**Title:** Spatial context of ovarian cancer tumor-infiltrating immune cells associates with improved survival

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**Running Title: Immune Cells Spatial Interactions in the TME**

**Key words:** spatial statistics, tumor microenvironment, Vectra Polaris, ovarian cancer, gynecological malignancy, high-grade serous carcinoma, cox proportional hazard models, Kaplan-Meier, tissue microarray

**Abstract**

Ovarian cancer is the deadliest gynecological malignancy and high-grade serous carcinoma (HGSOC) is the most common histotype. HGSOC is primarily deadly due to low early detection rates and the high prevalence of acquired therapy resistance. Multi-omics techniques have provided a platform for improved predictive modeling of therapy response and patient outcomes. Further, while HGSOC tumors are immunogenic and numerous studies have defined positive correlation to immune cell infiltration, immunotherapies have exhibited low efficacy rates in clinical trials. There is a significant need to better comprehend the role and composition of immune cells in mediating ovarian cancer therapeutic response and progression. We performed multiplex immunohistochemistry with an HGSOC tissue microarray (n=127) to characterize the immune cell composition within tumors. We analyzed the composition and spatial context of T cells (CD4/CD8), macrophages (CD68), and B cells (CD19) within the tumor. While CD8 T cell and macrophage tumor infiltration did not correlate with overall survival, we found that increased B cell and CD4 T cell presence did correlate with overall survival. More importantly, we observed that the close proximity between tumor-associated macrophages and B cells or CD4 T cells was correlated with overall survival. The results highlight the anti-tumor role of B cells and CD4 T cells, and that the spatial interactions between immune cell types are a novel predictor of therapeutic response and patient outcomes.

**Statement of Implication**

This is the first study to examine the spatial relationship of HGSOC tumors with clinical attributes like overall survival; we discovered that a “high” interaction between tumor-associated B cells and macrophages significantly correlates to survival.

**Introduction**

Ovarian cancer is the most lethal gynecologic malignancy, with over 295,400 new diagnoses and over 184,000 deaths per year, globally [1]. While most patients with high grade serous ovarian cancer (HGSOC) will respond to first-line treatment, a combination of cytoreductive surgery and platinum/taxane based chemotherapy, over 75% will recur and develop therapy-resistant disease [2]. More recently, advanced stage disease (stage IV) is treated with neoadjuvant chemotherapy (NACT) prior to cytoreductive surgery, to facilitate optimal tumor resection. The five-year overall survival rate of patients with advanced stage HGSOC is lower than 30% [3]. The ability of the immune system to mount an anti-tumor response directly correlates to patient outcomes [4]. However, current strategies to induce anti-tumor immunity in ovarian cancer (e.g., immune checkpoint blockade) have only had a minimal impact in HGSOC management [5]. Like most tumors, the microenvironment of HGSOC tumors is heterogeneous and complex, and the biological significance of spatial relationships between cell types is not well understood.

Tumor associated macrophages (TAMs) can convey an inflammatory microenvironment that leads to tumor progression and are the most prevalent immune cells in HGSOC tumors [6]. Elevated tumor infiltration of macrophages is a poor prognostic indicator in HGSOC ([7], [8]). TAMs secrete the cytokine IL-6, which binds to the IL-6 receptor to initiate The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway-mediated activation of the STAT3 transcription factor. IL-6 expression can lead to both inflammatory and anti-inflammatory responses [9]. Increased IL-6 expression and STAT3 activation in tumor cells have both been attributed to HGSOC disease progression and therapy resistance ([10], [11]). Currently, there is a significant research gap in understanding the tumor promoting properties of TAMs and defining strategies for TAM depletion in the HGSOC tumor microenvironment.

