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THE EFFECT OF THE PERITONEAL TUMOR MICROENVIRONMENT ON INVASION OF PERITONEAL METASTASES OF HIGH-GRADE SEROUS OVARIAN CANCER AND THE IMPACT OF NEOADJUVANT CHEMOTHERAPY

J.O.A.M. van Baal[1], C.A.R. Lok[1], E.S. Jordanova[1], H. Horlings[2], W.J. van Driel[1], F.C. Amant[1], K.K. Van de Vijver[2,3]

1 Department of Gynecology, Center for Gynecologic Oncology Amsterdam, P.O. Box 90203, 1006 BE, Amsterdam, The Netherlands.
2 The Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Division of Diagnostic Oncology & Molecular Pathology, Amsterdam, The Netherlands
3 Department of Pathology, Ghent University Hospital, Cancer Research Institute Ghent (CRIG), Ghent, Belgium

Corresponding author: J.O.A.M. van Baal
Address: Plesmanlaan 121, P.O. Box 90203, 1006 BE, Amsterdam, the Netherlands
Telephone: +3120512975, Fax: +31205126105, Electronic address: j.v.baal@nki.nl

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ABSTRACT

Introduction. Peritoneal metastases of high-grade serous ovarian cancer (HGSOC) are small-sized deposits with superficial growth towards the peritoneal cavity. It is unknown whether integrity of the peritoneal elastic lamina (PEL) correlates with the peritoneal tumor microenvironment (pTME) and whether neoadjuvant chemotherapy (NACT) affects the pTME. We explored integrity of PEL, composition of pTME, effects of NACT, and the prognostic implications in patients with extensive peritoneal metastases of HGSOC.

Methods. Peritoneal samples (n=69) were collected during cytoreductive surgery between 2003-2016. Clinical data were collected from medical charts. Integrity of PEL was evaluated with elastic stains. T-cell (CD3, CD8) and M2-macrophage markers (CD163) were scored using algorithms created in Definiens Tissue studio.

Results. Patients with a disrupted PEL (n=39; 57%), more often had residual disease after surgery (p=0.050), compared to intact PEL. An intact PEL was associated with increased intraepithelial (ie)CD8+ cells (p=0.032), but was not correlated with improved survival. After NACT, decreased stromal (s)CD3+ cells were shown, compared to no-NACT (p=0.044). Abundance of total CD3+ and CD8+ cells were associated with PFS (multivariate HR 0.40; 95%CI 0.23-0.69 and HR 0.49; 95%CI 0.29-0.83) and OS (HR 0.33; 95%CI 0.18-0.62 and HR 0.36; 95%CI 0.20-0.64). M2-macrophage infiltration was not correlated with survival.

Conclusion. NACT decreases abundance of sCD3+ cells in peritoneal metastases of HGSOC. Increase of CD3+ and CD8+ cells is associated with improved PFS and OS. This suggests that CD3+ and CD8+ cells may function as prognostic biomarkers. Their role as predictive biomarker for chemotherapy or immunotherapy response in HGSOC warrants further research.

KEYWORDS

Peritoneal elastic lamina, peritoneal tumor microenvironment, peritoneal metastases, high-grade serous ovarian cancer, tumor-infiltrating lymphocytes

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INTRODUCTION

Epithelial ovarian cancer (EOC) is the leading cause of death among gynecological malignancies. Prognosis is highly dependent on stage, histological subtype and tumor grade. High-grade serous ovarian carcinoma (HGSOC) is the most common histological subtype of EOC. The poor prognosis of HGSOC is primarily caused by the rapid and often unnoticed development of metastases, which are already present at diagnosis in approximately 80% of patients [1].

