**Supplementary methods**

**Digital Spatial Profiling**

Briefly, we deparaffined two slides derived from independent YTMA404 blocks, each block containing one non-adjacent tumor core per patient, and subjected them to antigen retrieval procedures with citrate pH 6 buffer (Sigma Aldrich, SKU C9999) at 100-112ºC for 15 min in a TintoRetriever pressure cooker (BioSB, BSB 7008), followed by 1-hour incubation with a blocking solution (Buffer W, NanoString). Subsequently, we co-incubated the slides overnight with three fluorescent-label visualization antibodies to detect tumor cells (pan-cytokeratin [CK]), all immune cells (CD45), and macrophages (CD68, clone KP1, Santa Cruz), together with a cocktail of 44 unique photocleavable oligonucleotide-labeled primary antibodies targeting immuno-oncology markers (Beta cores and modules, see supplementary table S1). Next, we applied 4 % paraformaldehyde (Thermo Scientific) for 30 minutes onto the slides, followed by nuclear staining with SYTO13 (NanoString) for 20 minutes.

Once the staining was completed, we loaded the slides in the prototype Beta version of the GeoMx DSP instrument (Rimm Lab at Yale), where they were scanned to produce a digital fluorescent image of the tissue. Next, we created individual regions of interest (ROIs) of a maximum of 600 μm2 in each TMA spot. Then, we created four molecularly-defined tissue compartments by fluorescent co-localization: tumor compartment (panCK+), immune cell compartment (CD45+), macrophage compartment (CD68+), and non-immune cell stroma compartment (panCK-/CD45-/CD68-/SYTO13+) **(Figure 1)**. Once each ROI was compartmentalized, a digital micromirror device sequentially and selectively directed UV light to the macrophage, immune cell, tumor, and finally non-immune cell stromal compartments. Upon UV-light exposure, the photocleaved oligos from specific compartments were collected via microcapillary aspiration and dispensed into a 96-well plate. These oligos were hybridized to 4-color, 6-spot optical barcodes and digitally counted in the nCounter system (NanoString).

Using the GeoMx software (NanoString), digitally counted barcodes corresponding to protein probes were first normalized to internal spike-in controls (ERCCs) to account for system hybridization variation. Then, we normalized the digital counts to the area of their compartment. Those compartments with less than 10 nuclei or an area of illumination (AOI) less than 100 μm2 were systematically excluded.

**Multiplexed NK cell panel staining protocol**

Briefly, after TMA sections were deparaffinized, we subjected them to antigen retrieval with EDTA pH 8 buffer at 97ºC for 20 min in a pressure boiling container (PT module, Lab Vision). Next, we incubated the slides with a solution of 0.3% hydrogen peroxide in methanol to inactivate endogenous peroxidase for 30 min, followed by another 30 min incubation with 0.3% bovine serum albumin with 0.05% tween-20 blocking solution. Subsequently, we performed a sequential multiplexed immunofluorescence staining. First, we incubated the slides with a primary monoclonal antibody against CD56 (clone 123C3, Cell Signaling, 1:200) overnight at 4ºC, followed by a primary monoclonal antibody against CD3 (clone SP7, Novus Biologicals, 1:100) for 1 h at room temperature (RT). After each of the primary antibodies, the slides were incubated with isotype-specific horseradish peroxidase (HRP)-conjugated secondary antibodies at RT for 1 h, followed by tyramide-based labelling for 10 min, followed by 1 mM benzoic hydrazide with 0.15% hydrogen peroxide for 7 min twice to eliminate HRP activity. The secondary antibodies were anti-mouse IgG1 (eBioscience, 1:100) and anti-rabbit EnVision reagent (Dako), respectively, and the substrates were TSA Plus Cy3 tyramide (PerkinElmer, 1:100), and Cy5 tyramide (PerkinElmer, 1:50), respectively. Finally, we incubated the tissue sections with a primary antibody against CK (rabbit polyclonal, Agilent, 1:100) for 1h at RT followed by another 1h RT incubation with secondary anti-rabbit Alexa Fluor 488 (Invitrogen, 1:100) to identify tumor cells. We used 4',6-diamidino-2-phenylindole (DAPI) to counterstain and visualize nuclei, and we mounted the slides with ProLong Gold Antifade reagent (Invitrogen).

