcomes and could also be a prognostic marker in ovarian cancer (219).

There are limited data published on the role of PD-L1 expression in EOC. The Keynote-100 study reported that a PD-L1 combined positive score (CPS) ≥10 and T-cell-inflamed 18-gene expression profile (T-cell-GEP) score were both associated with response to pembrolizumab (292). In the IMAGYN050 trial, less than 25% of patients had high numbers of (defined as >5%) PDL1-positive immune cells (265). Here, very little PD-L1 expression was seen. This may be due to small sample size but could also reflect the tumour heterogeneity of EOC. Additionally, in the 4 patients with paired samples, treatment with chemotherapy seems to have eliminated most PDL1+ tumour cells, suggesting that chemotherapy may have an impact on PD-L1 expression. In

NSCLC, one study reported that NACT increase PD-L1 expression on both immune and tumour cells resulting in a poorer response to chemotherapy and worse outcomes (371). However, another study in NSCLC demonstrated minimal change to PD-L1 expression post NACT, with no prognostic implication (372). There is also a question around whether decrease PD-L1 expression results in reduced efficacy of ICPIs (373).

In a study looking at omental biopsies and bloods from patients with HGSC taken pre- and post-chemotherapy, some T-cell subsets do change with neo-adjuvant treatment – specifically their functional orientation, activation status and density. In blood, levels of tumour-promoting cytokines were reduced following NACT (374). In order to establish the effect of NACT on the ovarian cancer TME, translational research on a larger number of patient samples is needed. It is imperative to look beyond PD-1 and PD-L1 in the ongoing pursuit of establishing whether immunotherapy has a role in the treatment of HGSC. Studies have shown that TAMS reduce the efficacy of and promote resistance to ICPIs (363), strengthening the argument to delve further into these cells to consider them as therapeutic targets.

Chapter 5: Final discussion

5.1 Conclusion

Treatment of HGSC remains challenging. Despite the successes of first line maintenance therapy with bevacizumab and/or PARP inhibitors in prolonging PFS (41,272,375,376), the majority of patients will ultimately progress and develop chemotherapy resistant disease. Over the years, there have been many clinical trials exploring different anti-cancer agents in relapsed ovarian cancer (377). However, treatments for platinum resistant disease are still limited and prognosis in this setting remains poor (378).

The initial aim for this MD (Res) project was to study the immune microenvironment of ovarian cancer, predominantly by analysing samples collected from patients recruited to the PROMPT trial to explore the effects of pembrolizumab on the ovarian cancer TME. I was the Clinical Fellow involved in writing the protocol and patient information sheet (PIS) for this study, supporting its set-up across 4 UK sites, processing of bloods and co-ordinating processing and storage of biopsy samples. Members of the Quezada laboratory at UCL were also involved in processing and storage of these samples. I am a member of the Trial Management Group (TMG) and presented the poster at the European Society of Gynaecological Oncology (ESGO) 2024 Congress in Barcelona (see appendix).

The rationale behind the PROMPT trial was to explore if maintenance immunotherapy improved PFS in patients with PROC, who have a very poor prognosis and where there is an urgent need to find better treatments. The survival data from PROMPT; PFS of 2.0 months and OS of 9.8 months, highlights the poor outcomes for these patients. This trial also confirms that maintenance treatment with a single agent anti-PD1 antibody (pembrolizumab) is not effective in enhancing the response to weekly paclitaxel, with 13/20 patients progressing after 3 cycles, and a disease response rate (DRR) of 5.0%.

This trial was faced with numerous challenges and recruitment was slower than anticipated. Reasons for this included the COVID-19 pandemic and the mandatory requirement for measurable and biopsiable disease. A number of patients had an excellent response to treatment, with no measurable or biopsiable disease and were

therefore ineligible. On the other extreme, many patients who were screened for the study had an initial response to weekly paclitaxel after 3 cycles but developed tumour progression prior to completing or at the end 6 cycles. Although recruitment picked up following the protocol amendment, which made the measurable disease and need for a biopsy optional, the total number of patients enrolled was 20, rather than the planned 28, as the trial closed early due to futility.

Another difficulty was obtaining samples for translational research. Baseline biopsies were infrequently performed following the amendment and many patients either declined or were too unwell for biopsies / blood samples at progression, which was prior to cycle 4 for a large proportion of patients. Another unfortunate incident was that some of stored samples were taken out of the HTA freezer in the lab, accidentally thawed overnight and had to be discarded.

Although the final numbers of samples available from this trial are small, the translational research is vital to further explore the effect of pembrolizumab on circulating immune cells and immune cells within the TME of ovarian cancer. These analyses should include identifying the types of cells, their proportions and their function, with additional focus on exploring immune cells beyond T-cells.

As a consequence of these challenges, translational research on these samples was not possible in the time frame required to complete this MD (Res) and the translational research shifted to investigate the immune microenvironment in patients with high grade EOC undergoing standard of care treatment. As described in detail in Chapter 4, Results, the next part of this project focused on exploring whether standard of care treatment with chemotherapy, surgery and maintenance PARPi, had any significant impact on circulating immune cells and whether the immune microenvironment in samples taken at first diagnosis exhibit any predictive biomarkers.

My data demonstrates definite changes seen within circulating immune cells when compared to HD and after treatment. However, more numbers are needed to determine the importance of these changes and whether peripheral blood reflects what is going in within the TME. My results show that the number of circulating Tregs are increased in peripheral blood, with a proportion of these cells expressing inhibitory checkpoints. This suggests that they may contribute to immunosuppression and pro-

tumour activity. Although the absolute number of Tregs does not change with systemic therapy, the function of these Tregs is unknown. Furthermore, TIM-3 and PD-1 expression is higher in diseased blood when compared to HD. Whilst Tregs are not abundant within the TME of these patient samples, there appears to be increased expression of PD-1 and TIM-3 on CD4+ TILs and the relevance of this is worth exploring further.

Another interesting finding is the reduction in circulating monocytes post systemic therapy. One could infer that these circulating monocytes have migrated into the TME, explaining the increased number of macrophages in patients with advanced disease. These monocytes develop into TAMs, which have definite prognostic implications. Analysis from these patient samples illustrates an increase in what appear to be 'M2-like' CD163+ macrophages in patients with a worse prognosis. A high density of CD163+ macrophages in the TME of EOC has been reported to be prognostic (379). TAMs are complex and play multiple different roles in the TME of cancer. They are associated with tumour progression, treatment resistance and worse clinical outcomes in a number of different solid tumours, such as breast, cervix, melanoma and NSCLC (360). They are versatile cells, and their functional phenotype and distribution are constantly changing in response to tissue- and tumour-specific stimuli within the TME. TAMS may play a role in ICPI resistance by modifying cytokines / chemokines involved in T-cell effector functions (380). Here, the data indicate that macrophages may contribute to tumour progression and poor prognosis.

In the tumour samples analysed here, there was minimal PD-L1 expression both on tumour cells and macrophages. Taken together, the increase in number and density of M2-like macrophages, minimal PDL1 expression and CD4+ TIM3+ PD1+ T-cells may contribute to the lack of efficacy of ICPI's in ovarian cancer, highlighting the need to look beyond ICPIs and T-cells.

TAMS may be an important therapeutic target in EOC and further research into their function should be considered. There are a variety of drugs targeting TAMs in development, which may lead to more effective immunotherapy agents becoming available for the treatment of solid tumours, including EOC (381).

Increase in TIM-3 expression on Tregs and on CD4+ T-cells within the TME may also be important and could represent another potential therapeutic target, although pre-clinical studies using TIM-3 antibodies either alone, or in combination with chemotherapy or anti- PD-L1 antibodies have not been effective (382,383). This is also reflected in an early phase study investigating Sabatolimab, an anti-TIM3 antibody, either alone or in combination with Spartalizumab, an anti-PD1 antibody, which reported no responses in the ovarian cancer cohort (384).

5.2 Limitations

In additional to the challenges with the PROMPT trial, there were a number of limitations to the translational component of my project, which I will summarise here.

A small number of patients were recruited from a single institution. Analysis of blood reveals clear differences in patient samples when compared to HDs, but no significant change following surgery or systemic therapy. If I were to do this again, I would have collected blood pre-operatively in the patients undergoing PCS in order to establish if upfront surgery impacts the type and levels of circulating immune cells, as well as taking bloods pre-operatively in the NACT patients who underwent ICS or DCS.

Fresh blood was processed into PBMCs, which were then frozen whilst the flow cytometry panels were being optimised. These samples were then analysed simultaneously once recruitment was complete. Freezing can affect the quality of the PMBCs and cause a loss of cells such as granulocytes and MDSCs. Analysis on fresh blood cells would have different limitations, including preventing simultaneous analysis of all samples and lack of consistency between experiments.

Despite collecting multiple tissue samples, many stained samples failed to pass the QC check and were therefore excluded from analysis. The small sample size makes it hard to draw any statistically valid conclusions. In future, at the time of biopsy, extra 16-guage cores should be taken specifically for research, which may improve the quality of tissue available for analysis. Repeat biopsies at the time of progression would also have allowed more robust analyses comparing the TME at diagnosis and if any significant changes occur at progression. Collection of ascites where possible would also provide additional tissue for analysis.

These results do not show any clear correlation between the findings in blood and the immune microenvironment in tumour, although this could be explained by the paucity of tumour samples. Another reason for this could be that circulating cells in peripheral blood do not provide a window into what is occurring within the tumour. Alternatively, we may need to look beyond immune cells to identify any potential biomarkers in blood or tumour.

In an attempt to establish whether there are any prognostic immune markers within the TME, the patients were split into patients who were disease free and those who had progressed at the time of data cutoff. Due to the small numbers, it was not feasible to split these patients into smaller groups, with differing prognostic factors, for example disease free interval. Additionally, within the available tissue samples, some were treatment naïve e.g. biopsy / primary surgery specimens, and some were post interval / delayed cytoreduction following NACT.

Additionally, the ovarian cancer TME is very complex and heterogeneous, with different sites of disease within the same patient displaying different characteristics (385). Obtaining multiple biopsies at one timepoint is invasive and further exploration of this heterogeneity is unlikely to be feasible. If the TME from omentum is so different to that of the primary tumour, which TME has the most influence on prognosis?

This project focused on CD4, CD8 and myeloid cells, which only account for some of the immune cells involved in the immunosuppressive microenvironment of EOC. The flow cytometry and mIF panels were designed to identify the phenotype of these cells but did not allow exploration of the function of these cells. B-cells, NK cells, macrophages and MDSCs may well be of importance and were not explored here.

5.3 Future directions

It remains unclear whether ICPIs will ever be effective in HGSC. Investigation into novel agents for the treatment of relapsed EOC are vital. Translational research on blood and tumour collected from patients with EOC is essential. Future research into the ovarian cancer immune TME should include more patients and sequential blood and biopsy samples taken at various timepoints, including diagnosis and at relapse. This is vital to explore whether there are any changes within the blood and TME following treatment and if so, is there anything that could predict relapse? If so, are

there any targetable checkpoints / immune cells / biomarkers with potential therapeutic options?

It may be that immune cells are truly not helpful in identifying predictive and prognostic biomarkers in ovarian cancer and future work should look beyond just the immune system and immunotherapy. Further work should combine approaches of looking at the immune microenvironment, and the role of genomic complexity through more novel high throughput technologies e.g. single cell RNA sequencing, multifunctional assays of immune cell components and gene expression signatures.

Analyses of the samples from the PROMPT trial and other translational projects are crucial to understand the changes in the immune microenvironment in EOC following treatment with ICPIs. An example of a large translational research study in ovarian cancer is BRITROC-1, which shows that the ovarian cancer genome is remarkably stable in samples taken at diagnosis and relapse (386). BRITROC-2 (387) is currently recruiting, with the goal to better understand the changes that occur within the tumour at diagnosis and relapse.

In conclusion, high grade EOC is a complex cancer. Over the last few years, PARPi have improved survival, particularly in women with BRCA mutations, with potential for cure in some patients (41). Additional work is needed to further improve outcomes. To do this, translational research studies are essential to help provide information for the development of personalised treatment.

References

1. Cancer Research UK Ovarian cancer statistics [Internet]. [cited 2021 Mar 23]. Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/ovarian-cancer/incidence - heading-Two.

2. The World Ovarian Cancer Coalition Atlas . https://worldovariancancercoalition.org/wp-content/uploads/2018/10/THE-WORLD-OVARIAN-CANCER-COALITION-ATLAS-2018.pdf.

3. Mills K, Fuh K. Recent Advances in Understanding, Diagnosing, and Treating Ovarian Cancer. F1000Res. 2017/02/12. 2017;6:84.

4. Cancer Stat Facts: Ovarian Cancer [Internet]. NIH National Cancer Institute: Surveillance, Epidemiology, and End Results Program.

5. Lee JM, Minasian L, Kohn EC. New strategies in ovarian cancer treatment. Cancer. 2019;125 Suppl:4623–9.

6. Berek JS, Kehoe ST, Kumar L, Friedlander M. Cancer of the ovary, fallopian tube, and peritoneum. International Journal of Gynecology & Obstetrics. 2018;143:59–78.

7. McGuire WP, Hoskins WJ, Brady MF, Kucera PR, Partridge EE, Look KY, et al. Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. N Engl J Med. 1996;334(1):1–6.

8. Ozols RF, Bundy BN, Greer BE, Fowler JM, Clarke-Pearson D, Burger RA, et al. Phase III Trial of Carboplatin and Paclitaxel Compared With Cisplatin and Paclitaxel in Patients With Optimally Resected Stage III Ovarian Cancer: A Gynecologic Oncology Group Study. Journal of Clinical Oncology. 2003;21(17):3194–200.

9. Winter WE, Maxwell GL, Tian C, Sundborg MJ, Rose GS, Rose PG, et al. Tumor Residual After Surgical Cytoreduction in Prediction of Clinical Outcome in Stage IV Epithelial Ovarian Cancer: A Gynecologic Oncology Group Study. Journal of Clinical Oncology. 2008;26(1):83–9.

10. Bois A du, Reuss A, Pujade-Lauraine E, Harter P, Ray-Coquard I, Pfisterer J. Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: A combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials. Cancer. 2009;115(6):1234–44.

11. Vergote I, Tropé CG, Amant F, Kristensen GB, Ehlen T, Johnson N, et al. Neoadjuvant Chemotherapy or Primary Surgery in Stage IIIC or IV Ovarian Cancer. N Engl J Med. 2010;363(10):943–53.