Peritoneal metastases are small-sized tumor depositions with only superficial invasion of the peritoneum, but they can be abundantly present in the peritoneal cavity. These tumor depositions grow towards the peritoneal cavity, rather than invading deeper layers of the abdominal wall. The peritoneum is composed of several structures, including the mesothelium, basal laminar and fibrovascular connective tissue [2]. Embedded within the connective tissue, the peritoneal elastic lamina (PEL) has been identified [2,3]. In patients with colon carcinoma, tumor invasion with an intact PEL has been associated with increased survival compared to tumor invasion with a fragmented PEL [4,5]. However, for peritoneal metastases of EOC, the prognostic relevance of the PEL and their correlation with depth of metastatic tumor invasion is unknown.

Apart from the interactions between EOC and distinctive peritoneal structures, the peritoneal tumor microenvironment (pTME) may play a role in the development and growth of peritoneal metastases of EOC [6]. CD3+ tumor infiltrating lymphocytes (TILs) and CD68+ macrophages have been shown to be abundantly present in peritoneal metastases [6]. Several studies indicate that high numbers of intraepithelial (ie)CD3+ and ieCD8+ cells in primary tumors of EOC are associated with a prolonged progression-free survival (PFS) and overall survival (OS) [7-9]. In contrast, increased CD163+ M2-macrophage numbers correlate with poor survival [10,11]. However, these studies focused on primary tumors rather than on peritoneal metastases. Whether pTME also influences the development and growth of peritoneal metastases and whether neoadjuvant chemotherapy (NACT) affects the integrity of PEL or the pTME, is yet unknown.

To understand the peculiar growth pattern of peritoneal metastases and its interaction with the pTME, improved knowledge of distinct peritoneal structures and their association with immune infiltrates in the presence of peritoneal metastases is needed. In the present study we explored the morphology of the PEL and the composition of pTME in presence of peritoneal metastases of HGSOC, and investigated the correlation with clinical characteristics.

MATERIALS AND METHODS

Patient selection

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Patients with extensive peritoneal carcinomatosis of HGSOC who were planned for cytoreductive surgery were eligible for our study. Following approval of the Institutional Review Board of the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital (NKI-AVL) and after patients' informed consent, 74 samples of peritoneal metastases were collected both prospectively and retrospectively during cytoreductive surgery between 2003-2016. All peritoneal biopsies were obtained from parietal peritoneal surfaces. Patients underwent either primary debulking surgery (PDS) or, if optimal cytoreduction seemed not feasible, were administered NACT followed by interval debulking surgery (NACT-IDS). All patients received adjuvant chemotherapy after cytoreductive surgery. Clinical data and patient characteristics were collected from patient hospital files. Data on vital status were extracted from the municipal population register on 25th of April, 2018.

Histochemical and immunohistochemical staining

On formalin-fixed and paraffin-embedded whole slide sections of peritoneal metastases of HGSOC, histochemical staining and immunohistochemical stainings were performed according to standard techniques.

For the purpose of histochemical staining, slides were deparaffinized and washed with 70% ethanol, after which they were incubated with Lawson solution at 37°C for 10 minutes. Slides were washed with 90% ethanol and demineralized water, after which they were incubated in Elastica Van Gieson (EVG) solution for 5 minutes. Slides were cleared in xylene and cover slipped.

Immunohistochemistry of the samples was performed on a BenchMark Ultra autostainer (Ventana Medical Systems). Briefly, paraffin sections were cut at 3 um, heated at 75°C for 28 minutes and deparaffinized in the instrument with EZ prep solution (Ventana Medical Systems). Heat-induced antigen retrieval was carried out using Cell Conditioning 1 (CC1, Ventana Medical Systems) for 32 minutes at 95°C (CD3, CD8), or 64 minutes at 95°C (CD163). CD3 was detected using clone SP7 (1/100 dilution, 32 minutes at 37°C, Spring / ITK), CD8 clone C8/144B (DAKO / Agilent) using 1/200 dilution 32 minutes at 37°C and CD163 was detected using clone MRQ-26 (Ready-to-use, 32 minutes at 36°C, Ventana Medical Systems). Bound antibody was detected using the UltraView DAB Detection kit (CD163) or OptiView DAB Detection Kit (CD3, CD8) (Ventana Medical Systems). Slides were counterstained with hematoxylin II and Bluing Reagent (Ventana Medical Systems).