**Cell count quantification**

We determined cell counts using the inForm Tissue Finder software (Akoya), on multispectral images acquired using a Vectra 3 system (PerkinElmer). Multispectral images were decomposed into their various components by spectral unmixing using a digital spectral library consisting of spectral profiles of each of the fluorophores. Automated tissue segmentation identified tumor and stroma regions. Cell segmentation within these regions identified individual cells and respective nuclei, cytoplasm, and membrane components using signal in the nucleus and membrane as internal and external

cell borders. Then cells were phenotyped for marker expression based on fluorescent marker intensity in the following phenotypes: CD56+/CD3- for NK cells, CD56+/CD3+ for NKT cells, CD56+/CK+ for CD56 expressing tumor cells, and CD56-/CD3+ T cells. Cell counts for each NSCLC case were calculated in terms of the number of cells positive for the marker of interest as a percentage of the cell population in the tumor compartment, the stromal compartment, and the entire TMA spot.

**Immunotherapy efficacy assessment**

We used response evaluation criteria in solid tumors (RECIST) v1.1 to assess treatment response to immune checkpoint blockade. We defined clinical benefit (CB) as having experienced partial response or stable disease lasting ≥ 6 months as best response, whereas non-clinical benefit (NCB) was defined as primary progressive disease or stable disease lasting < 6 months. Patients with stable disease who did not progress and were censored before 6 months of follow-up were non-evaluable. Overall survival (OS) and progression-free survival (PFS) were calculated from the treatment start date to the date of death or loss of follow-up, or the date of disease progression, death or loss of follow-up, respectively. For those patients who did not die or progress during the study period, the outcome was considered left-censored. Data cut-off date was June 19, 2018.

**Supplementary table S1. Human IO panel for protein detection used in this study**

|  |  |
| --- | --- |
| **Core/Module (for Beta program)** | **Target** |
| Immune Cell Profiling Core | Beta-2-microglobulin |
| CD11c |
| CD20 |
| CD3 |
| CD4 |
| CD45 |
| CD56 |
| CD68 |
| CD8 |
| CTLA4 |
| Granzyme B |
| Ki-67 |
| PD-1 |
| PD-L1 |
| Pan-cytokeratin |
| HLA-DR |
| SMA |
| Fibronectin |
| TGFB1 |
| 6 Controls (Histone H3, S6, GAPDH, Mouse IgG1, Mouse IgG2a, Rabbit IgG) |
| IO Drug Target Module | 4-1BB CD137 |
| LAG3 |
| OX40L |
| Tim-3 |
| VISTA |
| ARG1 |
| B7-H3 |
| IDO1 |
| STING |
| GITR |
| Immune Activation Status Module | CD127 |
| CD25 |
| CD80 |
| CD86 |
| ICOS |
| PD-L2 |
| CD40 |
| CD40L |
| CD27 |
| CD44 |

**Supplementary table S2. Markers significantly associated with CB under PD-1 checkpoint blockade**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Compartment** | **Marker** | **Cutpoint** | **OR (CI 95 %)** | ***p*** |
| **Tumor compartment** | VISTA | Top tertile | 0.09 (0.01-0.80) | 0.031 |
| CD127 | Top tertile | 0.08 (0.01-0.71) | 0.024 |
| **CD45 compartment** | CD56 | Top tertile | 6.70 (1.46-30.7) | 0.014 |
| CD4 | Median | 8.55 (1.54-47.40) | 0.014 |
| CTLA4 | Median | 0.13 (0.02-0.74) | 0.022 |
| OX40L | Median | 6.37 (1.16-34.9) | 0.033 |
| ICOS | Top tertile | 5.50 (1.23-24.5) | 0.025 |
| **CD68 compartment** | CD45 | Top tertile | 4.16 (1.05-16.4) | 0.042 |
| CD56 | Top tertile | 4.16 (1.05-16.4) | 0.042 |
| CD20 | Top tertile | 4.16 (1.05-16.4) | 0.042 |
| PD-L2 | Top tertile | 4.16 (1.05-16.4) | 0.042 |

