**Lo *et al.* Supplementary Materials:**

**Detailed Methods and Materials:**

***Patient cohort***

All tumor specimens and clinical information were obtained with informed consent (or a formal waiver of consent) with approval by the Research Ethics Boards of the BC Cancer Agency, University of British Columbia, and University Health Network. Three retrospective cohorts of HGSC cases were evaluated (**Table 1**). Cohort A consisted of 26 cases for which matched pre- and post-NACT tumor samples were available; these samples were collected at Vancouver General Hospital (VGH) from 2004-2012. Patients received 3-6 cycles (mean = 4 cycles) of NACT with carboplatin and paclitaxel. Following interval debulking, patients received an additional 3-4 cycles of NACT (or more). Where possible, pre- and post-NACT tumor samples from both extra-pelvic and pelvic sites were analyzed, and the average values were reported. Tissue areas containing viable tumor epithelium were identified from diagnostic slides (i.e., hematoxylin & eosin stained FFPE slides) and selected by a pathologist (B.A.C.) for construction of a tissue microarray (TMA; Beecher Instruments, Sun Prairie, WI) with 1 mm duplicate cores. Whole sections were used in cases where there was insufficient tumor material available for TMA construction.

Cohorts B and C consisted of 64 HGSC cases (18 cases from VGH, and 46 cases from Toronto General Hospital ) who underwent NACT similar to Cohort A but from whom only post-NACT tumor samples were available due to clinical or logistical barriers to obtaining pre-NACT samples for research purposes. **Table 1** provides clinicopathological information for cohorts A-C. BRCA1 and 2 mutation status was unknown for the vast majority of cases. **Suppl. Fig. S1** summarizes the criteria used to assemble the cohorts, including the numeric losses at each step.

***Immunohistochemistry***

For single-color IHC, serial sections from the TMA (or whole sections when necessary) were stained with antibodies against 15 immune and functional markers (**Suppl. Table S1**). Tissue sections were incubated overnight at 37°C, followed by deparaffinization in xylene and rehydration in graded ethanol. Single-color stains were performed using the Ventana Discovery XT autostainer and reagents (Ventana, Tucson, AZ) (except CD103, PD-1, and IDO-1, as described below). Antigen retrieval was performed using the standard cell conditioning 1 (CC1) protocol, followed by non-specific blocking (Discovery DABMap kit, Ventana, Tucson, AZ). Sections were incubated with primary antibodies **(Suppl. Table S1)** for 60 minutes at room temperature. Sections were incubated for 32 minutes at room temperature with cross-adsorbed biotinylated goat α-mouse immunoglobulin (IgG) secondary antibody (1:250) or goat α-rabbit immunoglobulin (IgG) secondary antibody (1:500) (Jackson Immunoresearch, West Grove, PA, USA), followed by 3, 3’-diaminobenzidine (DAB) detection. Sections were counterstained with hematoxylin and coverslipped. Human tonsil and liver tissues were used as positive controls.

Single-color IHC for CD103, PD-1, and IDO-1 was performed using an Intellipath FLX autostainer and reagents (Biocare Medical, Concord, CA, USA). Tissue sections were incubated overnight at 37°C, followed by deparaffinization in xylene and rehydration in graded ethanol. Following heat-induced epitope retrieval with Diva Decloaking reagent and blocking with Peroxidased-1 and Background Sniper, sections were incubated with primary antibodies (**Suppl. Table S1**) for 30 minutes at room temperature. Sections were incubated with MACH2 anti-rabbit HRP or MACH2 anti-mouse HRP polymer reagents for 30 minutes at room temperature. Secondary antibodies were detected with DAB reagent for 5 minutes at room temperature, after which sections were counterstained with hematoxylin and coverslipped.