Scoring of tumor morphology and integrity of PEL

Primary diagnosis of HGSOC were reviewed on hematoxylin and eosin (H&E) stained slides by a pathologist with expertise in gynecological malignancies (KVV). The following morphological characteristics were assessed; (1) predominant growth pattern, (2) mitotic rate, (3) presence of psammoma bodies, (4) lymphangio-invasive growth and (5) tertiary lymphoid structures. Using EVG histochemistry, presence and integrity of PEL was analyzed, categorizing all cases into either an intact PEL, or a disrupted PEL.

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Digital image analysis of CD3, CD8 and CD163 staining

Whole slide sections with peritoneal metastases stained for CD3, CD8 and CD163, were digitalized with a 20x magnification, using Leica Aperio AT2 Digital Pathology Slide Scanner (Leica Microsystems, Wetzlar, Germany). Digitalized slides were analyzed with a semi-automatic software, Definiens Tissue Studio (Munich, Germany). Within the Definiens software, regions of interest (ROIs) were indicated manually for each slide, according to recommendations of Hendry et al. [12,13]. On each slide, all tumor tissue and invasive margin of the tumor were selected. Stromal tissue other than stroma within tumor front, was excluded in further analyses. Necrotic tissue was avoided. The Tissue Studio software was trained to separate tumor regions and stroma regions, and to discriminate positively stained cells. After segmentation of all digitalized images, all detected cells were counted automatically by the Tissue Studio software and total number of positive cells within the tumor and within the stroma were calculated as number of total positive cells per mm^2.

Statistical analyses

CD3+, CD8+ and CD163+ cell counts were calculated as total number of positively stained cells per mm^2. Number of immune cells were analyzed both as continuous variables and as dichotomized variables using receiver-operating characteristic (ROC) curves to determine cut-off values to separate low density from high density of cells. Ratios of ieCD3+/CD8+ cells, sCD3+/CD8+ cells and total CD3+/CD8+ cells were calculated as number of CD3+ cells divided by number of CD8+ cells. Normally distributed continuous variables were analyzed with unpaired sample T-tests. Non-normally distributed continuous variables were analyzed with non-parametric Mann-Whitney U tests. For binary categorical variables, either Chi-square tests or Fisher's exact tests were used. For ordinal variables, linear-by-linear associations were performed. PFS was measured from date of the last chemotherapy course during primary treatment, until diagnosis of recurrent disease. Progression of disease was documented according to the Gynecological Cancer Intergroup (GCIG) criteria [14]: when patients had either symptomatic complaints in combination with increased serum CA125 levels, progressive disease documented by radiological imaging, or histocytological confirmation of recurrent disease. OS was calculated as duration of time between date of last chemotherapy course of initial treatment and date of death. Kaplan-Meier survival estimate curves were generated to demonstrate survival differences between patient cohorts. Univariate Cox regression analysis was carried out to assess relations between predictor variables and PFS and OS. In multivariable Cox regression analyses immune cell populations were analyzed after correction for NACT, FIGO stage and outcome of surgery. All statistical analyses were performed with IBM SPSS version 25.0 (SPSS Inc., Chicago, Illinois). P-values <0.05 were considered significant.

RESULTS

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Patients

Formalin-fixed, paraffin-embedded tissue of peritoneal metastasis was available from 74 patients. After central pathological review, five patients were excluded from further analyses due to histological diagnosis other than HGSOC. Mean age of the 69 remaining patients was 62 years (Table 1). NACT was administered to 44 patients (63.8%). Median follow-up duration of patients who were alive at the time of database closure, was 38.9 months (IQR 32.4-100.9). In total, 64 patients (92.8%) had recurrent disease and 54 patients (78.3%) had died by the end of the follow-up. The 44 patients who were not eligible for PDS and who received NACT-IDS, more often had FIGO stage IV disease (43% versus 4%; p<0.001; Table 1).