12. Kehoe S, Hook J, Nankivell M, Jayson GC, Kitchener H, Lopes T, et al. Primary chemotherapy versus primary surgery for newly diagnosed advanced ovarian cancer (CHORUS): an open-label, randomised, controlled, non-inferiority trial. The Lancet. 2015;386(9990):249–57.

13. Reuss A, Bois A du, Harter P, Fotopoulou C, Sehouli J, Aletti G, et al. TRUST: Trial of Radical Upfront Surgical Therapy in advanced ovarian cancer (ENGOT ov33/AGO-OVAR OP7). International Journal of Gynecologic Cancer. 2019;29(8):1327.

14. Khalique S, Hook JM, Ledermann JA. Maintenance therapy in ovarian cancer. Curr Opin Oncol. 2014;26(5):521–8.

15. Horowitz N, Matulonis UA. New biologic agents for the treatment of gynecologic cancers. Hematol Oncol Clin North Am. 2012;26(1):133–56.

16. Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, et al. Incorporation of Bevacizumab in the Primary Treatment of Ovarian Cancer. N Engl J Med. 2011;365(26):2473–83.

17. Perren TJ, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, Kristensen G, et al. A phase 3 trial of bevacizumab in ovarian cancer. New England Journal of Medicine. 2011;365(26):2484–96.

18. Tewari KS, Burger RA, Enserro D, Norquist BM, Swisher EM, Brady MF, et al. Final Overall Survival of a Randomized Trial of Bevacizumab for Primary Treatment of Ovarian Cancer. J Clin Oncol [Internet]. 2019;37(26):2317–28. Available from: https://www.ncbi.nlm.nih.gov/pubmed/31216226

19. Oza AM, Cook AD, Pfisterer J, Embleton A, Ledermann JA, Pujade-Lauraine E, et al. Standard chemotherapy with or without bevacizumab for women with newly diagnosed ovarian cancer (ICON7): overall survival results of a phase 3 randomised trial. Lancet Oncol. 2015;16(8):928–36.

20. Gonzalez Martin A, Oza AM, Embleton AC, Pfisterer J, Ledermann JA, Pujade-Lauraine E, et al. Exploratory outcome analyses according to stage and/or residual disease in the ICON7 trial of carboplatin and paclitaxel with or without bevacizumab for newly diagnosed ovarian cancer. Gynecol Oncol. 2019;152(1):53–60.

21. du Bois A, Kristensen G, Ray-Coquard I, Reuss A, Pignata S, Colombo N, et al. Standard first-line chemotherapy with or without nintedanib for advanced ovarian cancer (AGO-OVAR 12): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet Oncol. 2016 Jan;17(1):78–89.

22. McLachlan J, Banerjee S. Pazopanib in ovarian cancer. Expert Rev Anticancer Ther. 2015 Sep 2;15(9):995–1005.

23. Vergote I, du Bois A, Floquet A, Rau J, Kim JW, del Campo JM, et al. Overall survival results of AGO-OVAR16: A phase 3 study of maintenance pazopanib versus placebo in women who have not progressed after first-line chemotherapy for advanced ovarian cancer. Gynecol Oncol. 2019 Nov;155(2):186–91.

24. Ray-Coquard I, Cibula D, Mirza MR, Reuss A, Ricci C, Colombo N, et al. Final results from GCIG/ENGOT/AGO-OVAR 12, a randomised placebo-controlled phase III trial of nintedanib combined with chemotherapy for newly diagnosed advanced ovarian cancer. Int J Cancer. 2020 Jan 15;146(2):439–48.

25. Ferron G, De Rauglaudre G, Becourt S, Delanoy N, Joly F, Lortholary A, et al. Neoadjuvant chemotherapy with or without nintedanib for advanced epithelial ovarian cancer: Lessons from the GINECO double-blind randomized phase II CHIVA trial. Gynecol Oncol. 2023 Mar;170:186–94.

26. Lord CJ, Tutt AN, Ashworth A. Synthetic lethality and cancer therapy: lessons learned from the development of PARP inhibitors. Annu Rev Med. 2015;66:455–70.

27. Zhang S, Royer R, Li S, McLaughlin JR, Rosen B, Risch HA, et al. Frequencies of BRCA1 and BRCA2 mutations among 1,342 unselected patients with invasive ovarian cancer. Gynecol Oncol. 2011;121(2):353–7.

28. Ashour M, Ezzat Shafik H. Frequency of germline mutations in BRCA1 and BRCA2 in ovarian cancer patients and their effect on treatment outcome. Cancer Manag Res. 2019;11:6275–84.

29. Stewart MD, Merino Vega D, Arend RC, Baden JF, Barbash O, Beaubier N, et al. Homologous Recombination Deficiency: Concepts, Definitions, and Assays. Oncologist. 2022 Mar 11;27(3):167–74.

30. Moschetta M, George A, Kaye SB, Banerjee S. BRCA somatic mutations and epigenetic BRCA modifications in serous ovarian cancer. Ann Oncol. 2016;27(8):1449–55.

31. Bouberhan S, Philp L, Hill S, Al-Alem LF, Rueda B. Exploiting the Prevalence of Homologous Recombination Deficiencies in High-Grade Serous Ovarian Cancer. Cancers (Basel). 2020;12(5):1206.

32. Färkkilä A, Gulhan DC, Casado J, Jacobson CA, Nguyen H, Kochupurakkal B, et al. Immunogenomic profiling determines responses to combined PARP and PD-1 inhibition in ovarian cancer. Nat Commun. 2020 Dec 1;11(1).

33. Mullen MM, Kuroki LM, Thaker PH. Novel treatment options in platinum-sensitive recurrent ovarian cancer: A review. Gynecol Oncol [Internet].

2019;152(2):416–25. Available from: https://www.ncbi.nlm.nih.gov/pubmed/30409489

34. Ledermann JA, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Overall survival in patients with platinum-sensitive recurrent serous ovarian cancer receiving olaparib maintenance monotherapy: an updated analysis from a randomised, placebo-controlled, double-blind, phase 2 trial. Lancet Oncol. 2016;17(11):1579–89.

35. Friedlander M, Matulonis U, Gourley C, du Bois A, Vergote I, Rustin G, et al. Long-term efficacy, tolerability and overall survival in patients with platinum-sensitive, recurrent high-grade serous ovarian cancer treated with maintenance olaparib capsules following response to chemotherapy. Br J Cancer [Internet]. 2018;119(9):1075–85. Available from: https://www.ncbi.nlm.nih.gov/pubmed/30353045

36. Pujade-Lauraine E, Ledermann JA, Selle F, Gebski V, Penson RT, Oza AM, et al. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol. 2017/07/30. 2017;18(9):1274–84.

37. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med. 2012/03/29. 2012;366(15):1382–92.

38. Poveda A, Floquet A, Ledermann JA, Asher R, Penson RT, Oza AM, et al. Final overall survival (OS) results from SOLO2/ENGOT-ov21: A phase III trial assessing maintenance olaparib in patients (pts) with platinum-sensitive, relapsed ovarian cancer and a BRCA mutation. Journal of Clinical Oncology [Internet]. 2020 May 20;38(15_suppl):6002. Available from: https://doi.org/10.1200/JCO.2020.38.15_suppl.6002

39. Moore K, Colombo N, Scambia G, Kim BG, Oaknin A, Friedlander M, et al. Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. N Engl J Med. 2018;379(26):2495–505.

40. Friedlander ML, Moore K CN et al. Maintenance olaparib for patients (pts) with newly diagnosed, advanced ovarian cancer (OC) and a BRCA mutation (BRCAm): 5-year (y) follow-up (f/u) from SOLO1. In ESMO Asia Virtual Congress 2020; 2020.

41. DiSilvestro P, Banerjee S, Colombo N, Scambia G, Kim BG, Oaknin A, et al. Overall Survival With Maintenance Olaparib at a 7-Year Follow-Up in Patients With Newly Diagnosed Advanced Ovarian Cancer and a BRCA Mutation: The

SOLO1/GOG 3004 Trial. Journal of Clinical Oncology. 2023 Jan 20;41(3):609–17.

42. Mirza MR, Monk BJ, Herrstedt J, Oza AM, Mahner S, Redondo A, et al. Niraparib Maintenance Therapy in Platinum-Sensitive, Recurrent Ovarian Cancer. N Engl J Med. 2016;375(22):2154–64.

43. Matulonis UA, Herrstedt J, Tinker A, Marme F, Redondo A, Kalbacher E, et al. Long-term benefit of niraparib treatment of recurrent ovarian cancer (OC). Journal of Clinical Oncology. 2017 May 20;35(15_suppl):5534.

44. Matulonis U. Long-term safety and secondary efficacy endpoints in the ENGOT-OV16/NOVA phase III trial of niraparib in recurrent ovarian cancer. In.

45. González-Martín A, Pothuri B, Vergote I, Christensen RD, Graybill W, Mirza MR, et al. Niraparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. New England Journal of Medicine. 2019;381(25):2391–402.

46. Swisher EM, Lin KK, Oza AM, Scott CL, Giordano H, Sun J, et al. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. Lancet Oncol. 2017;18(1):75–87.

47. Coleman RL, Oza AM, Lorusso D, Aghajanian C, Oaknin A, Dean A, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017;390(10106):1949–61.

48. Ledermann JA, Oza AM, Lorusso D, Aghajanian C, Oaknin A, Dean A, et al. Rucaparib for patients with platinum-sensitive, recurrent ovarian carcinoma (ARIEL3): post-progression outcomes and updated safety results from a randomised, placebo-controlled, phase 3 trial. Lancet Oncol [Internet]. 2020;21(5):710–22. Available from: https://www.ncbi.nlm.nih.gov/pubmed/32359490

49. NICE guidance. Technology appraisal guidance. [cited 2021 Mar 30]. Niraparib for maintenance treatment of advanced ovarian, fallopian tube and peritoneal cancer after response to first-line platinum-based chemotherapy. Available from: https://www.nice.org.uk/guidance/ta673/resources/niraparib-for-maintenance-treatment-of-advanced-ovarian-fallopian-tube-and-peritoneal-cancer-after-response-to-firstline-platinumbased-chemotherapy-pdf-82609317907909

50. Liu JF, Barry WT, Birrer M, Lee JM, Buckanovich RJ, Fleming GF, et al. Combination cediranib and olaparib versus olaparib alone for women with recurrent platinum-sensitive ovarian cancer: a randomised phase 2 study. Lancet Oncol. 2014;15(11):1207–14.

51. Liu JF, Barry WT, Birrer M, Lee JM, Buckanovich RJ, Fleming GF, et al. Overall survival and updated progression-free survival outcomes in a randomized phase 2 study of combination cediranib and olaparib versus olaparib in relapsed platinum-sensitive ovarian cancer. Ann Oncol. 2019;30(4):551–7.

52. Mirza MR, Avall-Lundqvist E, Birrer MJ, Christensen R dePont, Nyvang GB, Malander S, et al. Combination of niraparib and bevacizumab versus niraparib alone as treatment of recurrent platinum-sensitive ovarian cancer: A randomized controlled chemotherapy-free study—NSGO-AVANOVA2/ENGOT-OV24. Journal of Clinical Oncology. 2019 May 20;37(15_suppl):5505.

53. Mirza MR, Nyvang GB, Lund B, Christensen R dePont, Werner TL, Malander S, et al. Final survival analysis of NSGO-AVANOVA2/ENGOT-OV24: Combination of niraparib and bevacizumab versus niraparib alone as treatment of recurrent platinum-sensitive ovarian cancer—A randomized controlled chemotherapy-free study. Journal of Clinical Oncology. 2020 May 20;38(15_suppl):6012.

54. Ray-Coquard I, Pautier P, Pignata S, Pérol D, González-Martín A, Berger R, et al. Olaparib plus Bevacizumab as First-Line Maintenance in Ovarian Cancer. New England Journal of Medicine. 2019;381(25):2416–28.

55. NICE final appraisal document [Internet]. [cited 2021 Mar 30]. Olaparib plus bevacizumab for maintenance treatment of advanced ovarian, fallopian tube or primary peritoneal cancer. Available from: https://www.nice.org.uk/guidance/gid-ta10526/documents/final-appraisal-determination-document

56. Wilson MK, Pujade-Lauraine E, Aoki D, Mirza MR, Lorusso D, Oza AM, et al. Fifth Ovarian Cancer Consensus Conference of the Gynecologic Cancer InterGroup: recurrent disease. Ann Oncol. 2017;28(4):727–32.

57. Markman M, Bookman MA. Second-line treatment of ovarian cancer. Oncologist. 2000;5(1):26–35.

58. Pignata S, Pisano C, Di Napoli M, Cecere SC, Tambaro R, Attademo L. Treatment of recurrent epithelial ovarian cancer. Cancer. 2019;125 Suppl:4609–15.

59. McGee J, Bookman M, Harter P, Marth C, McNeish I, Moore KN, et al. Fifth Ovarian Cancer Consensus Conference: individualized therapy and patient factors. Ann Oncol. 2017;28(4):702–10.

60. Colombo N, Sessa C, Bois AD, Ledermann J, McCluggage WG, McNeish I, et al. ESMO-ESGO consensus conference recommendations on ovarian cancer:

pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease. Int J Gynecol Cancer. 2019;

61. Pfisterer J, Plante M, Vergote I, du Bois A, Hirte H, Lacave AJ, et al. Gemcitabine plus carboplatin compared with carboplatin in patients with platinum-sensitive recurrent ovarian cancer: an intergroup trial of the AGO-OVAR, the NCIC CTG, and the EORTC GCG. J Clin Oncol. 2006;24(29):4699–707.

62. Wagner U, Marth C, Largillier R, Kaern J, Brown C, Heywood M, et al. Final overall survival results of phase III GCIG CALYPSO trial of pegylated liposomal doxorubicin and carboplatin vs paclitaxel and carboplatin in platinum-sensitive ovarian cancer patients. Br J Cancer. 2012;107(4):588–91.

63. Harter P, Sehouli J, Reuss A, Hasenburg A, Scambia G, Cibula D, et al. Prospective Validation Study of a Predictive Score for Operability of Recurrent Ovarian Cancer: The Multicenter Intergroup Study DESKTOP II. A Project of the AGO Kommission OVAR, AGO Study Group, NOGGO, AGO-Austria, and MITO. International Journal of Gynecologic Cancer. 2011 Jan 1;21(2):289–95.

64. Coleman RL, Spirtos NM, Enserro D, Herzog TJ, Sabbatini P, Armstrong DK, et al. Secondary Surgical Cytoreduction for Recurrent Ovarian Cancer. N Engl J Med. 2019;381(20):1929–39.

65. Bristow RE, Puri I, Chi DS. Cytoreductive surgery for recurrent ovarian cancer: a meta-analysis. Gynecol Oncol. 2009;112(1):265–74.

