Supplementary information

Supplementary table 1: All adverse events, regardless of attribution

Toxicity grade	1-2 N(%)	3-4 N(%)	All N(%)
Cardiovascular			
Cardiac troponin I increased	1(9)	0(0)	**1(9)**
Edema limbs	1(9)	0(0)	**1(9)**
Hypertension	2(18)	1(9)	**3(27)**
Sinus tachycardia	3(27)	0(0)	**3(27)**
Stroke	1(9)	0(0)	**1(9)**
Thromboembolic event	0(0)	2(18)	**2(18)**
Flushing	1(9)	0(0)	**1(9)**
Dermatologic			
Rash	3(27)	0(0)	**3(27)**
Dry skin	1(9)	0(0)	**1(9)**
Endocrine			
Hyperthyroidism	1(9)	0(0)	**1(9)**
Hypothyroidism	1(9)	0(0)	**1(9)**
Gastrointestinal			
Abdominal pain	4(36)	0(0)	**4(36)**
Anorexia	1(9)	2(18)	**3(27)**
Aspartate aminotransferase increased	6(55)	0(0)	**6(55)**
Blood bilirubin increased	2(18)	0(0)	**2(18)**
Constipation	3(27)	0(0)	**3(27)**
Diarrhea	4(36)	0(0)	**4(36)**
Gastrointestinal fistula	2(18)	0(0)	**2(18)**
Lipase increased	1(9)	0(0)	**1(9)**
Nausea	5(45)	0(0)	**5(45)**
Pancreatitis	1(9)	0(0)	**1(9)**
Alanine aminotransferase increased	5(45)	0(0)	**5(45)**
Alkaline phosphatase increased	3(27)	0(0)	**3(27)**
Dysgeusia	1(9)	0(0)	**1(9)**
Dyspepsia	1(9)	0(0)	**1(9)**
Gastrointestinal bleeding	0(0)	1(9)	**1(9)**
Vomiting	3(27)	0(0)	**3(27)**
General			
Fatigue	7(64)	0(0)	**7(64)**
Fever	4(36)	0(0)	**4(36)**
Hot flashes	1(9)	0(0)	**1(9)**
Pain	3(27)	1(9)	**4(36)**
Weight loss	2(18)	0(0)	**2(18)**
Dry Eye	1(9)	0(0)	**1(9)**
Hematologic			
Anemia	3(27)	3(27)	**6(55)**
Lymphocyte count decreased	0(0)	2(18)	**2(18)**
Neutrophil count decreased	1(9)	0(0)	**1(9)**
Platelet count decreased	4(36)	0(0)	**4(36)**
Activated partial thromboplastin time prolonged	0(0)	1(9)	**1(9)**
INR increased	1(9)	1(9)	**2(18)**
White blood cell decreased	3(27)	0(0)	**3(27)**