Peritoneal elastic lamina

Clinical characteristics

To study the relevance of depth of invasion of peritoneal metastases, elastic stains were evaluated. The PEL was found to be intact in 30 patients (53.5%) and was disrupted in 39 patients (56.5%) (Figure 1). Patients were stratified by integrity of PEL to explore the association with clinical characteristics. 21 patients (70%) who had an intact PEL, had no visible residual tumor (i.e. complete cytoreduction), whereas if the PEL was disrupted, only 17 patients (43.5%) had complete cytoreductive surgery (p=0.050; Table 1).

Morphological and immunological characteristics

Integrity of the PEL was not correlated with morphological characteristics including architectural growth pattern, mitotic rate, presence of psammoma bodies, lymphangio-invasive growth and tertiary lymphoid structures (Table 2). However, administration of NACT was associated with a low mitotic rate in peritoneal tumor cells (p<0.001).

With digital imaging analyses, we found high densities of ieCD8+ cells in the metastases with an intact PEL (median 210.5 cells; IQR 108.4-470.5), compared to peritoneal metastases with a disrupted PEL (105.3 cells; IQR 73.1-279.2; p=0.032; Table 3). Presence of other immune cell populations were similar between peritoneal metastases with an intact PEL and a disrupted PEL (Table 3).

To analyze the effect of NACT on the association between ieCD8+ cells and integrity of PEL, similar analyses were performed for patients who had either NACT-IDS or patients who underwent PDS. Of all patients with an intact PEL, those who had NACT-IDS showed similar number of ieCD8+ cells (median 278.3 cells; IQR 135.8-418.6) to patients who had PDS (229.9 cells; IQR 93.9-497.7; p=0.929). Similarly, patients with a fragmented PEL demonstrated no difference in ieCD8+ cells when stratified by treatment regimen. Median number of ieCD8+ cells after NACT-IDS was 121.2 cells (IQR 85.0-279.2) and 80.2 cells (IQR 35.3-412.5; p=0.270) after PDS.

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Peritoneal tumor microenvironment

Clinical characteristics

To explore the pTME in the presence of peritoneal metastases of HGSOC and its clinical consequences, various immune cell populations were studied with digital imaging analyses. Supplementary Table 1 summarizes numbers of CD3+, CD8+ and CD163+ cells and their correlations with various clinical characteristics. There was no correlation of any immune cell population with age, FIGO stage or outcome of surgery.

After NACT-IDS, lower densities of sCD3+ cells were observed (median 860.4 cells; IQR 453.1-1237.2) compared to those who had PDS (1265.5 cells; IQR 539.9-2075.8; p=0.044; Table 3). Furthermore, after NACT-IDS, ratio of sCD3+/sCD8+ cells were significantly lower than after PDS (1.3 cells; IQR 0.8-1.9 versus 1.8 cells; IQR 1.3-2.4; p=0.007).

Morphological characteristics

To explore the interaction between pTME and the morphology of peritoneal metastases, we analyzed correlations between various immune cell populations and morphological tumor characteristics. Tumors with a high mitotic rate showed higher densities of ieCD163+ (p=0.011) and sCD163+ cells (p=0.004) compared to tumors with a low mitotic rate. We found no other correlations between tumor morphology and pTME (Table 4).

Univariate and multivariable analyses for PFS and OS

For univariate and multivariable analyses, immune cells were dichotomized using ROC curves. The optimal number of immune cells cut-off values were 589 cells per mm^2 for ieCD3+ cells, 913 cells for sCD3+ cells, 1634 cells for total CD3+ cells, 152 cells for ieCD8+ cells, 522 cells for sCD8+ cells, 653 cells for total CD8+ cells, 783 cells for ieCD163+ cells, 1647 cells for sCD163+ cells, 2667 cells for total CD163+ cells to predict for vital status.

