Spatial Multiparametric Investigation of Mouse and Human Brain Tumor Microenvironment Identifies Critical Neuro-Oncological Processes in Brain Neoplasms

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NEURO-ONCOLOGY

An Imaging Mass Cytometry™ (IMC™) workflow with neuro-oncology panels enables high-quality neurological and immunological readouts of over **40 protein markers simultaneously without autofluorescence, tissue discohesion or signal spillover** in normal brain tissue and in brain tumors of glial, non-glial and mixed origin.

KEY TAKEAWAYS

- The use of 35-plus-parameter human or mouse antibody panels demonstrates high specificity of markers and signal intensity in human and mouse normal and diseased brain tissue.

- Spatial phenotyping with IMC reveals the presence or absence of primary brain cell constituents such as microglia, astrocytes, neurons, endothelial cells and oligodendrocytes.

- Spatial locations of both infiltrating and resident immune cells are demarcated within the tumor microenvironment (TME).

Background

Brain cancer research presents challenges that require comprehensive assessment of the structural and cellular organization of the TME.
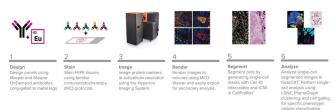
Here we present a deep phenotypic spatial analysis of various mouse and human brain tumors and identify cellular composition and activation of immuno-oncological processes within the TME.

Study Design

A 39-parameter human antibody panel and a 36-parameter mouse antibody panel were chosen to identify major cell populations in normal brain and the specific states of tumor and immune cell populations in the diseased brain.
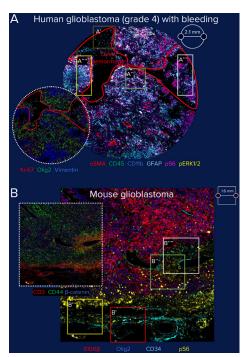
Tissue slides were prepared, stained and then imaged using the Hyperion™ Imaging System. Qualitative data analysis, multiplexed image rendering and single-channel image extractions were performed using MCD™ Viewer.

Results

- High-parameter neuro-oncology panels delineate tumor cell states and immune cell infiltration within the TME of human and mouse glioblastoma.

- The neuro-oncology panel allows deciphering of cell populations in various neoplasms in human and mouse diseased brain.

- Plasma membrane markers enhance cell segmentation capabilities to identify specific cell populations in the TME.
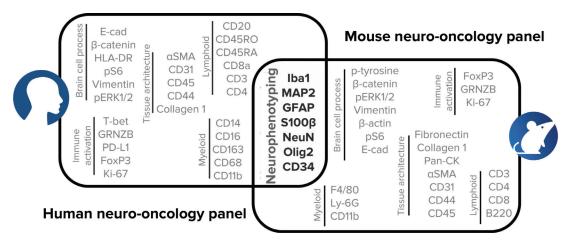
Imaging Mass Cytometry and single-cell analysis workflow

1 Design Design panels using Maxpar and Maxpar OnDemand antibodies conjugated to metal tags.

2 Stain Stain FFPE tissues using familiar immunohistochemistry (IHC) protocols.

3 Image Image protein markers at subcellular resolution using the Hyperion Imaging System.

4 Render Render images in minutes using MCD Viewer and easily export for secondary analysis.

5 Segment Segment cells by generating single-cell masks with Cell-ID Intercalator and ICSK in CellProfiler.

6 Analyze Analyze single-cell segmented images in histoCAT. Perform single-cell analysis using t-SNE, PhenoGraph clustering, and cell gating for specific phenotypic cellular classification.

IMC generated images of human glioblastoma (grade 4) with overt hemorrhage (bleeding) (A) and mouse glioblastoma (B) are shown.

Neuro-Oncology

Panels

Human neuro-oncology panel

Mouse neuro-oncology panel

Brain cell process: E-cad, β-catenin, HLA-DR, pS6, Vimentin, pERK1/2

Tissue architecture: αSMA, CD31, CD45, CD44, Collagen 1

Lymphoid: CD20, CD45RO, CD45RA, CD8a, CD3, CD4

Immune activation: T-bet, GRNZB, PD-L1, FoxP3, Ki-67

Myeloid: CD14, CD16, CD163, CD68, CD11b

Neurophenotyping: **Iba1, MAP2, GFAP, S100β, NeuN, Olig2, CD34**

Brain cell process: p-tyrosine, β-catenin, pERK1/2, Vimentin, β-actin, pS6, E-cad

Myeloid: F4/80, Ly-6G, CD11b

Tissue architecture: Fibronectin, Collagen 1, Pan-CK, αSMA, CD31, CD44, CD45

Immune activation: FoxP3, GRNZB, Ki-67

Lymphoid: CD3, CD4, CD8, B220

39-parameter human antibody panel and 36-parameter mouse antibody panel including two DNA and three ICSK (Maxpar® IMC Cell Segmentation Kit) channels designed to highlight central features of the TME. These panels are subdivided into six modules, each revealing critical insights about normal and tumor tissue composition, state and biology. The tissue architecture module identifies the underlying cellular and structural markers of the tumors. The brain cell process module identifies activation of signaling pathways, metabolism and growth in brain cells. The lymphoid and myeloid modules delineate lymphoid and myeloid cell subtypes of immune cell infiltrates in brain tissue. The immune activation module assesses the functional state of immune cells in brain tissue. E-cad — E-cadherin, GRNZB — granzyme B, Pan-CK — pan-cytokeratin

Ordering Information

Product Name	Product Number
Maxpar® Human Neuro-Oncology IMC™ Bundle	201508NO
Maxpar Neuro Phenotyping IMC Panel Kit	201337
Maxpar OnDemand™ Mouse Neuro-Oncology IMC Bundle	9100005NO
Maxpar IMC Cell Segmentation Kit	201500
Cell-ID™ Intercalator-Ir	201192B

See the **complete list of antibodies** used in this poster.

Scan the QR code to see the full digital version of the poster and supplemental data.