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

*Disease-free Survival Outcome*

In additional to our analysis of overall survival in the main manuscript, we conducted an analysis of disease-free survival and its association with B cell infiltrates and B cell and macrophage interactions. The results of this analysis are presented as Kaplan Meier curves (Supplemental Figure 1) and a table of median survival days (Supplemental Table 1). Supplemental Table 1 is also reproduced below. Neither B cell percentage (p = 0.83) nor B cell to macrophage interaction (p = 0.43) have a statistically significant effect on disease-free survival (Supplemental Figure 1). However, we see improvement in median survival time for the high vs. low B cell presence groups and for the high vs. low B cell – macrophage interaction groups for both survival outcomes. This indicates that the direction of the effect for disease-free survival is the same as the direction of the effect for overall survival.

*Sensitivity Analysis of Interaction Distance Cutoff*

Macrophages are typically 20-21 microns in diameter and B cells are typically 5-7 microns in diameter. Our 25-micron maximum interaction distance indicates that the outer edges of the interacting B cell and macrophage are within very close proximity (12-14 microns apart at most). We also performed a sensitivity analysis of interaction distance cutoffs of 20- and 30-micron distance cutoffs, which showed our findings are a robust choice of distance cutoff (Supplementary Table 2).

*Additional Cell Infiltrate Analysis*

In addition to CD4 T cells, CD19 B cells, and CD68 macrophages, the same cell infiltration analysis was conducted on CD8 T cells. The results of this analysis are presented in Kaplan-Meier curves (Supplementary Figure 3). Neither chemonaïve tumor samples (p = 0.14) nor chemonaïve and post-NACT tumor samples (p = 0.26) possessing high CD8 T cell infiltration differed from tumors with low CD8 T cell infiltration (Supplementary Figure 3).

*Additional Spatial Relationship Analysis*

Two additional spatial relationships not included in the main manuscript were analyzed: CD8 T cell-macrophage interaction and CD4 T cell-CD8 T cell interaction. The results of the analysis are presented in Kaplan-Meier curves. Chemonaïve tumor samples and the combination of chemonaïve and post-NACT tumor samples that had a higher interaction of CD8 T cells and macrophages within 25 microns of each other did not differ from those samples with a lower interaction (p = 0.63 and 0.47, respectively) (Supplementary Figure 4A and 4B). Additionally, chemonaïve tumor samples and the combination of chemonaïve and post-NACT tumor samples that had a higher interaction of CD8 T cells and CD4 T cells within 25 microns of each other did not differ from those samples with a lower interaction (p = 0.48 and 0.57, respectively) (Supplementary Figure 4C and 4D).

**Supplementary Figure 1:**

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**Supplementary Figure 1:** Kaplan-Meier curves of disease-free survival for B cell presence and B cell-macrophage interaction. **A**, Samples with a high percentage of B cell infiltration into tumor regions did not differ significantly in disease-free survival from the low infiltration group (p = 0.91). **B**, Samples with a high percentage of B cells (CD19+) near macrophages (CD68+) did not differ significantly in survival probability from the low interaction samples (p = 0.63).

**Supplementary Table 1:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Outcome** | **Variable** | **N** | | **Median Survival in Days (95% CI)** |
| **No event** | **event** |
| **Overall Survival** | B cell Presence High | 24 | 28 | 2608 (1795-3075) |
| B cell Presence Low | 11 | 39 | 1559 (1157-1698) |
| **Disease-free Survival** | B cell Presence High | 6 | 46 | 686 (604-884) |
| B cell Presence Low | 7 | 43 | 664 (569-864) |
| **Overall Survival** | B Cell – Macrophage Interaction High | 24 | 27 | 2301 (1884-2738) |
| B Cell – Macrophage Interaction Low | 11 | 40 | 1559 (1075-1698) |
| **Disease-free Survival** | B Cell – Macrophage Interaction High | 7 | 44 | 692 (604-943) |
| B Cell – Macrophage Interaction Low | 6 | 45 | 661 (510-853) |

**Supplementary Table 1:** Median survival times (in days) for overall survival and disease-free survival outcomes, split by high and low B cell presence and high and low B cell-macrophage interaction.

**Supplementary Table 2:**

| **Characteristic** | 20 Micron Threshold | | | 30 Micron Threshold | | |
| --- | --- | --- | --- | --- | --- | --- |
| **HR***1* | **95% CI***1* | **p-value** | **HR***1* | **95% CI***1* | **p-value** |
| **B Cell Macrophage Interaction** | 0.80 | 0.66, 0.96 | **0.002** | 0.86 | 0.76, 0.98 | **0.004** |
| **Age at Diagnosis** | 1.03 | 1.01, 1.06 | **0.008** | 1.03 | 1.01, 1.06 | **0.008** |
| **Treatment Effect** |  |  | 0.44 |  |  | 0.44 |
| *No Treatment* | — | — |  | — | — |  |
| *Platinum/taxane based chemotherapy* | 0.59 | 0.16, 2.13 |  | 0.59 | 0.16, 2.13 |  |
| **Debulking** |  |  | 0.21 |  |  | 0.19 |
| *Optimal* | — | — |  | — | — |  |
| *Interval* | 2.86 | 0.86, 9.54 |  | 2.84 | 0.85, 9.48 |  |
| *Suboptimal* | 1.42 | 0.76, 2.67 |  | 1.47 | 0.79, 2.76 |  |
| *1*HR = Hazard Ratio, CI = Confidence Interval | | | | | | |

**Supplementary Table 2:** Results from the Cox Proportional Hazards models for two different distance thresholds for B cell and macrophage interaction (20 and 30 microns). Coefficients have been exponentiated and interpreted as hazard ratios. The ‘B Cell Macrophage Interaction’ variable and age were statistically significant (p <0.05) in both models.

**Supplementary Figure 3**

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**Figure 3:** Kaplan-Meier curves for CD8 T cell infiltration into tumor regions. **A,** chemonaïve tumors with a high percentage of CD8 T cell infiltration into tumor regions did not differ significantly in survival probability from the low infiltration group (p=0.14). **B,** The combined chemonaïve and post-NACT tumors show the same direction as the chemonaïve only tumors but the high and low infiltration group still do not differ significantly (p=0.26)

**Supplementary Figure 4**

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**Figure 3.** Kaplan-Meier curves for different combinations of cell distance interactions in tumor regions. **A**, Chemonaïve tumors with high CD8+ T cell-to-macrophage interaction did not differ significantly in survival probability from the low interaction samples (p=0.63). **C,** Kaplan-Meier curves for different combinations of cell distance interactions in tumor regions. **A**, Chemonaïve tumors with high CD8 T cell-to-CD4 T cell interaction did not differ significantly in survival probability from the low interaction samples (p=0.48). **B, D,** include post-NACT Tumors andhave similar direction and interpretation as their chemonaïve only counterparts.