66. Harter P, Sehouli J, Vergote I, Ferron G, Reuss A, Meier W, et al. Randomized Trial of Cytoreductive Surgery for Relapsed Ovarian Cancer. New England Journal of Medicine. 2021 Dec 2;385(23):2123–31.

67. Davis A, Tinker A V, Friedlander M. “Platinum resistant” ovarian cancer: what is it, who to treat and how to measure benefit? Gynecol Oncol. 2014;133(3):624–31.

68. Aghajanian C, Blank S V, Goff BA, Judson PL, Teneriello MG, Husain A, et al. OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. Journal of Clinical Oncology. 2012;30(17):2039–45.

69. Aghajanian C, Goff B, Nycum LR, Wang Y V, Husain A, Blank S V. Final overall survival and safety analysis of OCEANS, a phase 3 trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent ovarian cancer. Gynecol Oncol [Internet]. 2015;139(1):10–6. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26271155

70. Coleman RL, Brady MF, Herzog TJ, Sabbatini P, Armstrong DK, Walker JL, et al. Bevacizumab and paclitaxel-carboplatin chemotherapy and secondary cytoreduction in recurrent, platinum-sensitive ovarian cancer (NRG Oncology/Gynecologic Oncology Group study GOG-0213): a multicentre, open-label, randomised, phase 3 trial. Lancet Oncol. 2017;18(6):779–91.

71. Pignata S, Lorusso D, Joly F, Gallo C, Colombo N, Sessa C, et al. Chemotherapy plus or minus bevacizumab for platinum-sensitive ovarian cancer patients recurring after a bevacizumab containing first line treatment: The randomized phase 3 trial MITO16B-MaNGO OV2B-ENGOT OV17. Journal of Clinical Oncology. 2018;36(15_suppl):5506.

72. Matulonis UA, Berlin S, Ivy P, Tyburski K, Krasner C, Zarwan C, et al. Cediranib, an oral inhibitor of vascular endothelial growth factor receptor kinases, is an active drug in recurrent epithelial ovarian, fallopian tube, and peritoneal cancer. J Clin Oncol. 2009;27(33):5601–6.

73. Ledermann JA, Embleton AC, Raja F, Perren TJ, Jayson GC, Rustin GJS, et al. Cediranib in patients with relapsed platinum-sensitive ovarian cancer (ICON6): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2016;387(10023):1066–74.

74. Ledermann JA, Embleton AC, Perren T, Jayson GC, Rustin GJS, Kaye SB, et al. Overall survival results of ICON6: A trial of chemotherapy and cediranib in relapsed ovarian cancer. Journal of Clinical Oncology. 2017;35(15_suppl):5506.

75. Elyashiv O, Ledermann J, Parmar G, Farrelly L, Counsell N, Feeney A, et al. ICON 9—an international phase III randomized study to evaluate the efficacy of maintenance therapy with olaparib and cediranib or olaparib alone in patients with relapsed platinum-sensitive ovarian cancer following a response to platinum-based chemotherapy. International Journal of Gynecologic Cancer [Internet]. 2021 Jan;31(1):134–8. Available from: https://ijgc.bmj.com/lookup/doi/10.1136/ijgc-2020-002073

76. Liu JF, Brady MF, Matulonis UA, Miller A, Kohn EC, Swisher EM, et al. Olaparib With or Without Cediranib Versus Platinum-Based Chemotherapy in Recurrent Platinum-Sensitive Ovarian Cancer (NRG-GY004): A Randomized, Open-Label, Phase III Trial. Journal of Clinical Oncology [Internet]. 2022 Mar 15;40(19):2138–47. Available from: https://doi.org/10.1200/JCO.21.02011

77. Lee J min, Moore RG, Ghamande SA, Park MS, Diaz JP, Chapman JA, et al. Cediranib in combination with olaparib in patients without a germline BRCA1/2 mutation with recurrent platinum-resistant ovarian cancer: Phase IIb CONCERTO trial. Journal of Clinical Oncology [Internet]. 2020 May

20;38(15_suppl):6056. Available from: https://doi.org/10.1200/JCO.2020.38.15_suppl.6056

78. Pujade-Lauraine E, Hilpert F, Weber B, Reuss A, Poveda A, Kristensen G, et al. Bevacizumab Combined With Chemotherapy for Platinum-Resistant Recurrent Ovarian Cancer: The AURELIA Open-Label Randomized Phase III Trial. Journal of Clinical Oncology [Internet]. 2014;32(13):1302–8. Available from: http://ascopubs.org/doi/abs/10.1200/JCO.2013.51.4489

79. Moore KN, Angelergues A, Konecny GE, García Y, Banerjee S, Lorusso D, et al. Mirvetuximab Soravtansine in FRα-Positive, Platinum-Resistant Ovarian Cancer. New England Journal of Medicine. 2023 Dec 7;389(23):2162–74.

80. Hanahan D, Weinberg RA. The Hallmarks of Cancer. Cell. 2000;100(1):57–70.

81. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity [Internet]. 2013;39(1):1–10. Available from: https://www.ncbi.nlm.nih.gov/pubmed/23890059

82. Mittica G, Genta S, Aglietta M, Valabrega G. Immune Checkpoint Inhibitors: A New Opportunity in the Treatment of Ovarian Cancer? Int J Mol Sci. 2016;17(7):1169.

83. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med [Internet]. 2004;10(9):942–9. Available from: https://www.ncbi.nlm.nih.gov/pubmed/15322536

84. Aristizábal B, González Á. Innate immune system. In: Anaya JM, Shoenfeld Y, Rojas-Villarraga A, et al., editors. Autoimmunity: From Bench to Bedside [Internet]. Bogota (Colombia): El Rosario University Press; 2013 Jul 18. Chapter 2. Available from: https: In.

85. Preston CC, Goode EL, Hartmann LC, Kalli KR, Knutson KL. Immunity and immune suppression in human ovarian cancer. Immunotherapy. 2011/04/06. 2011;3(4):539–56.

86. Cruse JM, Lewis RE, Wang H, Schreuder GMTh, Marsh SGE, Kennedy LJ. Immunology Guidebook. 2004;1–15.

87. Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. Chapter 24, The Adaptive Immune System. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21070/No Title. In.

88. Whitacre JM, Lin J, Harding A. T Cell Adaptive Immunity Proceeds through Environment-Induced Adaptation from the Exposure of Cryptic Genetic Variation. Front Genet. 2012;3:5.

89. Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. Allergy, Asthma & Clinical Immunology. 2018;14(Suppl 2):49.

90. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3(11):991.

91. Pennock GK, Chow LQ. The Evolving Role of Immune Checkpoint Inhibitors in Cancer Treatment. Oncologist. 2015/06/13. 2015;20(7):812–22.

92. Colvin EK. Tumor-associated macrophages contribute to tumor progression in ovarian cancer. Front Oncol. 2014/06/18. 2014;4:137.

93. Casey SC, Amedei A, Aquilano K, Azmi AS, Benencia F, Bhakta D, et al. Cancer prevention and therapy through the modulation of the tumor microenvironment. Semin Cancer Biol. 2015;35:S199–223.

94. Zou W, Chen L. Inhibitory B7-family molecules in the tumour microenvironment. Nat Rev Immunol. 2008;8(6):467–77.

95. Manjarrez-Orduno N, Menard LC, Kansal S, Fischer P, Kakrecha B, Jiang C, et al. Circulating T Cell Subpopulations Correlate With Immune Responses at the Tumor Site and Clinical Response to PD1 Inhibition in Non-Small Cell Lung Cancer. Front Immunol. 2018/08/21. 2018;9:1613.

96. Workman CJ, Szymczak-Workman AL, Collison LW, Pillai MR, Vignali DAA. The development and function of regulatory T cells. Cellular and Molecular Life Sciences. 2009 Aug 24;66(16):2603–22.

97. Coosemans A, Decoene J, Baert T, Laenen A, Kasran A, Verschuere T, et al. Immunosuppressive parameters in serum of ovarian cancer patients change during the disease course. Oncoimmunology. 2015;5(4):e1111505.

98. Wu JWY, Dand S, Doig L, Papenfuss AT, Scott CL, Ho G, et al. T-Cell Receptor Therapy in the Treatment of Ovarian Cancer: A Mini Review. Front Immunol. 2021 Apr 13;12.

99. Chester C, Dorigo O, Berek JS, Kohrt H. Immunotherapeutic approaches to ovarian cancer treatment. J Immunother Cancer. 2015/03/26. 2015;3:7.

100. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T Cells, Recurrence, and Survival in Epithelial Ovarian Cancer. New England Journal of Medicine. 2003;348(3):203–13.

101. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci U S A. 2005;102(51):18538–43.

102. Zsiros E, Tanyi J, Balint K, Kandalaft LE. Immunotherapy for ovarian cancer. Curr Opin Oncol. 2014;26(5):492–500.

103. Zhang S, Ke X, Zeng S, Wu M, Lou J, Wu L, et al. Analysis of CD8+ Treg cells in patients with ovarian cancer: a possible mechanism for immune impairment. Cell Mol Immunol. 2015;12(5):580–91.

104. Jiménez-Sánchez A, Memon D, Pourpe S, Veeraraghavan H, Li Y, Vargas HA, et al. Heterogeneous Tumor-Immune Microenvironments among Differentially Growing Metastases in an Ovarian Cancer Patient. Cell. 2017;170(5):927-938.e20.

105. Hensler M, Kasikova L, Fiser K, Rakova J, Skapa P, Laco J, et al. M2-like macrophages dictate clinically relevant immunosuppression in metastatic ovarian cancer. J Immunother Cancer. 2020 Aug 20;8(2).

106. Baci D, Bosi A, Gallazzi M, Rizzi M, Noonan DM, Poggi A, et al. The Ovarian Cancer Tumor Immune Microenvironment (TIME) as Target for Therapy: A Focus on Innate Immunity Cells as Therapeutic Effectors. Int J Mol Sci. 2020;21(9):3125.

107. Ignacio RMC, Lee ES, Wilson AJ, Beeghly-Fadiel A, Whalen MM, Son DS. Chemokine Network and Overall Survival in TP53 Wild-Type and Mutant Ovarian Cancer. Immune Netw. 2018;18(4):e29.

108. Sautes-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. Nat Rev Cancer. 2019;19(6):307–25.

109. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer. 2004;4(1):71–8.

110. Qian BZ, Pollard JW. Macrophage Diversity Enhances Tumor Progression and Metastasis. Cell. 2010;141(1):39–51.

111. Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J Pathol. 2002;196(3):254–65.

112. Okła K, Wertel I, Polak G, Surówka J, Wawruszak A, Kotarski J. Tumor-Associated Macrophages and Myeloid-Derived Suppressor Cells as Immunosuppressive Mechanism in Ovarian Cancer Patients: Progress and Challenges. Int Rev Immunol. 2016;35(5):1–14.

113. Balermpas P, Rödel F, Liberz R, Oppermann J, Wagenblast J, Ghanaati S, et al. Head and neck cancer relapse after chemoradiotherapy correlates with

CD163+ macrophages in primary tumour and CD11b+ myeloid cells in recurrences. Br J Cancer. 2014;111(8):1509.

114. Medrek C, Pontén F, Jirström K, Leandersson K. The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients. BMC Cancer. 2012;12(1):306.

115. Lee CH, Espinosa I, Vrijaldenhoven S, Subramanian S, Montgomery KD, Zhu S, et al. Prognostic Significance of Macrophage Infiltration in Leiomyosarcomas. Clinical Cancer Research. 2008;14(5):1423–30.

116. Chung F, Lee K, Wang C, Heh C, Chan Y, Chen H, et al. Tumor-associated macrophages correlate with response to epidermal growth factor receptor-tyrosine kinase inhibitors in advanced non-small cell lung cancer. Int J Cancer. 2012;131(3):E227–35.

117. Jensen TO, Schmidt H, Møller HJ, Høyer M, Maniecki MB, Sjoegren P, et al. Macrophage Markers in Serum and Tumor Have Prognostic Impact in American Joint Committee on Cancer Stage I/II Melanoma. Journal of Clinical Oncology. 2009 Jun 15;27(20):3330–7.

118. Lan C, Huang X, Lin S, Huang H, Cai Q, Wan T, et al. Expression of M2-Polarized Macrophages is Associated with Poor Prognosis for Advanced Epithelial Ovarian Cancer. Technol Cancer Res Treat. 2012;12(3):259–67.

119. Wanderley CW, Colon DF, Luiz JPM, Oliveira FF, Viacava PR, Leite CA, et al. Paclitaxel reduces tumor growth by reprogramming tumor-associated macrophages to an M1- profile in a TLR4-dependent manner. Cancer Res. 2018;78(20):canres.3480.2017.

120. Kawamura K, Komohara Y, Takaishi K, Katabuchi H, Takeya M. Detection of M2 macrophages and colony-stimulating factor 1 expression in serous and mucinous ovarian epithelial tumors. Pathol Int. 2009;59(5):300–5.

121. Condeelis J, Pollard JW. Macrophages: Obligate Partners for Tumor Cell Migration, Invasion, and Metastasis. Cell. 2006;124(2):263–6.

122. Gottlieb CE, Mills AM, Cross J V, Ring KL. Tumor-associated macrophage expression of PD-L1 in implants of high grade serous ovarian carcinoma: A comparison of matched primary and metastatic tumors. Gynecol Oncol. 2017;144(3):607–12.

123. Nola R De, Menga A, Castegna A, Loizzi V, Ranieri G, Cicinelli E, et al. The Crowded Crosstalk between Cancer Cells and Stromal Microenvironment in Gynecological Malignancies: Biological Pathways and Therapeutic Implication. Int J Mol Sci. 2019;20(10):2401.

124. Zhang Z, Liu S, Zhang B, Qiao L, Zhang Y, Zhang Y. T Cell Dysfunction and Exhaustion in Cancer. Front Cell Dev Biol. 2020;8:17.

125. No JH, Moon JM, Kim K, Kim YB. Prognostic Significance of Serum Soluble CD163 Level in Patients with Epithelial Ovarian Cancer. Gynecol Obstet Invest. 2013;75(4):263–7.

126. Monney L, Sabatos CA, Gaglia JL, Ryu A, Waldner H, Chernova T, et al. Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. Nature. 2002;415(6871):536–41.

127. Fourcade J, Sun Z, Benallaoua M, Guillaume P, Luescher IF, Sander C, et al. Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients. J Exp Med. 2010/09/06. 2010 Sep 27;207(10):2175–86.