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Infections and infestations			
Pneumonia	0(0)	1(9)	**1(9)**
Sepsis	0(0)	1(9)	**1(9)**
Upper respiratory infection	2(18)	0(0)	**2(18)**
Urinary tract infection	1(9)	0(0)	**1(9)**
Pelvic Infection	1(9)	0(0)	**1(9)**
Metabolic			
Hyperglycemia	6(55)	1(9)	**7(64)**
Hypernatremia	1(9)	0(0)	**1(9)**
Hypoalbuminemia	7(64)	0(0)	**7(64)**
Hypoglycemia	1(9)	0(0)	**1(9)**
Hypokalemia	3(27)	0(0)	**3(27)**
Hypomagnesemia	1(9)	0(0)	**1(9)**
Hyponatremia	3(27)	0(0)	**3(27)**
Musculoskeletal			
Arthralgia	2(18)	0(0)	**2(18)**
Back pain	2(18)	0(0)	**2(18)**
Muscle weakness	0(0)	1(9)	**1(9)**
Myalgia	2(18)	0(0)	**2(18)**
Pain in extremity	1(9)	0(0)	**1(9)**
Neurologic			
Depression	1(9)	0(0)	**1(9)**
Encephalopathy	0(0)	1(9)	**1(9)**
Headache	3(27)	0(0)	**3(27)**
Lethargy	1(9)	0(0)	**1(9)**
Meningitis	0(0)	1(9)	**1(9)**
Peripheral sensory neuropathy	1(9)	1(9)	**2(18)**
Arachnoiditis	0(0)	1(9)	**1(9)**
Confusion	1(9)	0(0)	**1(9)**
Dizziness	2(18)	0(0)	**2(18)**
Sensorineural hearing loss	0(0)	1(9)	**1(9)**
Ophthalmologic			
Conjunctivitis	1(9)	0(0)	**1(9)**
Renal			
Creatinine increased	1(9)	0(0)	**1(9)**
Reproductive and urinary tract			
Vaginal hemorrhage	1(9)	0(0)	**1(9)**
Gynecologic Hemorrhage	1(9)	0(0)	**1(9)**
Urinary tract pain	1(9)	0(0)	**1(9)**
Vaginal discharge	1(9)	0(0)	**1(9)**
Vaginal fistula	1(9)	0(0)	**1(9)**
Respiratory			
Cough	2(18)	0(0)	**2(18)**
Pneumonitis	1(9)	0(0)	**1(9)**
Respiratory failure	0(0)	1(9)	**1(9)**
Dyspnea	2(18)	0(0)	**2(18)**
Hoarseness	1(9)	0(0)	**1(9)**
Sore Throat	**1(9)**	**0(0)**	1(9)

Supplementary Table 2: Combined Positive Score (CPS) for PD-L1 expression.

Patient	7	1	2	4	8	9	3	5
PFS (months)	9.9	6.1	4.2	3.2	2.9	2.7	1.9	2
Clinical benefit	yes	yes	no	no	no	no	no	no
CPS	1.44	7.07	0.23	0.06	0.88	0	23.38	0

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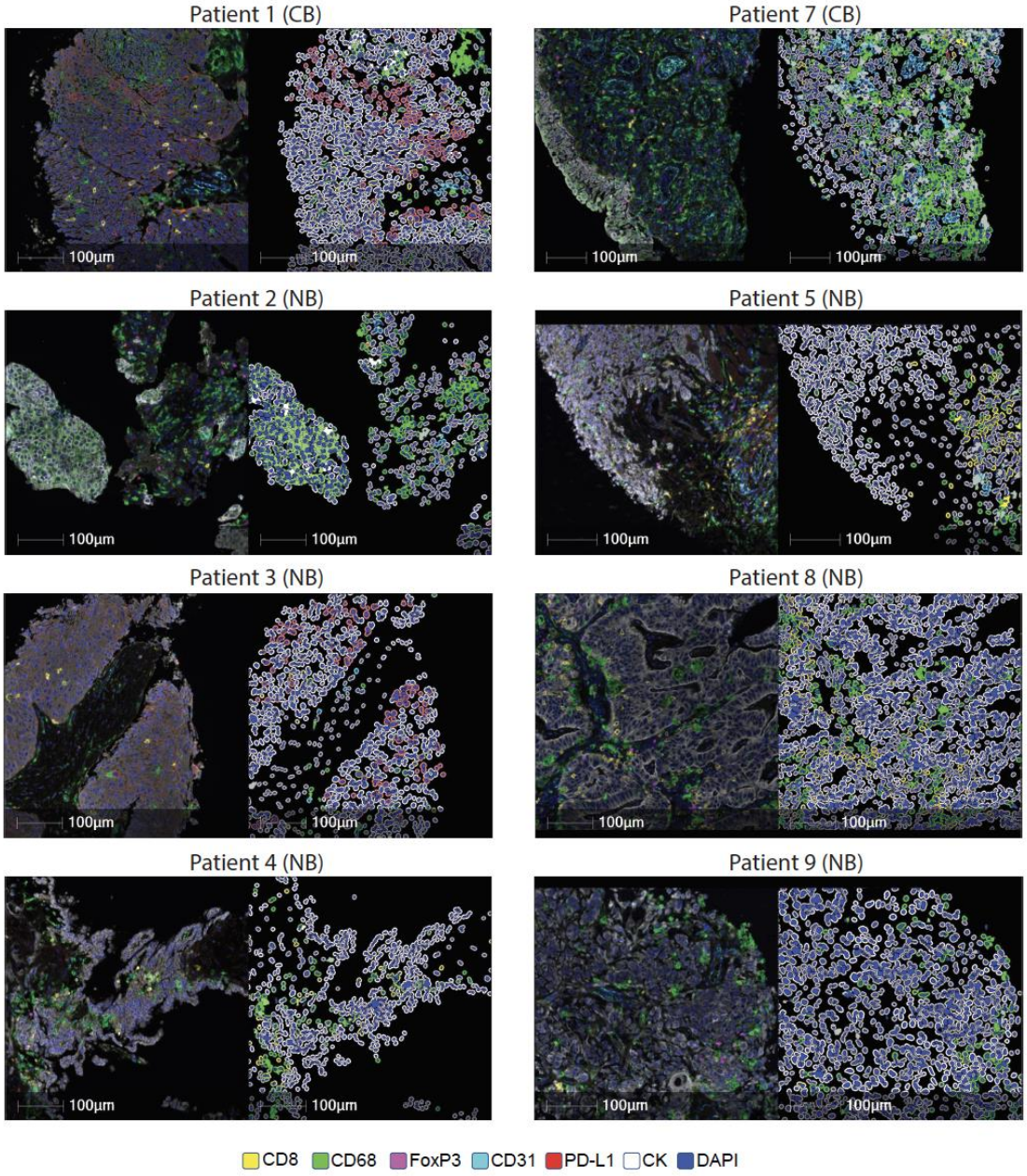
Supplementary Figure 1.