**Supplementary table S3. Subgroup analysis of markers significantly associated with PFS and/or OS benefit under PD-1 checkpoint blockade according to biopsy site and line of therapy**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Markers associated with PFS benefit according to biopsy site and line of therapy** | | | | | | |
| **Compartment** | **Marker** | **Cutpoint** | **Biopsy site** | | **Line of therapy** | |
| **Primary tumor or regional lymph nodes** | **Metastatic lesions** | **First line** | **Later line** |
| **Univariate**  **HR (CI 95%) (p)** | **Univariate**  **HR (CI 95%)** | **Univariate**  **HR (CI 95%)** | **Univariate**  **HR (CI 95%)** |
| **Tumor compartment** | VISTA | Top tertile | 2.52 (1.27-4.98)  (p = 0.008) | 2.27 (0.43-22.7)  (p = 0.25) | 306.6 (0.001-63653016.6) (p = 0.35) | 2.06 (1.01-4.18)  (p = 0.046) |
| CD127 | Top tertile | 2.65 (1.33-5.27)  (p = 0.005) | 3.16 (0.37-13.6)  (p = 0.36) | 306.6 (0.001-63653016.6) (p = 0.35) | 2.23 (1.11-4.46)  (p = 0.023) |
| **CD45 compartment** | CD56 | Top tertile | 0.39 (0.17-0.85)  (p = 0.019) | 0.006 (0.00-500061.2)  (p = 0.58) | 0.57 (0.06-5.25)  (p = 0.62) | 0.27 (0.11-0.65)  (p = 0.004) |
| CD4 | Median | 0.35 (0.16-0.74)  (p = 0.006) | 0.006 (0.00-500061.2)  (p = 0.58) | 0.44 (0.07-2.69)  (p = 0.37) | 0.32 (0.14-0.73)  (p = 0.007) |
| ARG1 | Median | 0.44 (0.21-0.90)  (p = 0.024) | 0.70 (0.04-11.7)  (p = 0.80) | 0.02 (0.00-34.83)  (p = 0.30) | 0.39 (0.18-0.85)  (p = 0.018) |
| **CD68 compartment** | CTLA4 | Top tertile | 1.76 (0.88-3.48)  (p = 0.10) | 4.21 (0.37-47.5)  (p = 0.24) | 1.38 (0.24-7.64)  (p = 0.71) | 2.15 (1.04-4.43)  (p = 0.038) |
| **Markers associated with OS benefit** | | | | | | |
| **Compartment** | **Marker** | **Cutpoint** | **Type of biopsy** | | **Line of therapy** | |
| **Primary tumor or regional lymph nodes** | **Metastatic lesions** | **First line** | **Later line** |
| **Univariate**  **HR (CI 95%) (p)** | **Univariate**  **HR (CI 95%)** | **Univariate**  **HR (CI 95%)** | **Univariate**  **HR (CI 95%)** |
| **Tumor compartment** | STING | Top tertile | 0.40 (0.18-0.90)  (p = 0.028) | 0.01 (0.00-30.20)  (p = 0.28) | 0.24 (0.02-2.36)  (p = 0.22) | 0.37 (0.15-0.87)  (p = 0.024) |