In contrast to TAMs, the role of B cells in HGSOC tumor biology is less understood. As such, there are several research articles with contradictory conclusions about the prognostic value of tumor infiltrating B cells [12]. For example, Yang, C. and colleagues have reported a median 113.3-month overall survival benefit in HGSOC tumors with low B cell (CD19+) infiltration [13]. In contrast, Milne, K. and colleagues found that B cell (CD20+) tumor infiltration promoted an approximately 60-month overall survival benefit [14]. The discrepancies are likely due to different subsets or developmental stages of B cells within the tumor, but additional research is needed. The primary function of B cells is antibody production, but B cells also function to secrete cytokines and initiate paracrine signaling with neighboring immune cells, such as macrophages. Interactions between B cells and macrophages are proposed to be both tumor promoting and tumor suppressive. Mechanisms of tumor-promoting B cell-macrophage associations are more established. For instance, secretion of IL-10 by B cells was shown to induce polarize macrophages to an M2-like, pro-tumor phenotype, and subsequently drive tumor progression [15]. In contrast, the tumor suppressive B cell-macrophage associations are not well understood. For example, depletion of macrophages blunts the anti-tumor response of anti-CD20 therapy in a lymphoma model [16]. With respect to HGSOC, in a mouse model B cell depletion led to a more aggressive progression, highlighting the anti-tumor properties of B cells [17]. Thus, to improve therapeutic response and patient outcome, in HGSOC tumors, there is a significant need to dissect B cell- and TAM-mediated tumor effects.

The spatial relationships between tumor cells and tumor associated immune cells are not well understood, but multispectral immunohistochemistry has provided a unique opportunity to assess the complex spatial organization within a single tumor section. In this study, we utilized a tissue microarray of HGSOC tumors and correlated the tumor composition (TAM, B cell, T cell) to clinical attributes. Specifically, in the cohort of HGSOC tumors, while TAM infiltration did not correlate with survival, the distance of TAMs to B cells or CD4 T cells did significantly correlate to improved survival. We also observed that B cell tumor infiltration was a positive predictor of survival.

**Methods**

*Regulatory Compliance*

The tissue microarray (TMA) was constructed with Institutional Review Board approval from the University of Colorado (COMIRB numbers 17-7788). A board-certified gynecologic pathologist (MDP), selected the tumor sections included on the array. Patient and clinical attributes of the tissues included on the TMA were previously published ([18], [19]).

*Data Collection/Attributes*

Tissue samples from tumor regions of 128 ovarian cancer patients were selected by a board-certified gynecologic pathologist (MDP). Primary tumor tissue samples from 103 patients were used for subsequent analyses. Following immunohistochemical analysis, one patient’s tumor was removed due to lack of an exact date for the “known alive as of” clinical variable. For Cox Proportional Analyses, one additional sample was removed due to lack of debulking data. All remaining samples are from patients with high grade serous ovarian cancer (n=101).

*Vectra Staining*

The TMA containing the high grade serous ovarian cancer tumors was previously analyzed via Vectra Automated Quantitative Pathology Systems (Akoya Biosciences) [18]. Sequential 5-micron slides from the TMA were stained with antibodies specific for CD8 (T cells, C8/144B, Agilent Technologies), CD68 (macrophages, KP1, Agilent Technologies), cytokeratin (CK, tumor cells, AE1/AE3, Agilent Technologies), CD3 (T cells, LN10, Leica), and CD19 (B cells, BT51E, Leica). The entire TMA core was imaged using the 20x objective on the Vectra 3.0 microscope (Akoya Biosciences) and image analysis was performed using inForm software version 2.3 (Akoya Biosciences), including tissue segmentation to define cells within the tumor regions (tumor-associated cells), cell segmentation to define cellular borders, and cell phenotyping.

*Statistical Analysis*

All statistical analyses were conducted in R Studio (version 1.3.1073) [20]. TMA slides were imaged, and cells classified by their phenotypes using Inform Software from Akoya Biosciences. Cells were classified using the following marker designations: B cell (CD19+), macrophage (CD68+), CD8 T cell (CD3+ CD8+), CD4 T cell (CD3+ CD8-), and tumor cell (CK+). Nearest neighbor distances were calculated using the “Phenoptr” package, which is a software provided by Akoya Biosciences to analyze images from their Vectra Polaris Imaging System [21] downstream of cell segmentation and phenotyping. Kaplan-Meier curves and Cox proportional hazards models were used to obtain p-values and hazard ratios. A p-value of less than 0.05 was considered significant. Due to the exploratory nature of this analysis, p-values were not adjusted for multiple comparisons ([22], [23], [24]).