128. Zhu L, Cao G, Hua D, Cui L, Zhao X, Wang S. The tumor immune microenvironment correlates with neoadjuvant chemotherapy response in high-grade serous ovarian cancer. Journal of Clinical Oncology. 2022 Jun 1;40(16_suppl):e17605–e17605.

129. Moroney JW, Powderly J, Lieu CH, Bendell JC, Eckhardt SG, Chang CW, et al. Safety and Clinical Activity of Atezolizumab Plus Bevacizumab in Patients with Ovarian Cancer: A Phase Ib Study. Clinical Cancer Research. 2020;26(21):5631–7.

130. Nixon AB, Schalper KA, Jacobs I, Potluri S, Wang IM, Fleener C. Peripheral immune-based biomarkers in cancer immunotherapy: can we realize their predictive potential? J Immunother Cancer. 2019;7(1):325.

131. Wolf AM, Wolf D, Steurer M, Gastl G, Gunsilius E, Grubeck-Loebenstein B. Increase of Regulatory T Cells in the Peripheral Blood of Cancer Patients. Clinical Cancer Research. 2003 Feb 1;9(2):606 LP – 612.

132. Li X, Ye DF, Xie X, Chen HZ, Lu WG. Proportion of CD4+CD25+ Regulatory T Cell is Increased in the Patients with Ovarian Carcinoma. Cancer Invest. 2009;23(5):399–403.

133. Ye S, Chen W, Zheng Y, Wu Y, Xiang L, Li T, et al. Peripheral lymphocyte populations in ovarian cancer patients and correlations with clinicopathological features. J Ovarian Res. 2022 Dec 11;15(1):43.

134. Wu J, Liu C, Qian S, Hou H. The Expression of Tim-3 in Peripheral Blood of Ovarian Cancer. DNA Cell Biol. 2013;32(11):648–53.

135. MacFarlane AW, Jillab M, Plimack ER, Hudes GR, Uzzo RG, Litwin S, et al. PD-1 Expression on Peripheral Blood Cells Increases with Stage in Renal Cell

Carcinoma Patients and Is Rapidly Reduced after Surgical Tumor Resection. Cancer Immunology. 2014;2(4):320–31.

136. Maine CJ, Aziz NH, Chatterjee J, Hayford C, Brewig N, Whilding L, et al. Programmed death ligand-1 over-expression correlates with malignancy and contributes to immune regulation in ovarian cancer. Cancer Immunol Immunother. 2014;63(3):215–24.

137. Massa C, Karn T, Denkert C, Schneeweiss A, Hanusch C, Blohmer JU, et al. Differential effect on different immune subsets of neoadjuvant chemotherapy in patients with TNBC. J Immunother Cancer. 2020 Nov 16;8(2):e001261.

138. Vermi W, Soncini M, Melocchi L, Sozzani S, Facchetti F. Plasmacytoid dendritic cells and cancer. J Leukoc Biol. 2011 Oct;90(4):681–90.

139. Wang PF, Song SY, Wang TJ, Ji WJ, Li SW, Liu N, et al. Prognostic role of pretreatment circulating MDSCs in patients with solid malignancies: A meta-analysis of 40 studies. Oncoimmunology. 2018 Jul 30;7(10):e1494113–e1494113.

140. Okła K, Czerwonka A, Wawruszak A, Bobiński M, Bilska M, Tarkowski R, et al. Clinical Relevance and Immunosuppressive Pattern of Circulating and Infiltrating Subsets of Myeloid-Derived Suppressor Cells (MDSCs) in Epithelial Ovarian Cancer. Front Immunol. 2019;10:691.

141. Santegoets SJAM, Groot AF de, Dijkgraaf EM, Simões AMC, Noord VE van der, Ham JJ van, et al. The blood mMDSC to DC ratio is a sensitive and easy to assess independent predictive factor for epithelial ovarian cancer survival. Oncoimmunology. 2018;7(8):0.

142. Krieg C, Nowicka M, Guglietta S, Schindler S, Hartmann FJ, Weber LM, et al. High-dimensional single-cell analysis predicts response to anti-PD-1 immunotherapy. Nat Med. 2018;24(2):144–53.

143. Wu L, Deng Z, Peng Y, Han L, Liu J, Wang L, et al. Ascites-derived IL-6 and IL-10 synergistically expand CD14 + HLA-DR -/low myeloid-derived suppressor cells in ovarian cancer patients. Oncotarget. 2014;5(0):76843–56.

144. Dillman RO. Cancer Immunotherapy. Cancer Biother Radiopharm. 2011 Feb;26(1):1–64.

145. Acharya N, Sabatos-Peyton C, Anderson AC. Tim-3 finds its place in the cancer immunotherapy landscape. J Immunother Cancer. 2020;8(1):e000911.

146. Rodriguez GM, Galpin KJC, McCloskey CW, Vanderhyden BC. The Tumor Microenvironment of Epithelial Ovarian Cancer and Its Influence on Response to Immunotherapy. Cancers (Basel). 2018;10(8).

147. Kelly E, Russell SJ. History of Oncolytic Viruses: Genesis to Genetic Engineering. Molecular Therapy. 2007;15(4):651–9.

148. Epenetos AA, Munro AJ, Stewart S, Rampling R, Lambert HE, McKenzie CG, et al. Antibody-guided irradiation of advanced ovarian cancer with intraperitoneally administered radiolabeled monoclonal antibodies. J Clin Oncol. 1987;5(12):1890–9.

149. Stewart JS, Hird V, Snook D, Dhokia B, Sivolapenko G, Hooker G, et al. Intraperitoneal yttrium-90-labeled monoclonal antibody in ovarian cancer. Journal of Clinical Oncology. 1990;8(12):1941–50.

150. Hird V, Maraveyas A, Snook D, Dhokia B, Soutter WP, Meares C, et al. Adjuvant therapy of ovarian cancer with radioactive monoclonal antibody. Br J Cancer. 1993;68(2):bjc1993349.

151. Epenetos AA, Hird V, Lambert H, Mason P, Coulter C. Long term survival of patients with advanced ovarian cancer treated with intraperitoneal radioimmunotherapy. International Journal of Gynecological Cancer. 2000;10(s1):44–6.

152. Oei AL, Verheijen RH, Seiden M V, Benigno BB, Lopes A, Soper JT, et al. Decreased intraperitoneal disease recurrence in epithelial ovarian cancer patients receiving intraperitoneal consolidation treatment with yttrium-90-labeled murine HMFG1 without improvement in overall survival. Int J Cancer. 2007;120(12):2710–4.

153. Bookman MA. Immunotherapy for ovarian cancer. Curr Opin Oncol. 1990/10/01. 1990;2(5):893–900.

154. Alberts DS, Mason-Liddil N, O'Toole R V, Abbott TM, Kronmal R, Hilgers RD, et al. Randomized phase III trial of chemoimmunotherapy in patients with previously untreated stage III, optimal disease ovarian cancer: a Southwest Oncology Group Study. Gynecol Oncol. 1989/01/01. 1989;32(1):16–21.

155. Alberts DS, Mason-Liddil N, O'Toole R V, Abbott TM, Kronmal R, Hilgers RD, et al. Randomized phase III trial of chemoimmunotherapy in patients with previously untreated stages III and IV suboptimal disease ovarian cancer: a Southwest Oncology Group Study. Gynecol Oncol. 1989/01/01. 1989;32(1):8–15.

156. Pujade-Lauraine E, Guastalla JP, Colombo N, Devillier P, Francois E, Fumoleau P, et al. Intraperitoneal recombinant interferon gamma in ovarian cancer patients with residual disease at second-look laparotomy. J Clin Oncol. 1996/02/01. 1996;14(2):343–50.

157. Windbichler GH, Hausmaninger H, Stummvoll W, Graf AH, Kainz C, Lahodny J, et al. Interferon-gamma in the first-line therapy of ovarian cancer: a randomized phase III trial. Br J Cancer. 2000/03/29. 2000;82(6):1138–44.

158. Hall GD, Brown JM, Coleman RE, Stead M, Metcalf KS, Peel KR, et al. Maintenance treatment with interferon for advanced ovarian cancer: results of the Northern and Yorkshire gynaecology group randomised phase III study. Br J Cancer. 2004 Aug 10;91(4):621–6.

159. Alberts DS, Marth C, Alvarez RD, Johnson G, Bidzinski M, Kardatzke DR, et al. Randomized phase 3 trial of interferon gamma-1b plus standard carboplatin/paclitaxel versus carboplatin/paclitaxel alone for first-line treatment of advanced ovarian and primary peritoneal carcinomas: results from a prospectively designed analysis of prog. Gynecol Oncol. 2008/03/04. 2008;109(2):174–81.

160. Jiang T, Zhou C, Ren S. Role of IL-2 in cancer immunotherapy. Oncoimmunology. 2016 Apr 25;5(6):e1163462–e1163462.

161. Choudhry H, Helmi N, Abdulaal WH, Zeyadi M, Zamzami MA, Wu W, et al. Prospects of IL-2 in Cancer Immunotherapy. Biomed Res Int. 2018 May 6;2018:9056173.

162. Dutcher JP, Creekmore S, Weiss GR, Margolin K, Markowitz AB, Roper M, et al. A phase II study of interleukin-2 and lymphokine-activated killer cells in patients with metastatic malignant melanoma. Journal of Clinical Oncology. 1989;7(4):477–85.

163. Parkinson DR, Abrams JS, Wiernik PH, Rayner AA, Margolin KA, Van Echo DA, et al. Interleukin-2 therapy in patients with metastatic malignant melanoma: a phase II study. J Clin Oncol. 1990;8(10):1650–6.

164. Rosenberg SA, Yang JC, Topalian SL, Schwartzentruber DJ, Weber JS, Parkinson DR, et al. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high-dose bolus interleukin 2. JAMA. 1994;271(12):907–13.

165. Grande C, Firvida JL, Navas V, Casal J. Interleukin-2 for the treatment of solid tumors other than melanoma and renal cell carcinoma. Anticancer Drugs. 2006;17(1):1–12.

166. Edwards RP, Gooding W, Lembersky BC, Colonello K, Hammond R, Paradise C, et al. Comparison of toxicity and survival following intraperitoneal recombinant interleukin-2 for persistent ovarian cancer after platinum: twenty-four-hour versus 7-day infusion. J Clin Oncol. 1997;15(11):3399–407.

167. Vlad AM, Budiu RA, Lenzner DE, Wang Y, Thaller JA, Colonello K, et al. A phase II trial of intraperitoneal interleukin-2 in patients with platinum-resistant or platinum-refractory ovarian cancer. Cancer Immunol Immunother. 2010;59(2):293–301.

168. Benedetti Panici P, Scambia G, Greggi S, Di Roberto P, Ragusa G, Perrone L, et al. Recombinant interleukin-2 continuous infusion in ovarian cancer patients with minimal residual disease at second-look. Cancer Treat Rev. 1989;16 Suppl A:123–7.

169. Wang YL, Peng HH, Su SY, Lin CT. Combined Immunotherapy (OK-432, IL-2) With Chemotherapy Decrease the Recurrence Rate in Advanced Ovarian Cancer. Reproductive Sciences. 2018;193371911876868.

170. Kaufman HL, Kohlhapp FJ, Zloza A. Oncolytic viruses: a new class of immunotherapy drugs. Nat Rev Drug Discov. 2015;14(9):642–62.

171. Hoare J, Campbell N, Carapuca E. Oncolytic virus immunotherapies in ovarian cancer: moving beyond adenoviruses. Porto Biomed J. 2018;3(1):e7.

172. Andtbacka RH, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, et al. Talimogene Laherparepvec Improves Durable Response Rate in Patients With Advanced Melanoma. J Clin Oncol. 2015;33(25):2780–8.

173. McNeish V; Jayson, G; Roxburg, P; Barretina Ginesta, M.P; Garcia-Donas, J; Anton Torres, A; Michael, A; Brown, R; Krige, D; Bendall, J; Di Genova, G; McElwaine-Johnn, H IM. OCTAVE: A phase I study of enadenotucirev, an oncolytic group B adenovirus, in combination with weekly paclitaxel in platinum-resistant epithelial ovarian cancer. Annals of Oncology. 2019;30(suppl_5:v421.

174. Zheng M, Huang J, Tong A, Yang H. Oncolytic Viruses for Cancer Therapy: Barriers and Recent Advances. Mol Ther Oncolytics. 2019;15:234–47.

175. Kawasaki T, Kawai T, Trendelenburg G. Toll-like receptor signaling pathways. 2014;

176. Monk BJ, Facciabene A, Brady WE, Aghajanian CA, Fracasso PM, Walker JL, et al. Integrative Development of a TLR8 Agonist for Ovarian Cancer Chemoimmunotherapy. Clinical Cancer Research. 2017;23(8):1955–66.

177. J O LA, Golenbock D, Bowie AG. The history of Toll-like receptors — redefining innate immunity. Nature Publishing Group. 2013.

178. Casares N, Pequignot MO, Tesniere A, Ghiringhelli F, Roux S, Chaput N, et al. Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. J Exp Med. 2005;202(12):1691–701.

179. Zitvogel L, Kepp O, Senovilla L, Menger L, Chaput N, Kroemer G. Immunogenic Tumor Cell Death for Optimal Anticancer Therapy: The Calreticulin Exposure Pathway. Clinical Cancer Research. 2010;16(12):3100–4.

180. Monk BJ, Brady MF, Aghajanian C, Lankes HA, Rizack T, Leach J, et al. A phase 2, randomized, double-blind, placebo- controlled study of chemo-immunotherapy combination using motolimod with pegylated liposomal doxorubicin in recurrent or persistent ovarian cancer: a Gynecologic Oncology Group partners study. Ann Oncol. 2017;28(5):996–1004.

181. Hornyák L, Dobos N, Koncz G, Karányi Z, Páll D, Szabó Z, et al. The Role of Indoleamine-2,3-Dioxygenase in Cancer Development, Diagnostics, and Therapy. Front Immunol. 2018;9:151.

182. WANG D, SAGA Y, MIZUKAMI H, SATO N, NONAKA H, FUJIWARA H, et al. Indoleamine-2,3-dioxygenase, an immunosuppressive enzyme that inhibits natural killer cell function, as a useful target for ovarian cancer therapy. Int J Oncol. 2011;40(4):929–34.

183. Mellor AL, Keskin DB, Johnson T, Chandler P, Munn DH. Cells Expressing Indoleamine 2,3-Dioxygenase Inhibit T Cell Responses. The Journal of Immunology. 2002;168(8):3771–6.

184. Uyttenhove C, Pilotte L, Theate I, Stroobant V, Colau D, Parmentier N, et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med. 2003;9(10):1269–74.