Univariate analyses showed that integrity of PEL was not correlated with PFS or OS (Supplementary Figure 1 and Table 5). In univariate analyses, FIGO stage IV, NACT and residual disease were significantly associated with poor PFS and OS. Abundance of CD3+ cells and CD8+ cells, either intraepithelial or stromal, but also total number of immune cells, were all associated with improved OS (Supplementary Figure 2 and Table 5).

Multivariable Cox-regression analyses for PFS and OS were performed adjusting for FIGO stage, NACT and outcome of surgery (Table 5). Density of both total CD3+ cells and total CD8+ cells were independently associated with PFS (HR 0.40; 95%CI 0.23-0.69 for CD3+ cells and HR 0.49; 95%CI 0.29-0.83 for CD8+ cells) and with OS (HR 0.33; 95%CI 0.18-0.62 for CD3+ cells and HR 0.36; 95%CI 0.20-0.64 for CD8+ cells).

DISCUSSION

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Peritoneal carcinomatosis is an ominous condition, often seen in recurrent disease, causing a high morbidity and mortality in women with EOC. The majority of these patients are not suitable for PDS and thus receive NACT-IDS to enhance chance of a surgical cytoreduction with no or <1 cm residual disease. Improved understanding of the biology and tumorigenesis of peritoneal metastases and their response to treatment is of paramount importance to understand clinical consequences and to develop new therapy strategies. In the present study we investigated the role of the PEL in peritoneal metastatic tumor growth and explored the pTME in patients with extensive peritoneal metastases of HGSOC who underwent either NACT-IDS or PDS. We found that high density of both CD3+ and CD8+ cells in peritoneal metastases of HGSOC are independently associated with improved PFS and OS.

We explored the integrity of the PEL and showed that in 57% of patients, the PEL was disrupted with tumor growth beyond the PEL. Remarkably, in patients with an intact PEL a complete cytoreduction was achieved more often compared to patients with a disrupted PEL (70% versus 44%). Possibly, a disrupted PEL reflects a more invasive growth pattern of metastatic HGSOC, which may be more difficult to remove completely during cytoreductive surgery. Complete cytoreduction in patients with advanced EOC is strongly associated with improved PFS and OS [15-17]. We demonstrated no survival difference between patients with an intact PEL and patients with a disrupted PEL. Therefore, the integrity of the PEL cannot be used as a prognostic marker as it is for colorectal cancer [18-22]. However, this study involved a relatively small patient cohort. Hypothetically, integrity of PEL demonstrated in a biopsy taken prior to treatment, could function as a biomarker to predict outcome of surgery and could help in the decision which patient would be eligible for PDS and which patient would benefit from NACT-IDS, as achieving a complete PDS may be less likely. However, this hypothesis will have to be investigated in a prospective study.

Infiltrating immune cells exhibit ambivalent functions in tumorigenesis [23,24]. CD8+ TILs, T-helper 1 cells and natural killer cells often correspond to a tumor-suppressive microenvironment associated with survival advantage. In contrast, T-helper 2 cells, regulatory T-cells and so-called polarized M2-macrophages are considered to create a tumor-supportive milieu which contributes to tumor growth and progression, and subsequent poor survival. Prognostic significance of TILs has been generally accepted. However, the exact location and characterization of these prognostic TILs remains ambiguous. A recent meta-analysis of Li et al. showed that in primary EOC presence of ieCD3+ TILs and ieCD8+ TILs is associated with improved survival [25]. Our results of TILs in peritoneal metastases of HGSOC are in line with previous findings in primary ovarian tumors, confirming the prognostic associations of intraepithelial, stromal and total CD3+ and CD8+ cells. Until now, data on immune landscape of EOC focuses mainly on primary ovarian tumors. Studies on the pTME of peritoneal metastases of EOC are scarce, and to our knowledge, no study focused on peritoneal metastases of HGSOC or differentiated between ieTILs and sTILs.