An interaction variable was derived for each pair of immune cell types. Using the B-cell to macrophage interaction for illustration, this variable was calculated as follows. Within each image, the total count of B cells that had at least one macrophage within 25 microns was counted towards the B cell-macrophage interaction variable. To avoid noise from B cell or macrophage cell presence alone, count was then divided by the total number of B cells and macrophages within the tumor region for a given image and multiplied by 100 (Figure 1C for representation of interaction variable between B cells and macrophages). To verify the robustness of our interaction variable we performed a sensitivity analysis with interaction cutoffs at 20 and 30 microns. Results of this sensitivity analysis reflect those at a 25-micron interaction cutoff and are provided in the supplementary material. For Kaplan-Meier curves, patients were classified as having a high interaction sample (Figure 1A) between two immune cells if they expressed above the median value of the interaction variable and a low interaction sample (Figure 1B), if below the median.

To construct Kaplan-Meier curves for high and low infiltration of immune cells, groups were split at the median percentage of a given immune cell in tumor regions within the tumor microenvironment (TME). Tumor-associated immune cell percentages were calculated as total immune cells divided by total number of cells within tumor regions for a given sample and given immune cell type. For example, a patient was classified as having a high macrophage (CD68+) presence if their percentage of macrophage cells in tumor regions was above the median for all samples of 2.69 % (Table 1A). To further the study of the TME and assist in reproducibility, data and code are provided in a public GitHub repository at the following link: <https://github.com/benjamin643/MCR_paper>.

**Results**

Our models examine how spatial interactions among different immune cells in the ovarian cancer TME are associated with overall survival. We analyzed the immune TME of over 100 HGSOC tumors using the Vectra-Polaris multi-spectral immunohistochemistry platform.

*Quantification of Immune Infiltration*

Immune cell composition across patients for B cells (CD19+), macrophages (CD68+), CD4 T cells (CD3+ CD8-), and CD8 T cells (CD3+ CD8+) is summarized in Table 1A. Average B cell presence across patients was only 0.286% of cells in tumor regions. Macrophages were more prevalent with a mean of 3.82%. Of the T cells, CD4 T cells and CD8 T cells made up, on average, 0.822% and 2.68%, respectively. Most of the cells in the tumor regions were CK+ cells with a mean of 81.9%.

Kaplan-Meier curves were constructed to assess the relationship between different immune cell types and overall survival for the 102 patients. Patients with high B cell infiltration in tumor regions expressed higher survival probability (p=0.005), with median survival for the high B cell presence group of 2608 days (95% CI: 1795, 3075) compared to 1559 days (95% CI: 1157, 1698) for the low group (Figure 2A). Additionally, patients with higher CD4 T cell infiltration expressed higher survival probability (p = 0.047), with median survival for the high CD4 T cell presence group of 2112 days (95% CI: 1658, 2738) compared to 1587 days (95% CI: 1157, 1804) for the low group (Figure 2C). The median subjects with high infiltration of macrophages and CD8 T cells in tumor regions did not differ in survival probability from low infiltration groups (p = 0.78 and 0.14, respectively) (Figure 2E and Supplemental Figure 3A). These analyses were also performed using both chemonaïve and post-NACT samples and the results followed the same direction as the chemonaïve only samples (Figures 2B, 2D and 2E). These findings indicate that B cell and CD4 T cell tumor infiltration positively correlate with improved survival.