185. Frumento G, Rotondo R, Tonetti M, Damonte G, Benatti U, Ferrara GB. Tryptophan-derived Catabolites Are Responsible for Inhibition of T and Natural Killer Cell Proliferation Induced by Indoleamine 2,3-Dioxygenase. J Exp Med. 2002;196(4):459–68.

186. Curti A, Pandolfi S, Valzasina B, Aluigi M, Isidori A, Ferri E, et al. Modulation of tryptophan catabolism by human leukemic cells results in the conversion of CD25− into CD25+ T regulatory cells. Blood. 2006;109(7):2871–7.

187. Munn DH, Mellor AL. IDO in the Tumor Microenvironment: Inflammation, Counter-Regulation, and Tolerance. Trends Immunol. 2016;37(3):193–207.

188. Smith C, Chang MY, Parker KH, Beury DW, DuHadaway JB, Flick HE, et al. IDO Is a Nodal Pathogenic Driver of Lung Cancer and Metastasis Development. Cancer Discov. 2012;2(8):722–35.

189. Okamoto A, Nikaido T, Ochiai K, Takakura S, Saito M, Aoki Y, et al. Indoleamine 2,3-Dioxygenase Serves as a Marker of Poor Prognosis in Gene Expression Profiles of Serous Ovarian Cancer Cells. 2005;

190. Inaba T, Ino K, Kajiyama H, Yamamoto E, Shibata K, Nawa A, et al. Role of the immunosuppressive enzyme indoleamine 2,3-dioxygenase in the progression of ovarian carcinoma. Gynecol Oncol. 2009;115(2):185–92.

191. Takao M, Okamoto A, Nikaido T, Urashima M, Takakura S, Saito M, et al. Increased synthesis of indoleamine-2,3-dioxygenase protein is positively associated with impaired survival in patients with serous-type, but not with other types of, ovarian cancer. Oncol Rep. 2007;

192. Sarivalasis A, Morotti M, Mulvey A, Imbimbo M, Coukos G. Cell therapies in ovarian cancer. Ther Adv Med Oncol. 2021 Jan 22;13:175883592110083.

193. Kandalaft LE, Odunsi K, Coukos G. Immunotherapy in Ovarian Cancer: Are We There Yet? Journal of Clinical Oncology. 2019 Sep 20;37(27):2460–71.

194. Pegalajar-Jurado A. Abstract TMIM-082: OVACURE - BRINGING PERSONALIZED TREATMENT TO WOMEN WITH OVARIAN CANCER. Clinical Cancer Research. 2019 Nov 15;25(22_Supplement):TMIM-082-TMIM-082.

195. Guo Q, Yang Q, Li J, Liu G, Nikoulin I, Jia S. Advanced clinical trials of dendritic cell vaccines in ovarian cancer. Journal of Investigative Medicine. 2020 Oct;68(7):1223–7.

196. Perez CR, De Palma M. Engineering dendritic cell vaccines to improve cancer immunotherapy. Nat Commun. 2019 Dec 27;10(1):5408.

197. Zhang X, He T, Li Y, Chen L, Liu H, Wu Y, et al. Dendritic Cell Vaccines in Ovarian Cancer. Front Immunol [Internet]. 2021 Jan 25;11. Available from: www.frontiersin.org

198. Fong L, Engleman EG. Dendritic Cells in Cancer Immunotherapy. Annu Rev Immunol. 2000 Apr;18(1):245–73.

199. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T Immunotherapy for Castration-Resistant Prostate Cancer. New England Journal of Medicine. 2010 Jul 23;363(5):411–22.

200. Cibula D, Rob L, Mallmann P, Knapp P, Klat J, Chovanec J, et al. Dendritic cell-based immunotherapy (DCVAC/OvCa) combined with second-line chemotherapy in platinum-sensitive ovarian cancer (SOV02): A randomized, open-label, phase 2 trial. Gynecol Oncol. 2021 Sep;162(3):652–60.

201. Rob L, Cibula D, Knapp P, Mallmann P, Klat J, Minar L, et al. Dendritic cell vaccine (DCVAC) combined with chemotherapy (CMT) in patients with newly diagnosed epithelial ovarian carcinoma (EOC) after primary debulking surgery (PDS): Biomarker exploratory analysis of a phase 2, open-label, randomized,

multicenter tri. Journal of Clinical Oncology. 2021 May 20;39(15_suppl):5521–5521.

202. Sabbatini P, Dupont J, Aghajanian C, Derosa F, Poynor E, Anderson S, et al. Phase I study of abagovomab in patients with epithelial ovarian, fallopian tube, or primary peritoneal cancer. Clin Cancer Res. 2006/09/27. 2006;12(18):5503–10.

203. Pfisterer J, du Bois A, Sehouli J, Loibl S, Reinartz S, Reuß A, et al. The anti-idiotypic antibody abagovomab in patients with recurrent ovarian cancer. A phase I trial of the AGO-OVAR. Annals of Oncology. 2006 Oct;17(10):1568–77.

204. Sabbatini P, Harter P, Scambia G, Sehouli J, Meier W, Wimberger P, et al. 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. J Clin Oncol. 2013;31(12):1554–61.

205. Pietragalla A, Duranti S, Daniele G, Nero C, Ciccarone F, Lorusso D, et al. Oregovomab: an investigational agent for the treatment of advanced ovarian cancer. Expert Opin Investig Drugs. 2021 Feb 1;30(2):103–10.

206. Rocconi RP, Grosen EA, Ghamande SA, Chan JK, Barve MA, Oh J, et al. Gemogenovatucel-T (Vigil) immunotherapy as maintenance in frontline stage III/IV ovarian cancer (VITAL): a randomised, double-blind, placebo-controlled, phase 2b trial. Lancet Oncol. 2020 Dec;21(12):1661–72.

207. Rocconi RP, Ghamande SA, Barve MA, Stevens EE, Aaron P, Stanbery L, et al. Maintenance vigil immunotherapy in newly diagnosed advanced ovarian cancer: Efficacy assessment of homologous recombination proficient (HRP) patients in the phase IIb VITAL trial. Journal of Clinical Oncology. 2021 May 20;39(15_suppl):5502–5502.

208. Rocconi RP, Stevens EE, Bottsford-Miller JN, Ghamande SA, Elder J, DeMars LL, et al. Proof of principle study of sequential combination atezolizumab and Vigil in relapsed ovarian cancer. Cancer Gene Ther. 2022 Mar 22;29(3–4):369–82.

209. Barve M, Aaron P, Manning L, Bognar E, Wallraven G, Horvath S, et al. Pilot Study of Combination Gemogenovatucel-T (Vigil) and Durvalumab in Women With Relapsed BRCA-wt Triple-Negative Breast or Ovarian Cancer. Clin Med Insights Oncol. 2022 Jan 6;16:117955492211105.

210. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nature Publishing Group. 2012;

211. Darvin P, Toor SM, Sasidharan Nair V, Elkord E. Immune checkpoint inhibitors: recent progress and potential biomarkers. Exp Mol Med. 2018/12/14. 2018;50(12):165.

212. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. Science (1979). 2015;348(6230):124–8.

213. Allen EM Van, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science (1979). 2015;350(6257):207–11.

214. Brunner-Weinzierl MC, Rudd CE. CTLA-4 and PD-1 Control of T-Cell Motility and Migration: Implications for Tumor Immunotherapy. Front Immunol. 2018;9:2737.

215. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol. 2013;13(4):227.

216. Zhang G, Liu C, Bai H, Cao G, Cui R, Zhang Z. Combinatorial therapy of immune checkpoint and cancer pathways provides a novel perspective on ovarian cancer treatment. Oncol Lett. 2019;17(3):2583–91.

217. Fan C (Ava), Reader J, Roque DM. Review of Immune Therapies Targeting Ovarian Cancer. Curr Treat Options Oncol. 2018;19(12):74.

218. Felice F De, Marchetti C, Palaia I, Musio D, Muzii L, Tombolini V, et al. Immunotherapy of Ovarian Cancer: The Role of Checkpoint Inhibitors. J Immunol Res. 2015;2015:191832.

219. Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K, et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. Proc Natl Acad Sci U S A. 2007/03/16. 2007;104(9):3360–5.

220. Webb JR, Milne K, Kroeger DR, Nelson BH. PD-L1 expression is associated with tumor-infiltrating T cells and favorable prognosis in high-grade serous ovarian cancer. Gynecol Oncol. 2016;141(2):293–302.

221. Paulsen EE, Kilvaer TK, Khanehkenari MR, Al-Saad S, Hald SM, Andersen S, et al. Assessing PD-L1 and PD-1 in Non-Small Cell Lung Cancer: A Novel Immunoscore Approach. Clin Lung Cancer. 2017;18(2):220-233 e8.

222. Darb-Esfahani S, Kunze CA, Kulbe H, Sehouli J, Wienert S, Lindner J, et al. Prognostic impact of programmed cell death-1 (PD-1) and PD-ligand 1 (PD-L1) expression in cancer cells and tumor-infiltrating lymphocytes in ovarian high grade serous carcinoma. Oncotarget. 2016;7(2):1486–99.

223. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer. N Engl J Med. 2012;366(26):2443–54.

224. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus Ipilimumab in Advanced Melanoma A BS TR AC T. N Engl J Med. 2013;369:122–55.

225. Hamanishi J, Mandai M, Ikeda T, Minami M, Kawaguchi A, Murayama T, et al. Safety and Antitumor Activity of Anti-PD-1 Antibody, Nivolumab, in Patients With Platinum-Resistant Ovarian Cancer. J Clin Oncol. 2015;33(34):4015–22.

226. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. N Engl J Med. 2010;363(8):711–23.

227. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, et al. Ipilimumab plus Dacarbazine for Previously Untreated Metastatic Melanoma. N Engl J Med. 2011;364(26):2517–26.

228. Callahan MK, Wolchok JD. At the Bedside: CTLA-4- and PD-1-blocking antibodies in cancer immunotherapy. J Leukoc Biol. 2013;94(1):41–53.

229. Vanpouille-Box C, Lhuillier C, Bezu L, Aranda F, Yamazaki T, Kepp O, et al. Trial watch: Immune checkpoint blockers for cancer therapy. Oncoimmunology. 2017;6(11):0.

230. Hinchcliff E, Hong D, Le H, Chisholm G, Iyer R, Naing A, et al. Characteristics and outcomes of patients with recurrent ovarian cancer undergoing early phase immune checkpoint inhibitor clinical trials. Gynecol Oncol. 2018;151(3):407–13.

231. Matulonis UA, Shapira-Frommer R, Santin AD, Lisyanskaya AS, Pignata S, Vergote I, et al. Antitumor Activity and Safety of Pembrolizumab in Patients with Advanced Recurrent Ovarian Cancer: Results from the Phase 2 KEYNOTE-100 Study. Annals of Oncology. 2019;

232. Varga A, Piha-Paul S, Ott PA, Mehnert JM, Berton-Rigaud D, Morosky A, et al. Pembrolizumab in patients with programmed death ligand 1-positive advanced ovarian cancer: Analysis of KEYNOTE-028. Gynecol Oncol. 2018/12/12. 2018;

233. Disis ML, Taylor MH, Kelly K, Beck JT, Gordon M, Moore KM, et al. Efficacy and Safety of Avelumab for Patients With Recurrent or Refractory Ovarian Cancer. JAMA Oncol. 2019;5(3).

234. Pujade-Lauraine, E, Fujiwara, K, Ledermann, J.A, Oza, A.M, Kristeleit, R.S, Roy-Coquard, I.L, Richardson, G.E, Sess, C, Yonemori, K, Banerjee, S, Leary,

A, Tinker, A.V, Jung, K.H, Madry, R, Park, S.Y, Anderson, C.K, Zohren, F, Stewart, R, Wei, C, Dychter, BJ. SGO-2019-Late-Breaking-Abstracts.pdf. In Honolulu, HI; 2019. p. 1.

235. Ray-Coquard IL, Savoye AM, Mouret-Reynier M ange, Chabaud S, Derbel O, Kalbacher E, et al. Efficacy and safety results from neopembrov study, a randomized phase II trial of neoadjuvant chemotherapy (CT) with or without pembrolizumab (P) followed by interval debulking surgery and standard systemic therapy ± P for advanced high-grade serous carci. Journal of Clinical Oncology. 2021 May 20;39(15_suppl):5500–5500.

236. Iida Y, Okamoto A, Hollis RL, Gourley C, Herrington CS. Clear cell carcinoma of the ovary: a clinical and molecular perspective. International Journal of Gynecologic Cancer. 2021 Apr;31(4):605–16.

237. Kristeleit R. Efficacy of pembrolizumab monotherapy (PM) for advanced clear cell gynaecological cancer (CGGC): Phase II PEACOCC trial. Annals of Oncology . 2022;33(suppl_7):S235–82.

238. O'Cearbhaill RE, Wolfer A, Disilvestro P, O'Malley DM, Sabbatini P, Shohara L, et al. A phase I/II study of chemo-immunotherapy with durvalumab (durva) and pegylated liposomal doxorubicin (PLD) in platinum-resistant recurrent ovarian cancer (PROC). Annals of Oncology. 2018 Oct;29:viii337.

239. Matulonis UA, Shapira R, Santin A, Lisyanskaya AS, Pignata S, Vergote I, et al. Final results from the KEYNOTE-100 trial of pembrolizumab in patients with advanced recurrent ovarian cancer. Journal of Clinical Oncology [Internet]. 2020 May 20;38(15_suppl):6005. Available from: https://doi.org/10.1200/JCO.2020.38.15_suppl.6005

240. Colombo I, Lien S, Yang C, Clouthier DL, Bonilla L, Cyriac S, et al. Immunologic and genomic characterization of high grade serous ovarian cancer (HGSOC) in patients (pts) treated with pembrolizumab (Pembro) in the phase II INSPIRE trial. Journal of Clinical Oncology. 2017 May 20;35(15_suppl):5581–5581.

241. Lee EK, Xiong N, Cheng SC, Barry WT, Penson RT, Konstantinopoulos PA, et al. Combined pembrolizumab and pegylated liposomal doxorubicin in platinum resistant ovarian cancer: A phase 2 clinical trial. Gynecol Oncol. 2020 Oct;159(1):72–8.

242. Hamanishi J, Takeshima N, Katsumata N, Ushijima K, Kimura T, Takeuchi S, et al. Nivolumab Versus Gemcitabine or Pegylated Liposomal Doxorubicin for Patients With Platinum-Resistant Ovarian Cancer: Open-Label, Randomized Trial in Japan (NINJA). Journal of Clinical Oncology. 2021 Nov 20;39(33):3671–81.

