Supplemental Online Content

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Imaging Mass Cytometry

Imaging mass cytometry was performed at the Flow Cytometry and Cellular Imaging Facility at The University of Texas MD Anderson Cancer Center. A tissue microarray was constructed with 6 representative 1-mm³ formalin-fixed, paraffin-embedded tissue samples: 2 samples obtained from the primary parotid tumor before the first vaccine dose, 2 samples obtained from pulmonary metastases before the first vaccine dose, and 2 samples obtained from pulmonary metastases after the second vaccine dose.

The tissue microarray was deparaffinized and rehydrated prior to heat-induced antigen retrieval performed by microwaving (MW014-MO, EZ-Retriever system, BioGenex) for 15 minutes at 95 °C in Ph 8.5 EZ-AR 2 (EDTA) buffer (HK522-XAK, BioGenex), blocking with 3% bovine serum albumin plus 1% horse serum in phosphate-buffered saline, and incubation with heavy metal-labelled antibodies specified in Supplementary Table 1 overnight at 4 °C. Slides were washed with TBS-T (TBS plus Tween 0.1%) followed by TBS and then incubated with 0.3125 µM Cell-ID Intercalator-Ir (Cat# 201192A, Fluidigm, 1:400 dilution) for the detection of nuclear DNA.

Metal-conjugated antibodies were detected with a Hyperion Imaging Mass Cytometer (Fluidigm). Tissue was laser ablated at 200 Hz. Data were analyzed by custom image analysis scripts. Cells were first segmented based on DNA signal after band-pass Gaussian filtering using Otsu’s method, and overlapping cells were divided by seeded watershed. Intensities for each antibody channel were corrected by lateral compensation as described. Marker combinations used to define cell populations are given in Supplementary Table 2. All analyses were performed in Matlab 2019a (Mathworks).

Tumor Molecular Profiling

Primary tumor molecular profiling was performed using a commercial tumor profiling service (CARIS Molecular Intelligence; CARIS Life Sciences, Texas, USA). Targeted-exome sequencing of the patient’s primary tumor was also performed using the Oncomine CDx Target test (Thermo Fisher Scientific).
## Supplementary Table 1. Antibodies and Metals Used for Imaging Mass Cytometry

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<th>Antibody</th>
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<th>Dilution</th>
<th>Vendor</th>
<th>Catalog No</th>
<th>Antibody Clone</th>
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*Bethyl Laboratories and Cell Signaling Technologies graciously provided antibodies to the MD Anderson Flow Cytometry and Cellular Imaging Facility for use in IMC.*
### Supplementary Table 2. Cell Populations Analyzed and Corresponding Markers

<table>
<thead>
<tr>
<th>CD45+ immune Cells</th>
<th>Markers</th>
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<tr>
<td>CD8 T cells</td>
<td>CD3+, CD8+</td>
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<tr>
<td>Memory CD8 T cells</td>
<td>CD45RO+ CD8 T cells</td>
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<td>Regulatory T cells</td>
<td>FoxP3+ CD4 T Cells</td>
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<td>All T cells</td>
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<td>NK cells</td>
<td>CD56+, CD3-</td>
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<td>Cytolytic cells</td>
<td>Granzyme B+ and CD56+ or CD8+</td>
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**Other cells**

- Tumor cells: Pan-cytokeratin+ or p63+, CD31-, non-immune cells
- Endothelial cells: CD31+, pan-cytokeratin-, p63-, non-immune cells
- Fibroblasts: Vimentin+ or αSMA+, CD31-, pan-cytokeratin-, p63-, non-immune cells

**Functional markers**

- Proliferating cells: Ki67
- T cell function: TIM3, TIGIT
Supplementary Figure 1. Representative hematoxylin and eosin–stained sections.

(A-B) Sections of primary parotid tumor at 4x (A) and 10x (B) magnification. Morphologically, the carcinoma is composed of relatively monotonous round cells with prominent nucleoli and eosinophilic cytoplasm; several areas have a more basophilic and other areas have a rhabdoid/plasmacytic-type appearance of the neoplastic cells. The phenotype is suggestive of a dominant myoepithelial carcinoma. Very rare, scattered mononuclear/lymphocytic elements are noted at the tumor border. (C-D) Section of pre-vaccination lung biopsy specimen at 10x and 4x magnification. The morphology is that of metastatic myoepithelial carcinoma, and entirely resembles the morphology of the primary tumor, including with respect to the immune environment, which has a rare mononuclear/lymphocytic inflammatory infiltrate. (E-F) Sections of post-vaccination lung biopsy specimens at 4x (E) and 10x (F) magnification. There is a massive inflammatory infiltrate with embedded scant tumor clusters (<5%). The inflammatory infiltrate consists of admixed mononuclear cells (lymphocytes, plasma cells, and monocytes) and a significant number of histiocytes.

A.
IMAGING MASS CYTOMETRY IMAGES

Supplementary Figure 2. Imaging mass cytometry images showing expression of antibody markers in representative lung biopsy specimens. (A) Pre-vaccine biopsy specimen. All images are of the same region of the same tumor (X, Y: 310, 130; height and width, 750 pixels). Scale bars, 100 μm. (B) Post-vaccine biopsy specimen. All images are of the same region of the same tumor (X, Y: 5, 85; height and width, 750 pixels). Scale bars, 100 μm.

A.
Reference