*Immune Cell Spatial Relationships*

We examined the distance relationships between the different immune cell types and correlated these spatial parameters with overall patient survival. Spatial relationships between cell types were represented through our derived cell type interaction variables (see Figure 1C for pictorial representation). Kaplan Meier curves and unadjusted Cox proportional hazards models were used to assess overall survival and show that the “high” and “low” interaction groups did not differ in survival probability for the CD8 to CD68 cell relationship (Supplemental Figure 4A, p = 0.63), for the CD4 to CD68 relationship (Figure 3C, p = 0.27), or for the CD4 to CD8 relationship (Supplemental Figure 4C, p = 0.84). Kaplan-Meier curves were also constructed to assess the relationship between disease-free survival and B cell infiltrates (Supplemental Figure 1). Subjects with high B cell infiltration and high B cell – Macrophage interaction expressed higher median disease-free survival, though results were not statistically significant (Supplemental Table 1).

Cox proportional hazards models similarly found that most cell type interactions failed to predict overall survival. Specifically, no statistically significant relationships with overall survival were found for the CD8 to CD4 T cell interaction (p = 0.23) or the CD8 T cell to macrophage interaction (0.25) variables. B cell interaction with CD4 and CD8 T cells was low across samples, with over 50 percent of samples not containing any B cells within 25 microns of a CD4 or CD8 T cell. Cox regression models were still implemented and found to be not statistically significant with p-values of 0.39 and 0.08, respectively.

*B Cell and Macrophage Spatial Relationship*

Given the discrepant literature on the role of B cells within the HGSOC TME, we hypothesized that B cell interaction with different immune cells, rather than B cell presence alone, is an important indicator of overall survival. Subjects with high B cell-to-macrophage interactions (median survival of 2301 days, 95% CI: 1884, 2738) exhibited a higher survival probability compared to subjects with low B cell-to-macrophage interactions (median survival of 1559 days, 95% CI: 1075, 1698) (Figure 3A). The unadjusted hazard ratio for overall survival, comparing the high B cell-to-macrophage interaction group to the low high B cell-to-macrophage interaction group is 0.54 (95% CI: 0.33, 0.88). This analysis was also performed using both chemonaïve and post-NACT samples and the results were statistically significant and followed the same direction as the chemonaïve only samples (Figure 3B).

Cox proportional hazards models, adjusted for clinical covariates presented in Table 1A, were used to compare B cell presence alone versus B cell-to-macrophage interaction as predictors of overall survival. In both the unadjusted model and the covariate adjusted model, the total B cell percentage variable was not a significant predictor of overall survival (p=0.31 and 0.28, respectively). The B cell-to-macrophage interaction variable was a significant predictor of overall survival in both the unadjusted model and model adjusted for clinical attributes (p = 0.004 and 0.003, respectively). The adjusted hazard ratio for the B cell-to-macrophage interaction variable is 0.84 (95% CI: 0.73, 0.97), suggesting a decreased hazard of death for increased B cell-to-macrophage interaction. Hazard ratios, confidence intervals, and p-values for all covariates in the adjusted B cell-to-macrophage interaction model are presented in Table 1B.

*CD4 T Cell and Macrophage Spatial Relationship*

In addition to the relationship between B Cells and macrophages, we also found a meaningful spatial relationship between CD4 T cells and macrophages. Splitting subjects into binary groups at the median interaction level, results were not statistically significant (p = 0.15). Subjects with high CD4 T cell-to-macrophage interactions (median survival of 2072 days, 95% CI: 1644, 2609) exhibited a higher survival probability, compared to subjects with low CD4 T cell-to-macrophage interactions (median survival of 1587 days, 95% CI: 1130, 2491) (Figure 3C). When the analysis was performed using both chemonaïve and post-NACT samples, the effect was in the same direction but not statistically significant (Figure 3D). However, when analyzed using Cox-Proportional Hazard Models adjusting for the clinical covariates in table 1A, the interaction between CD4 T cells and macrophages was found to be statistically significant. The CD4 T cell-to-macrophage interaction variable was a significant predictor of overall survival in both the unadjusted model and model adjusted for clinical attributes (p = 0.0459 and 0.0314, respectively). The adjusted hazard ratio for the CD4 T cell-to-macrophage interaction variable is 0.9549 (95% CI: 0.92, 0.99), suggesting a decreased hazard of death for increased CD4 T cell-to-macrophage interaction. Hazard ratios, confidence intervals, and p-values for all covariates in the adjusted CD4 T cell-to-macrophage interaction model are presented in Table 1C.