243. Ledermann, J.A, Colombo, N, Oza, A.M, Fujiwara, K, Birrer, M.J, Randall, L.M, Poddubskaya, E.V, Scambia, G, Shparyk, Y.V, Lim M.C, Bhoola, S.M, Sohn, J, Yonemori, K, Stewart, R.A, Zhang, X, Zohren, F, Linn, C, Monk BJ. SGO-2020-Annual-Meeting-Oral-Abstracts.pdf. In 2020. p. 21.

244. Tan DSP, Choi CH, Ngoi N, Sun H, Heong V, Ow SGW, et al. A multicenter phase II randomized trial of durvalumab (D) versus physician's choice chemotherapy (PCC) in patients (pts) with recurrent ovarian clear cell adenocarcinoma (MOCCA/APGOT-OV2/GCGS-OV3). Journal of Clinical Oncology. 2022 Jun 1;40(16_suppl):5565–5565.

245. Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G. Immunological Effects of Conventional Chemotherapy and Targeted Anticancer Agents. Cancer Cell. 2015;28(6):690–714.

246. Tada Y, Togashi Y, Kotani D, Kuwata T, Sato E, Kawazoe A, et al. Targeting VEGFR2 with Ramucirumab strongly impacts effector/ activated regulatory T cells and CD8+ T cells in the tumor microenvironment. J Immunother Cancer. 2018;6(1):106.

247. Zheng F, Zhang Y, Chen S, Weng X, Rao Y, Fang H. Mechanism and current progress of Poly ADP-ribose polymerase (PARP) inhibitors in the treatment of ovarian cancer. Biomedicine & Pharmacotherapy. 2020 Mar;123:109661.

248. Strickland KC, Howitt BE, Shukla SA, Rodig S, Ritterhouse LL, Liu JF, et al. Association and prognostic significance of BRCA1/2-mutation status with neoantigen load, number of tumor-infiltrating lymphocytes and expression of PD-1/PD-L1 in high grade serous ovarian cancer. Oncotarget. 2016;7(12):13587–98.

249. Musacchio L, Cicala CM, Camarda F, Ghizzoni V, Giudice E, Carbone MV, et al. Combining PARP inhibition and immune checkpoint blockade in ovarian cancer patients: a new perspective on the horizon? ESMO Open. 2022 Aug;7(4):100536.

250. Maiorano BA, Lorusso D, Maiorano MFP, Ciardiello D, Parrella P, Petracca A, et al. The Interplay between PARP Inhibitors and Immunotherapy in Ovarian Cancer: The Rationale behind a New Combination Therapy. Int J Mol Sci. 2022 Mar 31;23(7):3871.

251. Samson N, Ablasser A. The cGAS–STING pathway and cancer. Nat Cancer. 2022 Dec 12;3(12):1452–63.

252. Konstantinopoulos PA, Waggoner S, Vidal GA, Mita M, Moroney JW, Holloway R, et al. Single-Arm Phases 1 and 2 Trial of Niraparib in Combination With Pembrolizumab in Patients With Recurrent Platinum-Resistant Ovarian Carcinoma. JAMA Oncol. 2019;5(8).

253. list of authors A, affiliations appears at the end of the paper their. Signatures of mutational processes in human cancer. 2013;

254. Drew Y, Jonge M de, Hong SH, Park YH, Wolfer A, Brown J, et al. An open-label, phase II basket study of olaparib and durvalumab (MEDIOLA): Results in germline BRCA-mutated (gBRCAm) platinum-sensitive relapsed (PSR) ovarian cancer (OC). 2018;149.

255. Drew Y, Kaufman B, Banerjee S, Lortholary A, Hong SH, Park YH, et al. 1190PD Phase II study of olaparib + durvalumab (MEDIOLA): Updated results in germline BRCA-mutated platinum-sensitive relapsed (PSR) ovarian cancer (OC). Annals of Oncology. 2019;30:v485–6.

256. Drew Y, Penson RT, O'Malley DM, Kim JW, Zimmermann S, Roxburgh P, et al. 814MO Phase II study of olaparib (O) plus durvalumab (D) and bevacizumab (B) (MEDIOLA): Initial results in patients (pts) with non-germline BRCA-mutated (non-gBRCAm) platinum sensitive relapsed (PSR) ovarian cancer (OC). Annals of Oncology. 2020 Sep;31:S615–6.

257. Landen C. Association of BRCA1/2, homologous recombination deficiency, and PD-L1 with clinical outcomes in patients receiving atezolizumab versus placebo combined with carboplatin, paclitaxel, and bevacizumab for newly diagnosed ovarian cancer: Exploratory analyses. In: SGO Annual Virtual Meeting. 2021.

258. Moore KN, Bookman M, Sehouli J, Miller A, Anderson C, Scambia G, et al. Atezolizumab, Bevacizumab, and Chemotherapy for Newly Diagnosed Stage III or IV Ovarian Cancer: Placebo-Controlled Randomized Phase III Trial (IMagyn050/GOG 3015/ENGOT-OV39). J Clin Oncol. 2021;39(17):1842–55.

259. Kurtz JE, Pujade-Lauraine E, Oaknin A, Belin L, Leitner K, Cibula D, et al. Atezolizumab Combined With Bevacizumab and Platinum-Based Therapy for Platinum-Sensitive Ovarian Cancer: Placebo-Controlled Randomized Phase III ATALANTE/ENGOT-ov29 Trial. Journal of Clinical Oncology. 2023 Oct 20;41(30):4768–78.

260. Michels J, Ghiringhelli F, Frenel JS, Brard C, You B, Floquet A, et al. Pembrolizumab in combination with bevacizumab and pegylated liposomal doxorubicin in patients with platinum-resistant epithelial ovarian cancer. Journal of Clinical Oncology. 2021 May 20;39(15_suppl):5522–5522.

261. González-Martín A, Rubio-Pérez MJ, Heitz F, Christensen RD, Colombo N, Gorp T Van, et al. 403 Primary results from ENGOT-Ov41/GEICO 69-O/ANITA, a double-blind placebo-controlled phase 3 trial evaluating atezolizumab combined with platinum-based chemotherapy and maintenance

niraparib for recurrent ovarian cancer with a platinum-free interval >6 months. In: Oral Sessions. BMJ Publishing Group Ltd; 2024. p. A35–6.

262. Randall LM, O'Malley DM, Monk BJ, Coleman RL, Gaillard S, Adams SF, et al. MOONSTONE/GOG-3032: Interim analysis of a phase 2 study of niraparib + dostarlimab in patients (pts) with platinum-resistant ovarian cancer (PROC). Journal of Clinical Oncology. 2022 Jun 1;40(16_suppl):5573–5573.

263. Park J, Lee JB, Lim MC, Kim BG, Kim JW, Kim S, et al. Phase II study of durvalumab and tremelimumab with front-line neoadjuvant chemotherapy in patients with advanced-stage ovarian cancer: primary analysis in the original cohort of KGOG3046/TRU-D. J Immunother Cancer. 2023 Oct 21;11(10):e007444.

264. Oaknin A, Vergote I, Ray-Coquard I, Leary A, Rodriguez Freixinos V, Concin N, et al. Vanucizumab (VAN) in combination with atezolizumab (ATEZO) for platinum-resistant recurrent ovarian cancer (PROC): Results from a single arm extension phase of the phase I study BP28179. Annals of Oncology. 2017 Sep;28:v335.

265. Moore KN, Bookman M, Sehouli J, Miller A, Anderson C, Scambia G, et al. LBA31 Primary results from IMagyn050/GOG 3015/ENGOT-OV39, a double-blind placebo (pbo)-controlled randomised phase III trial of bevacizumab (bev)-containing therapy +/- atezolizumab (atezo) for newly diagnosed stage III/IV ovarian cancer (OC). Annals of Oncology. 2020 Sep;31:S1161–2.

266. Kurtz JE, Pujade-Lauraine E, Oaknin A, Belin L, Tsibulak I. Phase III ATALANTE/ov29 trial: Atezolizumab (Atz) versus placebo with platinum-based chemotherapy (Cx) plus bevacizumab (bev) in patients (pts) with platinum-sensitive relapse (PSR) of epithelial ovarian cancer (OC). Annals of Oncology. 2022;33(Suppl_7):S808–69.

267. Vergote I, Sehouli J, Salutari V, Zola P, Madry R, Wenham RM, et al. ENGOT-OV43/KEYLYNK-001: A phase III, randomized, double-blind, active- and placebo-controlled study of pembrolizumab plus chemotherapy with olaparib maintenance for first-line treatment of BRCA-nonmutated advanced epithelial ovarian cancer. Journal of Clinical Oncology. 2019 May 20;37(15_suppl):TPS5603–TPS5603.

268. Hardy-Bessard AC, Moore KN, Mirza MR, Asselain B, Redondo A, Pfisterer J, et al. ENGOT-OV44/FIRST study: a randomized, double-blind, adaptive, phase III study of standard of care (SOC) platinum-based therapy ± dostarlimab followed by niraparib ± dostarlimab maintenance as first-line (1L) treatment of stage 3 or 4 ovarian cancer (OC). Journal of Clinical Oncology. 2020 May 20;38(15_suppl):TPS6101–TPS6101.

269. Lee YJ, Lim MC, Kim BG, Ngoi NYL, Choi CH, Park SY, et al. A single-arm phase II study of olaparib maintenance with pembrolizumab and bevacizumab in BRCA non-mutated patients with platinum-sensitive recurrent ovarian cancer (OPEB-01). J Gynecol Oncol. 2021 Mar;32(2).

270. Harter P, Marmé F, Pautier P, Reuss A, Lindemann K, Kurzeder C, et al. AGO-OVAR 2.29 (ENGOT-ov34): Atezolizumab in combination with bevacizumab and chemotherapy versus bevacizumab and chemotherapy in recurrent ovarian cancer (ROC). Journal of Clinical Oncology. 2022 Jun 1;40(16_suppl):TPS5611–TPS5611.

271. Musacchio L, Salutari V, Pignata S, Braicu E, Cibula D, Colombo N, et al. Randomized phase III trial on niraparib-TSR-042 (dostarlimab) versus physician's choice chemotherapy in recurrent ovarian, fallopian tube, or primary peritoneal cancer patients not candidate for platinum retreatment: NItCHE trial (MITO 33). International Journal of Gynecologic Cancer. 2021 Oct 4;31(10):1369–73.

272. Monk BJ, Parkinson C, Lim MC, O'Malley DM, Oaknin A, Wilson MK, et al. A Randomized, Phase III Trial to Evaluate Rucaparib Monotherapy as Maintenance Treatment in Patients With Newly Diagnosed Ovarian Cancer (ATHENA–MONO/GOG-3020/ENGOT-ov45). Journal of Clinical Oncology. 2022 Dec 1;40(34):3952–64.

273. Monk BJ, Coleman RL, Fujiwara K, Wilson MK, Oza AM, Oaknin A, et al. ATHENA (GOG-3020/ENGOT-ov45): a randomized, phase III trial to evaluate rucaparib as monotherapy (ATHENA–MONO) and rucaparib in combination with nivolumab (ATHENA–COMBO) as maintenance treatment following frontline platinum-based chemotherapy in ovarian cancer. International Journal of Gynecologic Cancer. 2021 Dec;31(12):1589–94.

274. Press Release. https://www.pharmaand.com/wp-content/uploads/2024/05/Rubraca-ATHENA-COMBO-Topline-Data-News-Release-FINAL-.pdf.

275. Marth C, Park-Simon TW, Aghajanian C, Reuss A, Nishio S, Lim MC, et al. #1120 Durvalumab with paclitaxel/carboplatin + bevacizumab then maintenance durvalumab, bevacizumab + olaparib in patients with newly diagnosed advanced ovarian cancer without a tumour BRCA1/2 mutation: results from the DUO-O/ENGOT-Ov46/AGO-OVAR 23/GOG-3025 trial. In: Late Breaking Abstracts. BMJ Publishing Group Ltd; 2023. p. A428.3-A429.

276. Manoharan S, Bressel M, James P, Ko YA, Hamilton AL, Scott CL, et al. Beacon: A phase II study of bevacizumab, atezolizumab, and cobimetinib in patients with recurrent, platinum resistant, high grade serous ovarian cancer. Journal of Clinical Oncology . 2024;42(Number 16 suppl).

277. González-Martín A, Chung HC, Saada-Bouzid E, Yanez E, Senellart H, Cassier PA, et al. Lenvatinib plus pembrolizumab for patients with previously treated advanced ovarian cancer: Results from the phase 2 multicohort LEAP-005 study. Gynecol Oncol. 2024 Jul;186:182–90.

278. Hagos YB, Akarca AU, Ramsay A, Rossi RL, Pomplun S, Moioli A, et al. DeepMIF: Deep Learning Based Cell Profiling for Multispectral Immunofluorescence Images with Graphical User Interface. In 2022. p. 140–9.

279. Hagos YB, Narayanan PL, Akarca AU, Marafioti T, Yuan Y. ConCORDe-Net: Cell Count Regularized Convolutional Neural Network for Cell Detection in Multiplex Immunohistochemistry Images. In 2019. p. 667–75.

280. (https://worldovariancancercoalition.org/about-ovarian-cancer/key-stats/#:~:text=According%20to%20Globocan's%202022%20projections,of%20almost%2070%25%20from%202022 [Internet]. [cited 2024 Apr 4]. Available from: (https://worldovariancancercoalition.org/about-ovarian-cancer/key-stats/#:~:text=According%20to%20Globocan's%202022%20projections,of%20almost%2070%25%20from%202022

281. Osman MA, Elkady MS, Nasr KE. Weekly Paclitaxel versus Three-Weekly Paclitaxel in Recurrent Platinum-Resistant Epithelial Ovarian and Peritoneal Cancers: A Phase III Study. Clin Med Insights Oncol. 2016 Jan 25;10:CMO.S38204.

282. McNeish IA, Ledermann JA, Webber L, James L, Kaye SB, Hall M, et al. A randomised, placebo-controlled trial of weekly paclitaxel and saracatinib (AZD0530) in platinum-resistant ovarian, fallopian tube or primary peritoneal cancer. Annals of Oncology. 2014 Oct;25(10):1988–95.

283. Li J, Zou G, Wang W, Yin C, Yan H, Liu S. Treatment options for recurrent platinum-resistant ovarian cancer: A systematic review and Bayesian network meta-analysis based on RCTs. Front Oncol. 2023 Apr 11;13.

284. Odunsi K. Immunotherapy in ovarian cancer. Annals of Oncology. 2017 Nov;28:viii1–7.

285. Principe DR, Kamath SD, Korc M, Munshi HG. The immune modifying effects of chemotherapy and advances in chemo-immunotherapy. Pharmacol Ther. 2022 Aug;236:108111.