| **CD45 compartment** | CD45 | Median | 0.32 (0.14-0.69)  (p = 0.004) | 2.00 (0.12-31.97)  (p = 0.62) | NE | 0.41 (0.18-0.93)  (p = 0.033) |
| CD56 | Top tertile | 0.40 (0.17-0.95)  (p = 0.04) | 0.50 (0.03-7.99)  (p = 0.62) | 0.03 (0.00-1734.7)  (p = 0.54) | 0.39 (0.17-0.89)  (p = 0.027) |
| PD-L1 | Median | 0.48 (0.23-1.02)  (p = 0.058) | 0.02 (0.00-5748.1)  (p = 0.56) | 0.70 (0.09-5.09)  (p = 0.73) | 0.41 (0.18-0.94)  (p = 0.037) |
| CD68 | Top tertile | 0.40 (0.17-0.91)  (p = 0-31) | 0.50 (0.03-7.99)  (p = 0.62) | NE | 0.19 (0.07-0.51)  (p = 0.001) |
| CD4 | Median | 0.29 (0.13-0.64)  (p = 0.002) | 0.50 (0.03-7.99)  (p = 0.62) | 0.87 (0.08-8.51)  (p = 0.90) | 0.19 (0.07-0.50)  (p = 0.001) |
| B2M | Median | 0.27 (0.12-0.62)  (p = 0.002) | 0.02 (0.00-5748.1)  (p = 0.56) | 0.17 (0.01-1.71)  (p = 0.13) | 0.35 (0.15-0.80)  (p = 0.013) |
| CD20 | Median | 0.35 (0.16-0.78)  (p = 0.011) | 2.00 (0.12-31.97)  (p = 0.62) | 0.13 (0.01-1.50)  (p = 0.10) | 0.48 (0.21-1.09)  (p = 0.08) |
| CD3 | Median | 0.20 (0.08-0.47)  (p<0.001) | 38.04 (0.00-8479604)  (p = 0.56) | 0.13 (0.01-1.50)  (p = 0.10) | 0.27 (0.11-0.64)  (p = 0.003) |
| CD8 | Top tertile | 0.40 (0.17-0.91)  (p = 0.031) | NE | 0.53 (0.05-5.28)  (p = 0.59) | 0.30 (0.12-0.78)  (p = 0.014) |
| TIM3 | Median | 0.30 (0.12-0.71)  (p = 0.006) | 2.00 (0.12-31.97)  (p = 0.62) | 0.35 (0.05-2.59)  (p = 0.31) | 0.35 (0.14-0.86)  (p = 0.023) |
| CD40 | Median | 0.49 (0.23-1.03)  (p = 0.06) | NE | 0.87 (0.08-8.51)  (p = 0.90) | 0.42 (0.18-0.97)  (p = 0.044) |
| ICOS | Top tertile | 0.34 (0.15-0.79)  (p = 0.013) | 0.02 (0.00-5748.1)  (p = 0.56) | 0.53 (0.07-3.91)  (p = 0.54) | 0.22 (0.11-0.76)  (p = 0.011) |
| **CD68 compartment** | CD45 | Top tertile | 0.31 (0.14-0.72)  (p = 0.006) | NE | 0.84 (0.11-6.01)  (p = 0.86) | 0.19 (0.06-0.57)  (p = 0.003) |
| PD-L1 | Top tertile | 0.41 (0.18-0.92)  (p = 0.033) | 1.64 (0.14-18.23)  (p = 0.68) | 1.60 (0.22-11.46)  (p = 0.63) | 0.27 (0.11-0.69)  (p = 0.007) |
| CD20 | Top tertile | 0.28 (0.12-0.66)  (p = 0.004) | 1.64 (0.14-18.23)  (p = 0.68) | 0.41 (0.05-3.03)  (p =0.38) | 0.31 (0.12-0.84)  (p = 0.021) |
| GNZB | Top tertile | 0.32 (0.13-0.80)  (p = 0.015) | 4.85 (0.42-54.93)  (p = 0.20) | 0.35 (0.03-3.52)  (p =0.37) | 0.38 (0.15-0.95)  (p = 0.039) |