**Discussion**

The cellular composition of HGSOC tumors provides critical insight into how patients may respond to primary chemotherapy. Each of the 127 tumor samples in the TMA were obtained from patients who received the standard of care: a combination of surgical debulking and chemotherapy (platinum/taxane-based treatment). Thus, the overall survival benefits and positive immune cell correlations observed in our study may be a direct reflection as to how effective the standard-of-care is within the patient cohort. We observed that, within the TME, the interactions between B cells (CD19+) and macrophages (CD68+), as well as interactions between CD4 T cells and macrophages, were associated with better overall survival outcomes. Higher infiltration of B cells into tumor regions was also associated with better survival outcomes. Using Cox proportional hazards models, interactions between macrophages and B cells or CD4 T cells were shown to be a better predictors of survival probability than the presence of any immune cell type alone. Taken together, elevated macrophage-B cell and macrophage-CD4 T cell interactions within tumors suggest a more favorable response to chemotherapy.

Previous studies have defined how chemotherapy remodels the HGSOC TME. Notably, chemotherapy often induces a robust IL-6 mediated inflammatory response that may directly remodel the TME ([18], [25]). In HGSOC, IL-6 induction is mainly described as tumor promoting; thus, dampening inflammatory or IL-6-driven responses may ultimately be beneficial. While the mechanisms driving the improved survival benefit from B cell/macrophage interactions are not well understood, we speculate that B cell-dependent paracrine signaling via immunomodulatory cytokines, such as IL-10, serves to attenuate inflammation. Moreover, macrophages can present antigens to B cells, potentially enriching for tumor-specific antigen antibody production, leading to a more robust anti-tumor immune response following chemotherapy. Dissecting these B cell/macrophage anti-tumor mechanisms will be the subject of future investigations.

Small populations of immune cells, as well as intratumoral heterogeneity, can obfuscate investigations into the TME by making it difficult to ascertain whether results will replicate to other similar data sets. Larger studies with explicit *a priori* hypothesis about immune cell reactions in the TME are needed to confirm these findings. In addition, while we did not observe CD8+ T cells were significantly associated with improved survival, there was a trend towards improved survival that may be observable in future, larger studies. To better understand the relationships between different immune cells within the TME, further research will also need to be conducted with larger TME samples and with stronger phenotyping of immune cells (for example, double positive or double negative T cells were not a focus of this study). Regardless, based on previous studies ([17], [18], [25]), there is reason to believe that immune cell interaction within the TME leads to better overall survival outcomes, as demonstrated by the “interaction” of B cells (CD19+) and macrophages (CD68+) in this data set. Understanding which immune cell interactions are most important in the TME may lead to more accurate diagnoses and the development of novel therapy techniques.

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**Visual Overview**

**Bubble chart

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**Figure 1:**

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**Figure 1.** Tissue microarray (TMA) slides of ovarian tumor samples stained with antibodies specific for CD4 (T cells, 4B12, Leica Biosystems), CD8 (T cells, C8/144B, Agilent Technologies), CD68 (macrophages, KP1, Agilent Technologies), and cytokeratin (tumor cells, AE1/AE3, Agilent Technologies). **A**, TMA slides exhibiting high values for the interaction variable for B cells and macrophages (classified as high if greater than the median for all samples). **B**, TMA slide exhibiting low values for the interaction variable for B cells and macrophages (classified as low if less than the median for all samples). **C**, Explanatory diagram of how the B cell (CD19+) and macrophage (CD68+) interaction variable was calculated. The interaction variable was calculated by taking the total number of B cells that had a macrophage within 25 microns of it, dividing by the total number of B cells and macrophages for that sample, and multiplying by 100. The other cell interaction variables were calculated in the same manner.