286. Lim PT, Goh BH, Lee WL. Taxol: Mechanisms of action against cancer, an update with current research. In: Paclitaxel. Elsevier; 2022. p. 47–71.

287. Zhu L, Chen L. Progress in research on paclitaxel and tumor immunotherapy. Cell Mol Biol Lett. 2019 Dec 13;24(1):40.

288. Eric PL, Hilpert F, Weber B, Reuss A, Poveda A, Kristensen G, et al. AURELIA: A randomized phase III trial evaluating bevacizumab (BEV) plus chemotherapy (CT) for platinum (PT)-resistant recurrent ovarian cancer (OC). Journal of Clinical Oncology [Internet]. 2012;1). Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed11&AN=71012660

289. Ortiz M, Wabel E, Mitchell K, Horibata S. Mechanisms of chemotherapy resistance in ovarian cancer. Cancer Drug Resistance. 2022;

290. Grunewald T, Tang M, Chen J, Lord S, Friedlander M, Lee CK. Where have we gone wrong? Phase II trials (Ph2t) do not inform the results of phase III trials (Ph3t) in platinum resistant ovarian cancer (PROC). Journal of Clinical Oncology. 2016 May 20;34(15_suppl):5559–5559.

291. Pujade-Lauraine E, Fujiwara K, Ledermann JA, Oza AM, Kristeleit R, Ray-Coquard IL, et al. Avelumab alone or in combination with chemotherapy versus chemotherapy alone in platinum-resistant or platinum-refractory ovarian cancer (JAVELIN Ovarian 200): an open-label, three-arm, randomised, phase 3 study. Lancet Oncol. 2021 Jul;22(7):1034–46.

292. Matulonis UA, Shapira-Frommer R, Santin AD, Lisyanskaya AS, Pignata S, Vergote I, et al. Antitumor activity and safety of pembrolizumab in patients with advanced recurrent ovarian cancer: results from the phase II KEYNOTE-100 study. Annals of Oncology. 2019 Jul;30(7):1080–7.

293. Muroyama Y, Wherry EJ. Memory T-Cell Heterogeneity and Terminology. Cold Spring Harb Perspect Biol. 2021 Oct;13(10):a037929.

294. Manjarrez-Orduño N, Menard LC, Kansal S, Fischer P, Kakrecha B, Jiang C, et al. Circulating T Cell Subpopulations Correlate With Immune Responses at the Tumor Site and Clinical Response to PD1 Inhibition in Non-Small Cell Lung Cancer. Front Immunol. 2018;9:1613.

295. Kamphorst AO, Pillai RN, Yang S, Nasti TH, Akondy RS, Wieland A, et al. Proliferation of PD-1+ CD8 T cells in peripheral blood after PD-1–targeted therapy in lung cancer patients. Proceedings of the National Academy of Sciences. 2017 May 9;114(19):4993–8.

296. Fanale D, Dimino A, Pedone E, Brando C, Corsini LR, Filorizzo C, et al. Prognostic and Predictive Role of Tumor-Infiltrating Lymphocytes (TILs) in Ovarian Cancer. Cancers (Basel). 2022 Sep 6;14(18):4344.

297. Gonzalez H, Hagerling C, Werb Z. Roles of the immune system in cancer: from tumor initiation to metastatic progression. Genes Dev. 2018 Oct 1;32(19–20):1267–84.

298. Gong J, Chehrazi-Raffle A, Reddi S, Salgia R. Development of PD-1 and PD-L1 inhibitors as a form of cancer immunotherapy: a comprehensive review of registration trials and future considerations. J Immunother Cancer. 2018 Dec 23;6(1):8.

299. Dumitru A, Dobrica EC, Croitoru A, Cretoiu SM, Gaspar BS. Focus on PD-1/PD-L1 as a Therapeutic Target in Ovarian Cancer. Int J Mol Sci. 2022 Oct 11;23(20):12067.

300. Lozano-Ojalvo D, López-Fandiño R, López-Expósito I. PBMC-Derived T Cells. In: The Impact of Food Bioactives on Health. Cham: Springer International Publishing; 2015. p. 169–80.

301. Reading JL, Gálvez-Cancino F, Swanton C, Lladser A, Peggs KS, Quezada SA. The function and dysfunction of memory CD8 $^{+}$ T cells in tumor immunity. Immunol Rev. 2018 May;283(1):194–212.

302. Thornton AM, Shevach EM. CD4+CD25+ Immunoregulatory T Cells Suppress Polyclonal T Cell Activation In Vitro by Inhibiting Interleukin 2 Production. Journal of Experimental Medicine. 1998 Jul 20;188(2):287–96.

303. Homma Y, Taniguchi K, Nakazawa M, Matsuyama R, Mori R, Takeda K, et al. Changes in the immune cell population and cell proliferation in peripheral blood after gemcitabine-based chemotherapy for pancreatic cancer. Clinical and Translational Oncology. 2014 Mar 17;16(3):330–5.

304. Peng DJ, Liu R, Zou W. Regulatory T Cells in Human Ovarian Cancer. J Oncol. 2012;2012:1–7.

305. Saleh R, Elkord E. FoxP3+ T regulatory cells in cancer: Prognostic biomarkers and therapeutic targets. Cancer Lett. 2020 Oct;490:174–85.

306. Goda N, Sasada S, Shigematsu H, Masumoto N, Arihiro K, Nishikawa H, et al. The ratio of CD8 + lymphocytes to tumor-infiltrating suppressive FOXP3 + effector regulatory T cells is associated with treatment response in invasive breast cancer. Discover Oncology. 2022 Dec 19;13(1):27.

307. Baras AS, Drake C, Liu JJ, Gandhi N, Kates M, Hoque MO, et al. The ratio of CD8 to Treg tumor-infiltrating lymphocytes is associated with response to cisplatin-based neoadjuvant chemotherapy in patients with muscle invasive urothelial carcinoma of the bladder. Oncoimmunology. 2016 May 3;5(5):e1134412.

308. Preston CC, Maurer MJ, Oberg AL, Visscher DW, Kalli KR, Hartmann LC, et al. The Ratios of CD8+ T Cells to CD4+CD25+ FOXP3+ and FOXP3- T Cells Correlate with Poor Clinical Outcome in Human Serous Ovarian Cancer. PLoS One. 2013 Nov 14;8(11):e80063.

309. Patel AA, Zhang Y, Fullerton JN, Boelen L, Rongvaux A, Maini AA, et al. The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. Journal of Experimental Medicine. 2017 Jul 3;214(7):1913–23.

310. Olingy CE, Dinh HQ, Hedrick CC. Monocyte heterogeneity and functions in cancer. J Leukoc Biol. 2019 Jul 25;106(2):309–22.

311. Gardner A, de Mingo Pulido Á, Ruffell B. Dendritic Cells and Their Role in Immunotherapy. Front Immunol. 2020 May 21;11.

312. Napoletano C, Bellati F, Landi R, Pauselli S, Marchetti C, Visconti V, et al. Ovarian cancer cytoreduction induces changes in T cell population subsets reducing immunosuppression. J Cell Mol Med. 2010 Dec 6;14(12):2748–59.

313. Ye S, Chen W, Zheng Y, Wu Y, Xiang L, Li T, et al. Peripheral lymphocyte populations in ovarian cancer patients and correlations with clinicopathological features. J Ovarian Res. 2022 Dec 11;15(1):43.

314. Cassar E, Kartikasari AER, Plebanski M. Regulatory T Cells in Ovarian Carcinogenesis and Future Therapeutic Opportunities. Cancers (Basel). 2022 Nov 8;14(22):5488.

315. Valverde CV. Infiltration of Regulatory T Lymphocytes Impair Clinical Outcome in Ovarian Cancer Patients. MOJ Immunol. 2016 Dec 9;4(5).

316. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med. 2004 Sep 22;10(9):942–9.

317. Liu M, Starenki D, Scharer CD, Silva-Sanchez A, Molina PA, Pollock JS, et al. Circulating Tregs Accumulate in Omental Tumors and Acquire Adipose-Resident Features. Cancer Immunol Res. 2022 May 3;10(5):641–55.

318. Kang DH, Chung C, Sun P, Lee DH, Lee SI, Park D, et al. Circulating regulatory T cells predict efficacy and atypical responses in lung cancer patients treated with PD-1/PD-L1 inhibitors. Cancer Immunology, Immunotherapy. 2022 Mar 18;71(3):579–88.

319. Verma C, Eremin JM, Robins A, Bennett AJ, Cowley GP, El-Sheemy MA, et al. Abnormal T regulatory cells (Tregs: FOXP3+, CTLA-4+), myeloid-derived suppressor cells (MDSCs: monocytic, granulocytic) and polarised T helper cell profiles (Th1, Th2, Th17) in women with large and locally advanced breast cancers undergoing neoadjuvant chemotherapy (NAC) and surgery: failure of abolition of abnormal treg profile with treatment and correlation of treg levels with pathological response to NAC. J Transl Med. 2013 Dec 15;11(1):16.

320. Wu M, Chen X, Lou J, Zhang S, Zhang X, Huang L, et al. Changes in regulatory T cells in patients with ovarian cancer undergoing surgery: Preliminary results. Int Immunopharmacol. 2017 Jun;47:244–50.

321. He Q, Li G, Ji X, Ma L, Wang X, Li Y, et al. Impact of the immune cell population in peripheral blood on response and survival in patients receiving neoadjuvant chemotherapy for advanced gastric cancer. Tumor Biology. 2017 May 5;39(5):101042831769757.

322. Sánchez-Margalet V, Barco-Sánchez A, Vilariño-García T, Jiménez-Cortegana C, Pérez-Pérez A, Henao-Carrasco F, et al. Circulating regulatory T cells from breast cancer patients in response to neoadjuvant chemotherapy. Transl Cancer Res. 2019 Feb;8(1):59–65.

323. Toker A, Nguyen LT, Stone SC, Yang SYC, Katz SR, Shaw PA, et al. Regulatory T Cells in Ovarian Cancer Are Characterized by a Highly Activated Phenotype Distinct from that in Melanoma. Clinical Cancer Research. 2018 Nov 15;24(22):5685–96.

324. Tan CL, Kuchroo JR, Sage PT, Liang D, Francisco LM, Buck J, et al. PD-1 restraint of regulatory T cell suppressive activity is critical for immune tolerance. Journal of Experimental Medicine. 2021 Jan 4;218(1).

325. Banerjee H, Nieves-Rosado H, Kulkarni A, Murter B, McGrath K V., Chandran UR, et al. Expression of Tim-3 drives phenotypic and functional changes in Treg cells in secondary lymphoid organs and the tumor microenvironment. Cell Rep. 2021 Sep;36(11):109699.

326. Gianchecchi E, Fierabracci A. Inhibitory Receptors and Pathways of Lymphocytes: The Role of PD-1 in Treg Development and Their Involvement in Autoimmunity Onset and Cancer Progression. Front Immunol. 2018 Oct 17;9.

327. Gambichler T, Schröter U, Höxtermann S, Susok L, Stockfleth E, Becker JC. Decline of programmed death-1-positive circulating T regulatory cells predicts more favourable clinical outcome of patients with melanoma under immune checkpoint blockade. British Journal of Dermatology. 2020 May 27;182(5):1214–20.

328. Kang DH, Chung C, Sun P, Lee DH, Lee SI, Park D, et al. Circulating regulatory T cells predict efficacy and atypical responses in lung cancer patients treated with PD-1/PD-L1 inhibitors. Cancer Immunology, Immunotherapy. 2022 Mar 18;71(3):579–88.

329. Martens A, Wistuba-Hamprecht K, Foppen MG, Yuan J, Postow MA, Wong P, et al. Baseline Peripheral Blood Biomarkers Associated with Clinical Outcome

of Advanced Melanoma Patients Treated with Ipilimumab. Clinical Cancer Research. 2016 Jun 15;22(12):2908–18.

330. Simeone E, Gentilcore G, Giannarelli D, Grimaldi AM, Caracò C, Curvietto M, et al. Immunological and biological changes during ipilimumab treatment and their potential correlation with clinical response and survival in patients with advanced melanoma. Cancer Immunology, Immunotherapy. 2014 Jul 3;63(7):675–83.

331. Ye S, Chen W, Zheng Y, Wu Y, Xiang L, Li T, et al. Peripheral lymphocyte populations in ovarian cancer patients and correlations with clinicopathological features. J Ovarian Res. 2022 Apr 11;15(1):43.

332. Yang C, Lee H, Jove V, Deng J, Zhang W, Liu X, et al. Prognostic significance of B-cells and pSTAT3 in patients with ovarian cancer. PLoS One. 2013;8(1):e54029.

333. Wolf NK, Kissiov DU, Raulet DH. Roles of natural killer cells in immunity to cancer, and applications to immunotherapy. Nat Rev Immunol. 2023 Feb 30;23(2):90–105.

334. Henriksen JR, Nederby L, Donskov F, Waldstrøm M, Adimi P, Jakobsen A, et al. Blood natural killer cells during treatment in recurrent ovarian cancer. Acta Oncol (Madr). 2020 Nov 1;59(11):1365–73.

335. Henriksen JR, Donskov F, Waldstrøm M, Jakobsen A, Hjortkjaer M, Petersen CB, et al. Favorable prognostic impact of Natural Killer cells and T cells in high-grade serous ovarian carcinoma. Acta Oncol (Madr). 2020 Jun 2;59(6):652–9.

336. Hirooka S, Yanagimoto H, Satoi S, Yamamoto T, Toyokawa H, Yamaki S, et al. The role of circulating dendritic cells in patients with unresectable pancreatic cancer. Anticancer Res. 2011 Nov;31(11):3827–34.

337. Carenza C, Franzese S, Calcaterra F, Mavilio D, Della Bella S. Comprehensive Phenotyping of Dendritic Cells in Cancer Patients by Flow Cytometry. Cytometry Part A. 2021 Mar 11;99(3):218–30.

338. Bol KF, Schreibelt G, Rabold K, Wculek SK, Schwarze JK, Dzionek A, et al. The clinical application of cancer immunotherapy based on naturally circulating dendritic cells. J Immunother Cancer. 2019 Dec 18;7(1):109.

339. Yanagimoto H, Takai S, Satoi S, Toyokawa H, Takahashi K, Terakawa N, et al. Impaired function of circulating dendritic cells in patients with pancreatic cancer. Clinical Immunology. 2005 Jan;114(1):52–60.