NE: not evaluable

**Supplementary table S4. Markers significantly associated with PFS and/or OS benefit under PD-1 checkpoint blockade**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Markers associated with PFS benefit** | | | | | | | | | |
| **Compartment** | **Marker** | **Cutpoint** | **Univariate**  **HR (CI 95%)** | ***p*** | **HR adjusted by biopsy site**  **(CI 95%)** | ***p*** | **HR adjusted by lines of therapy**  **(CI 95%)** | ***p*** |
| **Tumor compartment** | VISTA | Top tertile | 2.60  (1.37-4.92) | 0.003 | 2.58  (1.36-4.90) | 0.004 | 2.49  (1.29-4.80) | 0.006 |
| CD127 | Top tertile | 2.65  (1.41-4.98) | 0.002 | 2.71  (1.44-5.12) | 0.002 | 2.69  (1.41-5.11) | 0.003 |
| **CD45 compartment** | CD56 | Top tertile | 0.38  (0.18-0.80) | 0.011 | 0.37  (0.17-0.79) | 0.011 | 0.28  (0.12-0.64) | 0.002 |
| CD4 | Median | 0.33  (0.16-0.67) | 0.002 | 0.32  (0.15-0.67) | 0.003 | 0.33  (0.16-0.70) | 0.004 |
| ARG1 | Median | 0.43  (0.21-0.86) | 0.018 | 0.43  (0.21-0.87) | 0.019 | 0.36  (0.17-0.74) | 0.006 |
| **CD68 compartment** | CTLA4 | Top tertile | 1.95  (1.01-3.77) | 0.044 | 1.93  (1.00-3.72) | 0.047 | 2.06  (1.06-4.02) | 0.033 |
| **Markers associated with OS benefit** | | | | | | | | | |
| **Tumor compartment** | STING | Top tertile | 0.31  (0.14-0.69) | 0.004 | 0.35  (0.16-0.75) | 0.007 | 0.33  (0.14-0.74) | 0.007 |
| **CD45 compartment** | CD45 | Median | 0.35  (0.16-0.73) | 0.005 | 0.34  (0.16-0.73) | 0.005 | 0.38  (0.17-0.85) | 0.020 |
| CD56 | Top tertile | 0.44  (0.20-0.97) | 0.044 | 0.42  (0.19-0.94) | 0.035 | 0.37  (0.17-0.84) | 0.018 |
| PD-L1 | Median | 0.48  (0.23-0.99) | 0.049 | 0.49  (0.24-1.00) | 0.052 | 0.48  (0.23-1.00) | 0.052 |
| CD68 | Top tertile | 0.43  (0.20-0.93) | 0.033 | 0.41  (0.19-0.90) | 0.027 | 0.24  (0.10-0.59) | 0.002 |
| CD4 | Median | 0.31  (0.15-0.66) | 0.002 | 0.34  (0.15-0.65) | 0.002 | 0.31  (0.14-0.67) | 0.003 |
| B2M | Median | 0.28  (0.12-0.61) | 0.002 | 0.28  (0.13-0.62) | 0.002 | 0.30  (0.13-0.65) | 0.003 |
| CD20 | Median | 0.38  (0.18-0.82) | 0.014 | 0.39  (0.18-0.83) | 0.015 | 0.43  (0.19-0.94) | 0.036 |
| CD3 | Median | 0.24  (0.11-0.53) | <0.001 | 0.24  (0.11-0.53) | <0.001 | 0.26  (0.11-0.58) | 0.001 |
| CD8 | Top tertile | 0.38  (0.17-0.87) | 0.023 | 0.38  (0.16-0.88) | 0.024 | 0.36  (0.15-0.83) | 0.017 |
| TIM3 | Median | 0.32  (0.14-0.72) | 0.006 | 0.32  (0.14-0.72) | 0.007 | 0.35  (0.15-0.79) | 0.012 |
| CD40 | Median | 0.48  (0.24-0.99) | 0.049 | 0.48  (0.23-1.00) | 0.052 | 0.52  (0.25-1.08) | 0.082 |
| ICOS | Top tertile | 0.35  (0.16-0.78) | 0.010 | 0.35  (0.16-0.78) | 0.010 | 0.37  (0.16-0.82) | 0.015 |
| **CD68 compartment** | CD45 | Top tertile | 0.33  (0.15-0.74) | 0.008 | 0.30  (0.13-0.69) | 0.005 | 0.32  (0.14-0.74) | 0.008 |
| PD-L1 | Top tertile | 0.45  (0.21-0.98) | 0.045 | 0.44  (0.20-0.96) | 0.040 | 0.41  (0.18-0.90) | 0.027 |
| CD20 | Top tertile | 0.33  (0.14-0.74) | 0.007 | 0.31  (0.14-0.71) | 0.006 | 0.37  (0.16-0.85) | 0.020 |
| GNZB | Top tertile | 0.42  (0.18-0.93) | 0.032 | 0.41  (0.18-0.92) | 0.031 | 0.42  (0.19-0.95) | 0.039 |