**Figure 2:**

Application

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**Figure 2.** Kaplan-Meier curves for immune cell infiltration in tumor regions. **A**, Chemonaïve tumors with a high percentage of B cell infiltration into tumor regions had higher overall survival probability compared to the low infiltration group (p=0.005). The Hazard ratio of 0.5 suggests that the samples with a high presence of B cells in tumor regions had 0.5 times the risk of hazard (death) compared to the low infiltration group (95% CI: 0.31, 0.82). **C**, Chemonaïve tumors with a high percentage of CD4 cell infiltration into tumor regions had higher overall survival probability compared to the low infiltration group (p=0.047). The Hazard ratio of 0.61 suggests that the samples with a high presence of B cells in tumor regions had 0.61 times the risk of hazard (death) compared to the low infiltration group (95% CI: 0.38, 0.99). **E**, Chemonaïve tumors with a high percentage of Macrophage (CD68+) infiltration into tumor regions did not differ significantly in survival probability from the low infiltration group (p=0.78). **B, D, F** include post-NACT Tumors andhave similar direction and interpretation as their chemonaïve only counterparts.

**Figure 3:**

Chart

Description automatically generated with low confidence **Figure 3.** Kaplan-Meier curves for different combinations of cell distance interactions in tumor regions. **A**, Chemonaïve tumors with a high percentage of B cells (CD19+) near macrophages (CD68+) had higher overall survival probability compared to the low interaction group (p=0.01). The Hazard ratio of 0.54 suggests the samples with high B cell-macrophage interaction had 0.54 times the risk of hazard (death) compared to the low interaction samples (95% CI: 0.33, 0.88). **C**, Chemonaïve tumors with high CD4 T cell-to-macrophage interaction did not differ significantly in survival probability from the low interaction samples (p=0.27). **B, D** include post-NACT tumors andhave similar direction and interpretation as their chemonaïve only counterparts.

**Table 1A**

|  | **Alive (N=35)** | **Death (N=67)** | **Overall (N=102)** |
| --- | --- | --- | --- |
| **B Cell Macrophage Interaction Variable** |  |  |  |
| Mean (SD) | 3.24 (4.75) | 0.872 (2.38) | 1.68 (3.54) |
| Median [Min, Max] | 1.20 [0, 19.4] | 0 [0, 16.7] | 0.0919 [0, 19.4] |
| **CD19+ Percent** |  |  |  |
| Mean (SD) | 0.462 (0.881) | 0.194 (0.849) | 0.286 (0.866) |
| Median [Min, Max] | 0.130 [0, 4.54] | 0.0200 [0, 6.62] | 0.0300 [0, 6.62] |
| **CD68+ Percent** |  |  |  |
| Mean (SD) | 4.50 (4.99) | 3.46 (3.40) | 3.82 (4.02) |
| Median [Min, Max] | 2.51 [0.0600, 21.9] | 2.86 [0.0400, 14.6] | 2.69 [0.0400, 21.9] |
| **CD3+ CD8+ Percent** |  |  |  |
| Mean (SD) | 3.02 (4.56) | 2.50 (4.10) | 2.68 (4.25) |
| Median [Min, Max] | 1.53 [0.0600, 19.9] | 0.900 [0, 21.8] | 1.09 [0, 21.8] |
| **CD3+ CD8- Percent** |  |  |  |
| Mean (SD) | 1.45 (3.17) | 0.495 (1.09) | 0.822 (2.09) |
| Median [Min, Max] | 0.190 [0, 16.7] | 0.0900 [0, 7.15] | 0.125 [0, 16.7] |
| **CK+ Percent** |  |  |  |
| Mean (SD) | 80.1 (16.1) | 82.8 (15.7) | 81.9 (15.8) |
| Median [Min, Max] | 86.5 [29.5, 96.3] | 88.6 [30.0, 97.2] | 88.2 [29.5, 97.2] |
| **Age at Diagnosis** |  |  |  |
| Mean (SD) | 56.5 (10.7) | 61.5 (9.92) | 59.8 (10.4) |
| Median [Min, Max] | 54.0 [38.0, 76.0] | 62.0 [39.0, 80.0] | 60.0 [38.0, 80.0] |
| **Grade** |  |  |  |
| 3 | 32 (91.4%) | 62 (92.5%) | 94 (92.2%) |
| 2 | 3 (8.6%) | 5 (7.5%) | 8 (7.8%) |
| **Treatment Effect** |  |  |  |
| *No Treatment* | 28 (80.0%) | 56 (83.6%) | 84 (82.4%) |
| *Platinum/taxane based chemotherapy* | 7 (20.0%) | 11 (16.4%) | 18 (17.6%) |
| **Debulking** |  |  |  |
| Optimal | 26 (74.3%) | 38 (56.7%) | 64 (62.7%) |
| Interval | 8 (22.9%) | 14 (20.9%) | 22 (21.6%) |
| Suboptimal | 1 (2.9%) | 14 (20.9%) | 15 (14.7%) |
| Missing | 0 (0%) | 1 (1.5%) | 1 (1.0%) |