Both platinum-based and taxane-based chemotherapy are capable to reduce the immunosuppressive mechanisms induced by EOC and on the other hand augment anti-tumor immune

responses. In our study, we evaluated the effect of NACT on the pTME. After NACT, we observed a significant decrease of sCD3+ cells and ratio sCD3+/CD8+ cells, suggesting that NACT particularly has an impact on CD3+CD8- immune cells. In literature, conflicting data is reported on the presence of TILs after NACT on primary EOC. Mesnage et al. [26] analyzed 83 paired primary tumors of EOC, pre- and post-NACT, and reported an increase in sTILs but no change in ieTILs after the administration of NACT. Remarkably, 51% showed an increase of sTILs, but 25% demonstrated a decrease and 24% showed stable amounts of sTILs after NACT. Furthermore, from two studies performed on paired samples of HGSOC, Lo et al. [27] and Bohm et al. [28] showed no differences in CD3+ or CD8+ cells in either primary tumors or omental metastases after administration of NACT. In contrast, Polcher et al. [29] and Peng et al. [30] showed increased numbers of CD8+ cells in EOC after administration of NACT. Whether these results on primary tumors and omental metastases are similar to the effects of NACT on the pTME is yet unclear. However, together with these studies, our results imply that NACT has a versatile effect on the immune cell composition in both primary and peritoneal metastatic HGSOC, which probably reflects the heterogeneity of HGSOC.

Besides the tumor-suppressive immune cells, we analyzed immune cells which are considered to support the tumor growth. Our results on CD163+ cells demonstrated that high density of these immune cells is associated with significant higher mitotic rate of tumor cells in peritoneal metastases. The high mitotic rate which generally characterizes HGSOC, is negatively affected by chemotherapy. The correlation between increased mitotic rate in presence of high density of CD163+ cells therefore could be correlated with NACT-response. However, we could not demonstrate survival effects of abundance of CD163+ cells.

EOC is a malignancy with a high mortality rate, which has hardly changed over the past decades. New treatment strategies are urgently necessary. Ideally, TILs could function as biomarkers to select optimal treatment strategy for patients with EOC. For melanoma patients, presence of sCD8+ TILs predicted response to immunotherapy with checkpoint inhibitor pembroluzimab accurately [31]. It would be of great interest to investigate in future immunotherapy trials, whether HGSOC patients with presence of high numbers of TILs in peritoneal metastases are more likely to respond to immunotherapy as well.

In our study, samples of peritoneal biopsies were collected from parietal peritoneum, from preferred localizations of metastatic EOC, including the pelvic peritoneum or the paracolic gutter. However, difference of composition of immune cell infiltrates or aspect of the PEL may differ between various areas in the peritoneal cavity. For example, the PEL in the peritoneal cavity is shown to exhibit a variable thickness, depending on movements of underlying organs [3]. Whether thickness of the PEL influences the integrity is unknown.

In literature, various scoring methodology have been used to quantify immune cell populations and optimal quantification of the immune landscape remains a matter of debate. Li et al. [25] demonstrated the various techniques that are used in different large studies on immune landscape and

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prognosis of EOC. HGSOC is characterized by molecular and immunological heterogeneity. To study immune cell infiltrates in HGSOC, whole-slide analyses is preferred over tissue-microarray. In our study immune cells were scored on whole slides by digital imaging. Digital imaging analysis minimizes bias due to interobserver variance. Several studies confirmed the accuracy of digital imaging analysis for objectifying number of immune cells on immunohistochemically stained formalin-fixed slides [32,33]. In our study, we found that digitally analyzed immune cells were significantly correlated with survival, Therefore, our data supports the use of digital imaging analyses for scoring various immune cell populations.