340. Principe DR, Chiec L, Mohindra NA, Munshi HG. Regulatory T-Cells as an Emerging Barrier to Immune Checkpoint Inhibition in Lung Cancer. Front Oncol. 2021 Jun 1;11.

341. Mabuchi S, Sasano T, Komura N. Targeting Myeloid-Derived Suppressor Cells in Ovarian Cancer. Cells. 2021 Feb 5;10(2):329.

342. Kotsakis A, Harasymczuk M, Schilling B, Georgoulias V, Argiris A, Whiteside TL. Myeloid-derived suppressor cell measurements in fresh and cryopreserved blood samples. J Immunol Methods. 2012 Jul;381(1–2):14–22.

343. Komura N, Mabuchi S, Shimura K, Yokoi E, Kozasa K, Kuroda H, 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. 2020 Dec 19;69(12):2477–99.

344. Li X, Wang J, Wu W, Gao H, Liu N, Zhan G, et al. Myeloid-derived suppressor cells promote epithelial ovarian cancer cell stemness by inducing the CSF2/p-STAT3 signalling pathway. FEBS J. 2020 Dec 17;287(23):5218–35.

345. Santegoets SJAM, de Groot AF, Dijkgraaf EM, Simões AMC, van der Noord VE, van Ham JJ, et al. The blood mMDSC to DC ratio is a sensitive and easy to assess independent predictive factor for epithelial ovarian cancer survival. Oncoimmunology. 2018 May 2;e1465166.

346. Okła K. Myeloid-Derived Suppressor Cells (MDSCs) in Ovarian Cancer—Looking Back and Forward. Cells. 2023 Jul 22;12(14):1912.

347. Tay RE, Richardson EK, Toh HC. Revisiting the role of CD4+ T cells in cancer immunotherapy—new insights into old paradigms. Cancer Gene Ther. 2021 Feb 27;28(1–2):5–17.

348. Nagl S, Haas M, Lahmer G, Büttner-Herold M, Grabenbauer GG, Fietkau R, et al. Cell-to-cell distances between tumor-infiltrating inflammatory cells have the potential to distinguish functionally active from suppressed inflammatory cells. Oncoimmunology. 2016 May 3;5(5):e1127494.

349. Accogli T, Bruchard M, Végran F. Modulation of CD4 T Cell Response According to Tumor Cytokine Microenvironment. Cancers (Basel). 2021 Jan 20;13(3):373.

350. Cheng X, Wang H, Wang Z, Zhu B, Long H. Tumor-associated myeloid cells in cancer immunotherapy. J Hematol Oncol. 2023 Jul 6;16(1):71.

351. Sun D, Luo T, Dong P, Zhang N, Chen J, Zhang S, et al. CD86 $^{+}$ /CD206 $^{+}$ tumor-associated macrophages predict prognosis of patients with intrahepatic cholangiocarcinoma. PeerJ. 2020 Jan 22;8:e8458.

352. Fitzsimmons TS, Singh N, Walker TDJ, Newton C, Evans DGR, Crosbie EJ, et al. Immune checkpoint inhibitors efficacy across solid cancers and the utility of PD-L1 as a biomarker of response: a systematic review and meta-analysis. Front Med (Lausanne). 2023 May 12;10.

353. Lawson BC, Euscher ED, Bassett RL, Liu J, Ramalingam P, Zhong Y, et al. A 3-Tier Chemotherapy Response Score for Ovarian/Fallopian Tube/Peritoneal High-grade Serous Carcinoma. American Journal of Surgical Pathology. 2020 Feb;44(2):206–13.

354. Clayton KL, Haaland MS, Douglas-Vail MB, Mujib S, Chew GM, Ndhlovu LC, et al. T Cell Ig and Mucin Domain–Containing Protein 3 Is Recruited to the Immune Synapse, Disrupts Stable Synapse Formation, and Associates with Receptor Phosphatases. The Journal of Immunology. 2014 Jan 15;192(2):782–91.

355. Sawada M, Goto K, Morimoto-Okazawa A, Haruna M, Yamamoto K, Yamamoto Y, et al. PD-1+ Tim3+ tumor-infiltrating CD8 T cells sustain the potential for IFN-γ production, but lose cytotoxic activity in ovarian cancer. Int Immunol. 2020 May 30;32(6):397–405.

356. Fucikova J, Rakova J, Hensler M, Kasikova L, Belicova L, Hladikova K, et al. TIM-3 Dictates Functional Orientation of the Immune Infiltrate in Ovarian Cancer. Clinical Cancer Research. 2019 Aug 1;25(15):4820–31.

357. Cai L, Li Y, Tan J, Xu L, Li Y. Targeting LAG-3, TIM-3, and TIGIT for cancer immunotherapy. J Hematol Oncol. 2023 Sep 5;16(1):101.

358. Yan J, Zhang Y, Zhang JP, Liang J, Li L, Zheng L. Tim-3 Expression Defines Regulatory T Cells in Human Tumors. PLoS One. 2013 Mar 5;8(3):e58006.

359. Accogli T, Bruchard M, Végran F. Modulation of CD4 T Cell Response According to Tumor Cytokine Microenvironment. Cancers (Basel). 2021 Jan 20;13(3):373.

360. Shao S, Miao H, Ma W. Unraveling the enigma of tumor-associated macrophages: challenges, innovations, and the path to therapeutic breakthroughs. Front Immunol. 2023 Nov 14;14.

361. Feng Y, Xiao M, Cao G, Liu H, Li Y, Wang S, et al. Human monocytes differentiate into tumor-associated macrophages upon SKOV3 cells coculture and/or lysophosphatidic acid stimulation. J Inflamm. 2022 Dec 16;19(1):11.

362. Cotechini T, Atallah A, Grossman A. Tissue-Resident and Recruited Macrophages in Primary Tumor and Metastatic Microenvironments: Potential Targets in Cancer Therapy. Cells. 2021 Apr 20;10(4):960.

363. Gao J, Liang Y, Wang L. Shaping Polarization Of Tumor-Associated Macrophages In Cancer Immunotherapy. Front Immunol. 2022 Jun 30;13.

364. Pittet MJ, Michielin O, Migliorini D. Clinical relevance of tumour-associated macrophages. Nat Rev Clin Oncol. 2022 Jun 30;19(6):402–21.

365. Zhang J, Li S, Liu F, Yang K. Role of CD68 in tumor immunity and prognosis prediction in pan-cancer. Sci Rep. 2022 May 12;12(1):7844.

366. Blanc-Durand F, Clemence Wei Xian L, Tan DSP. Targeting the immune microenvironment for ovarian cancer therapy. Front Immunol. 2023 Dec 18;14.

367. Davis AA, Patel VG. The role of PD-L1 expression as a predictive biomarker: an analysis of all US Food and Drug Administration (FDA) approvals of immune checkpoint inhibitors. J Immunother Cancer. 2019 Dec 26;7(1):278.

368. Li H, van der Merwe PA, Sivakumar S. Biomarkers of response to PD-1 pathway blockade. Br J Cancer. 2022 Jun 1;126(12):1663–75.

369. Abiko K, Mandai M, Hamanishi J, Yoshioka Y, Matsumura N, Baba T, et al. PD-L1 on Tumor Cells Is Induced in Ascites and Promotes Peritoneal Dissemination of Ovarian Cancer through CTL Dysfunction. Clinical Cancer Research. 2013 Mar 15;19(6):1363–74.

370. Alwosaibai K, Aalmri S, Mashhour M, Ghandorah S, Alshangiti A, Azam F, 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 Jan 5;23(1):13.

371. Guo L, Song P, Xue X, Guo C, Han L, Fang Q, et al. Variation of Programmed Death Ligand 1 Expression After Platinum-based Neoadjuvant Chemotherapy in Lung Cancer. Journal of Immunotherapy. 2019 Jul;42(6):215–20.

372. Zens P, Bello C, Scherz A, von Gunten M, Ochsenbein A, Schmid RA, et al. The effect of neoadjuvant therapy on PD-L1 expression and CD8+lymphocyte density in non-small cell lung cancer. Modern Pathology. 2022 Dec;35(12):1848–59.

373. Moldvay J, Rojkó L, Téglási V, Fábián K, Pipek O, Vágvölgyi A, et al. P2.04-08 Platinum-Based Chemotherapy is Associated with Altered PD-L1 Expression in Lung Cancer. Journal of Thoracic Oncology. 2018 Oct;13(10):S733.

374. Böhm S, Montfort A, Pearce OMT, Topping J, Chakravarty P, Everitt GLA, et al. Neoadjuvant Chemotherapy Modulates the Immune Microenvironment in Metastases of Tubo-Ovarian High-Grade Serous Carcinoma. Clinical Cancer Research. 2016 Jun 15;22(12):3025–36.

375. Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, et al. Incorporation of Bevacizumab in the Primary Treatment of Ovarian Cancer. New England Journal of Medicine. 2011 Dec 29;365(26):2473–83.

376. González-Martín A, Pothuri B, Vergote I, Graybill W, Lorusso D, McCormick CC, et al. Progression-free survival and safety at 3.5 years of follow-up: results from the randomised phase 3 PRIMA/ENGOT-OV26/GOG-3012 trial of niraparib maintenance treatment in patients with newly diagnosed ovarian cancer. Eur J Cancer. 2023 Aug;189:112908.

377. Baert T, Ferrero A, Sehouli J, O'Donnell DM, González-Martín A, Joly F, et al. The systemic treatment of recurrent ovarian cancer revisited. Annals of Oncology. 2021 Jun;32(6):710–25.

378. Lheureux S, Gourley C, Vergote I, Oza AM. Epithelial ovarian cancer. The Lancet. 2019 Mar;393(10177):1240–53.

379. Lan C, Huang X, Lin S, Huang H, Cai Q, Wan T, et al. Expression of M2-Polarized Macrophages is Associated with Poor Prognosis for Advanced Epithelial Ovarian Cancer. Technol Cancer Res Treat. 2013 Jun 1;12(3):259–67.

380. Truxova I, Cibula D, Spisek R, Fucikova J. Targeting tumor-associated macrophages for successful immunotherapy of ovarian carcinoma. J Immunother Cancer. 2023 Feb 23;11(2):e005968.

381. Li M, He L, Zhu J, Zhang P, Liang S. Targeting tumor-associated macrophages for cancer treatment. Cell Biosci. 2022 Dec 7;12(1):85.

382. Berckmans Y, Vankerckhoven A, Caro AA, Kempeneers J, Ceusters J, Thirion G, et al. TIM3 Checkpoint Inhibition Fails to Prolong Survival in Ovarian Cancer-Bearing Mice. Cancers (Basel). 2024 Mar 14;16(6):1147.

383. Guo Z, Cheng D, Xia Z, Luan M, Wu L, Wang G, et al. Combined TIM-3 blockade and CD137 activation affords the long-term protection in a murine model of ovarian cancer. J Transl Med. 2013;11(1):215.

384. Curigliano G, Gelderblom H, Mach N, Doi T, Tai D, Forde PM, et al. Phase I/Ib Clinical Trial of Sabatolimab, an Anti–TIM-3 Antibody, Alone and in Combination with Spartalizumab, an Anti–PD-1 Antibody, in Advanced Solid Tumors. Clinical Cancer Research. 2021 Jul 1;27(13):3620–9.

385. Roberts, Cardenas, Tedja. The Role of Intra-Tumoral Heterogeneity and Its Clinical Relevance in Epithelial Ovarian Cancer Recurrence and Metastasis. Cancers (Basel). 2019 Jul 30;11(8):1083.

386. Smith P, Bradley T, Gavarró LM, Goranova T, Ennis DP, Mirza HB, et al. The copy number and mutational landscape of recurrent ovarian high-grade serous carcinoma. Nat Commun. 2023 Jul 20;14(1):4387.

387. BriTROC-2 [Internet]. https://ovarian.org.uk/our-research/research-we-fund/britroc-2/.

388. Grunewald T, Miller R, Tookman L, Mcneish I, Roux R, Farrelly L, et al. 110 PROMPTS: phase II trial of maintenance pembrolizumab following weekly paclitaxel for recurrent ovarian, fallopian tube or peritoneal cancer. In: Poster and E-Posters. BMJ Publishing Group Ltd; 2024. p. A292.2-A292.

Author contribution statement

Contributors to the PROMPT study, in particular, Professor Jonathan Ledermann, Chief Investigator and all members of the TMG, CTC – in particular Laura Hughes, Trials co-ordinator and Nicholos Counsell, Statistician, who provided the paragraphs explaining the statistical analysis, all the survival and AE data and the KM curves.

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Appendix

PROMPT Abstract (388)

PROMPT: Phase II trial of maintenance pembrolizumab following weekly paclitaxel for recurrent ovarian, fallopian tube or peritoneal cancer

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Introduction/Background

Most women with advanced ovarian cancer eventually develop platinum-resistant disease, which has a poor outcome. The median progression-free survival (PFS) is around 3-4 months and overall survival (OS) approximately 12 months. We have explored the addition of maintenance pembrolizumab to extend PFS in patients who do not progress on weekly paclitaxel.

Methodology

Patients were enrolled in a phase II single-arm study with response or stable disease after a minimum of 4 cycles of weekly paclitaxel. They were treated with pembrolizumab 200mg IV q21 days until progression, unacceptable toxicity, patient or clinician decision. Primary endpoint was 6-month PFS from start of pembrolizumab, targeting a rate of 65% whilst <40% would be of no further interest (N=28). Secondary endpoints included OS, disease response (RECIST v1.1), toxicity and compliance.

Results

Twenty patients with high grade serous carcinoma were enrolled and evaluable. Median age 61 years (range 41-78), ECOG 0/1 50%/50%. 85% of patients had stage III/IV at diagnosis and had received a median of 5.5 prior cycles (4-17) weekly paclitaxel, with 35% achieving partial response and 65% stable disease. A median of 3.5 cycles (2-18) of pembrolizumab were given; 19 patients (95%) stopped due to progression and 1 discontinued due to a treatment-related adverse event (TRAE) of hepatitis. Two (10%) patients had 3 grade 3 TRAEs: rash; hepatitis and diarrhoea. There were no grade 4/5 TRAEs. After a median follow-up of 16.5 months, median PFS from the start of pembrolizumab was 2.0 months (95%CI: 1.8-3.6) and median OS was 9.8 months (95%CI: 6.2-20.7). As the 6-month PFS rate was 5.0% (95%CI: 0.3-20.5) the trial stopped early due to futility.

Conclusion

Maintenance pembrolizumab did not improve PFS in patients with platinum-resistant ovarian cancer with non-progressive disease after paclitaxel. Translational research is crucial to understand why most patients with ovarian cancer do not benefit from immune checkpoint inhibitors.