**Table 1B**

| **Characteristic** | **HR***1* | **95% CI***1* | **p-value** |
| --- | --- | --- | --- |
| **B Cell Macrophage Interaction** | 0.84 | 0.73, 0.97 | **0.003** |
| **Age at Diagnosis** | 1.03 | 1.01, 1.06 | **0.008** |
| **Treatment Effect** |  |  | 0.45 |
| *No treatment* | Reference |  |  |
| *Platinum/taxane based chemotherapy* | 0.59 | 0.16, 2.14 |  |
| **Debulking** |  |  | 0.18 |
| *Optimal* | Reference |  |  |
| *Interval* | 2.85 | 0.86, 9.51 |  |
| *Suboptimal* | 1.49 | 0.80, 2.79 |  |
| *1*HR = Hazard Ratio, CI = Confidence Interval | | | |

**Table 1C**

| **Characteristic** | **HR***1* | **95% CI***1* | **p-value** |
| --- | --- | --- | --- |
| **CD4 T Cell Macrophage Interaction** | 0.95 | 0.92, 1.00 | **0.014** |
| **Age at Diagnosis** | 1.03 | 1.01, 1.06 | **0.006** |
| **Treatment Effect** |  |  | 0.321 |
| *No treatment* | Reference |  |  |
| *Platinum/taxane based chemotherapy* | 0.50 | 0.14, 1.80 |  |
| **Debulking** |  |  | 0.181 |
| *Optimal* | Reference |  |  |
| *Interval* | 2.83 | 0.85, 9.44 |  |
| *Suboptimal* | 1.50 | 0.80, 2.82 |  |
| *1*HR = Hazard Ratio, CI = Confidence Interval | | | |

**Table 1. A**, Descriptive table of variables included in analyses split by whether a patient was still alive or dead at the last follow up. Cell percentages are calculated as total cells of a specified type divided by total cells in tumor regions for a given sample. Medians of cell percentages were used to split up samples into high or low infiltration groups referenced in Figure 2. **B**, Results from the Cox proportional hazards model where coefficients have been exponentiated and interpreted as hazard ratios. The *‘B Cell Macrophage Interaction’* variable and *age* were statistically significant (p <0.05). Formula for the model was (Survival Time, Death) ~ B Cell Macrophage Interaction + Age at Diagnosis + Treatment Effect + Debulking. **C**, Results from the cox proportional hazards model where coefficients have been exponentiated and interpreted as hazard ratios. The *‘CD4 T Cell Macrophage Interaction’* variable and *age* were statistically significant (p <0.05). Formula for the model was (Survival Time, Death) ~ B Cell Macrophage Interaction + Age at Diagnosis + Treatment Effect + Debulking. Numbers for Tables 1A, B, and C were computed for subset of data only including chemonaïve tumors. The sample size for the Kaplan-Meier curves and Table 1A was 102. One sample was removed from cox proportional hazard models due to a missing clinical variable. The samples size for cox-proportional hazard models presented in Table 1B and C was then 101 patients with a total of 66 events.

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