In conclusion, knowledge of the pathophysiology of peritoneal metastases is of utmost importance, yet understanding of pTME in peritoneal metastases is limited. The present study provides novel insights into the interplay between metastatic HGSOC and the peritoneum. Our study shows that sCD3+ cells in peritoneal metastases are decreased after NACT compared to chemo-naive patients. Furthermore, high density of both CD3+ and CD8+ cells in peritoneal metastases of HGSOC are independently associated with improved OS and PFS. Therefore, these TILs may create a tumor-suppressive milieu and theoretically function as a prognostic biomarker, and potentially as a biomarker for response to immunotherapy. Further refinement of TILs-profiles after NACT may optimize selection of patients for immunotherapy trials.

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COMPLIANCE WITH ETHICAL STANDARDS

The present study was approved by the Institutional Review Board of the NKI-AVL (PTC15.0324/M14BBB).

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed to research goals and study design. JB, HH and KV performed histopathological revision and immune scoring of samples. JB and EJ performed digital imaging analyses. JB, EJ, HH and KV contributed to interpretation of data. Acquisition of data, analyses and writing first draft of the paper was performed by JB and CL. All authors have read and approved the final version of the manuscript.

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FIGURE CAPTIONS

Fig. 1

Histopathological images of peritoneal metastases of HGSOC

A. and C. Hematoxylin and eosin staining of peritoneum demonstrating peritoneal metastasis of HGSOC (PM). **B.** Elastica van Gieson staining demonstrating a continuous intact peritoneal elastic lamina (arrows). Growth of tumor cells is not beyond the elastic lamina. **D.** Elastica van Gieson staining demonstrates a disrupted PEL and tumor growth beyond the PEL (arrows).

Supplementary Fig. 1

Kaplan-Meier survival estimate curve demonstrating OS of patients with an intact PEL and patients with a disrupted PEL

Events of death between tumors with an intact PEL (n=21; 70.0%) and tumors with a disrupted PEL (n=33; 84.6%; p=0.238) were similar. Patients with an intact PEL showed a median OS of 31.7 months

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(95%CI 17.3-46.0), compared with 28.0 months (95%CI 20.7-35.3; Log Rank 1.683; p=0.195) for patients with a disrupted PEL. After stratification for high density of ieCD8+ cells, OS was similar between patients with an intact PEL (31.3 months; 95%CI 28.9-33.6) and patients with a disrupted PEL (32.6 months; 95%CI 18.6-46.5; Log Rank 0.055; p=0.814)

Supplementary Fig. 2

Kaplan-Meier survival estimate curves for OS depicting differences between immune cell populations

Kaplan-Meier survival curves for OS showed a significant improved survival of all patients with high densities of intraepithelial, stromal and total CD3+ and CD8+ cells compared to low densities. Patients with high density ieCD3+ cells showed median OS of 39.9 months (95%CI 9.8-69.9) compared to 27.3 months (95%CI 18.5-36.1) in patients with low density ieCD3+ cells. Patients with high sCD3+ cells showed an OS of 41.4 months (95%CI 15.2-67.5) compared with 27.3 months (95%CI 19.0-35.7) in those with low density sCD3+ cells. High total CD3+ cells corresponded with an OS of 41.4 months (95%CI 14.2-68.6) compared with 27.3 months (95%CI 18.0-36.7) for low density total CD3+ cells. Patients with high ieCD8+ cells showed an OS of 32.6 months (95%CI 23.3-41.9) compared with 24.1 months (95%CI 13.6-34.6) in patients with low density ieCD8+ cells. Patients with high sCD8+ cells demonstrated an OS of 41.4 months (15.3-67.5) compared with 21.1 months (13.2-29.0) for patients with low sCD8+ cells. High total CD8+ cells corresponded with an OS of 38.1 months (24.9-51.3) compared to 21.1 months (11.4-30.8) for low density total CD8+ cells. No survival differences were observed in patients with different numbers of CD163+ cells in the peritoneal metastases.