Multi-color IHC was performed using the Intellipath FLX autostainer and reagents (Biocare Medical, Concord, CA, USA). All steps prior to primary antibody incubation were performed as above. Sections were incubated with primary antibodies **(Suppl. Table S2)** (30 minutes, room temperature), followed by MACH2 anti-mouse HRP, MACH2 Double Stain 1, or MACH2 Double Stain 2 polymer reagents (30 minutes, room temperature) and detected with chromogens **(Suppl. Table S2)**. For the three- and four-colored IHC, antibody denaturation was performed at 50°C for 45 minutes in an SDS-glycine solution with periodic agitation ([1](#_ENREF_1)). Sections were then incubated with a second round of primary antibodies **(Suppl. Table S2)** (30 minutes, room temperature), followed by incubation with MACH2 Double Stain 2 polymer reagent (30 minutes, room temperature) and chromogen detection. Sections were counterstained with hematoxylin and coverslipped. Human tonsil and liver tissue served as positive controls.

***Quantitative IHC analysis***

For single-color IHC, slides were scanned using the Aperio (Leica Biosystems, Germany), Pannoramic MIDI (3DHistech, Budapest, Hungary), or Vectra (Perkin Elmer, Waltham, MA, USA) imaging systems. For multicolor IHC, slides were scanned and analysed using the Vectra automated imaging system and inForm software (Perkin Elmer, Waltham, MA, USA). Manual scoring of TIL subsets in multi-color IHC was performed using unmixed images (i.e., individual images showing each chromogen separately).

Except for IDO-1 (see below), single-color stained slides were reviewed by two pathologists (B.A.C. & S.S.), who were blinded to clinical data and treatment status. Immune cells in both the tumor epithelium and stroma were quantified. For whole sections, the equivalent of two 1 mm tumor-positive regions were scored for intraepithelial and stromal immune infiltrates. The total number of tumor cells in each core was manually estimated. IDO-1 was manually scored on a 0-3 scale (0 = no IDO-1+ cells; 1 = 1-30% IDO-1+ cells, 2 = 31-70% IDO-1+ cells, and 3 = >70% IDO-1+ cells across the entire core). PC density was scored using a 4-point scale ([2](#_ENREF_2)). TIL expressing CD3, CD8, TIA-1, and PD-1 in multi-color IHC were manually scored using ImageJ. PD-L1 expression was quantified across the entire core using the cell phenotyping function in inForm 2.1.1 (Perkin Elmer, Waltham, MA, USA). Intraepithelial TIL densities for single- and multi-color IHC were reported as the number of positive cells per 100 tumor cells. PD-L1+ densities were reported as the number of positive cells per mm2. For all markers, the values for duplicate cores were averaged.

***Statistical analysis***

Statistical analyses were performed using GraphPad Prism 6.05 (GraphPad, La Jolla, CA). The Wilcoxon matched pairs test with Pratt’s method was used to compare change in cell densities between matched tumor samples. Multiple comparisons testing was performed using the false discovery rate controlled by the Benjamini-Hochberg ([3](#_ENREF_3)) procedure for single-color markers, and *P* values were adjusted accordingly. Survival analyses were performed on the combined post-NACT TIL data from cohorts A-C using the Cox proportional hazard regression model with multivariable testing that was stratified by cohort and adjusted for the 6 biomarkers with the statistical software R (v 3.1.1). The reported p values for the Cox proportional hazard regression model were based on the omnibus likelihood ratio test for each biomarker (all markers vs all markers minus CD3, all markers minus CD3 vs all markers minus CD8, and so on). All hypothesis tests were two-sided and *P*-values ≤0.05 were considered significant. Unsupervised hierarchical clustering of standard deviation-scaled marker values was performed using the gplots package with Euclidean distance calculations.

***References***

1. Pirici D, Mogoanta L, Kumar-Singh S, Pirici I, Margaritescu C, Simionescu C, et al. Antibody elution method for multiple immunohistochemistry on primary antibodies raised in the same species and of the same subtype. J Histochem Cytochem 2009;57:567-75.

2. Lohr M, Edlund K, Botling J, Hammad S, Hellwig B, Othman A, et al. The prognostic relevance of tumour-infiltrating plasma cells and immunoglobulin kappa C indicates an important role of the humoral immune response in non-small cell lung cancer. Cancer Lett 2013;333:222-8.

3. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Statist Soc B 1995;57:289-300.