Supplementary Figure 1. Tumor microenvironment in pre-treatment samples. Representative immunofluorescence images and segmentation masks from each patient with clinical benefit (CB) or no benefit (NB) are shown.

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Supplemental Methods: Multiplexed Immunofluorescence

The 4 µm FFPE tissue sections were baked for 3 hrs. at 62 degrees Celsius with subsequent deparaffinization performed on the Leica Bond RX followed by 6 sequential cycles of staining with each round including a 30-minute combined block and primary antibody incubation (PerkinElmer antibody diluent/block ARD1001). For all antibodies other than CD68, detection was performed using a secondary horseradish peroxidase (HRP)-conjugated polymer (PerkinElmer Opal polymer HRP Ms + Rb ARH1001; 10-minute incubation). Detection of the CD68 primary antibody was performed using a goat anti-mouse Poly HRP secondary antibody (Invitrogen B40961; 10-minute incubation). PanCK, CK7 and Cam5.2 primary antibodies were used as a cocktail. After 6 sequential rounds of staining, sections were stained with Hoechst (Invitrogen 33342) to visualize nuclei and mounted with ProLong Gold antifade reagent mounting medium (Invitrogen P36930).

Seven color multiplex stained slides were imaged using the Vectra Multispectral Imaging System version 3 (Perkin Elmer). The antibody panel included CD68 (PG-M1, Dako), FOXP3 (236A/E7, Biocare), PD-L1 (E1L3N, Cell Signaling), CD31 (Pecam, Biocare), CD8 (4B11, Leica) and a panel of cytokeratin antibodies [PanCK (AE1/AE3, Dako), CK7 (OV-TL-12/30, Abcam), Cam5.2 (Cam5.2, Becton Dickinson)]. Scanning was performed at 20X (200X final magnification). Filter cubes used for multispectral imaging were DAPI, FITC, Cy3, Texas Red and Cy5. A spectral library containing the emitted spectral peaks of the fluorophores in this study was created using the Vectra image analysis software (Perkin Elmer). Using multispectral images from single-stained slides for each marker, the spectral library was used to separate each multispectral cube into individual components (spectral unmixing) allowing for identification of the seven marker channels of interest using Inform 2.4 image analysis software. Images were exported to Indica Labs Halo image analysis platform and cell segmentation and signal thresholding was performed separately on each case using a supervised algorithm. Quantification was performed using entire images.

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