Orion™: 14-plex clinical sample imaging of liver cancer modalities using one-step staining and imaging

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Overview

This liver section exhibits extensive infiltration by metastatic moderately-differentiated adenocarcinoma with high Ki-67 proliferation index and central dirty necrosis morphologically consistent with primary tumor from the colon.

The tumor microenvironment was studied using a one-step 14-plex biomarker immunofluorescence stain and scan, followed by H&E staining and brightfield imaging of the same section. This allowed the traditional pathology analysis to be complimented by same-cell phenotyping.

The histopathological pattern of the immune microenvironment is divided into 3 categories; tumor with dirty necrosis (left), desmoplastic stroma (right) and invasive front (lower right) (figure 1a).

In the dirty necrosis region, tumor glands contain immune cells, mainly CD68+CD163- macrophage aggregates and rare T lymphocytes, which are responsible for cleaning the necrotic debris.

The desmoplastic stroma surrounding the tumor cells is densely populated with immune cells; predominantly clusters of CD68-CD163+ macrophages, scattered T lymphocytes (mixture of CD4+ T Helper cells as well as CD8+ cytotoxic T cells) and very rare, scattered B cells.

The immune cells at the invasive front are predominantly aggregates of CD4+ T Helper cells with high expression of PD-1, which are responsible for preventing further tumor invasion in the liver parenchyma.

Neoplastic glands can be visualized (outlined in cyan) with histiocytes (yellow) scavenging necrotic material. The glands are surrounded by extensive immune infiltrate (T cells, white). Many of the metastatic epithelial cancer cells are proliferating (nuclei, magenta) (figure 1b,1c).

H&E imaging shows significant replacement of liver parenchyma by metastatic tumor cell nodules. Examination shows that normal liver cells are surrounded by lymphocytic infiltration and neoplastic glands are surrounded by necrotic areas infiltrated by histiocytes. (figure 1d).

Hepatocytes

Hepatocytes (Autofluorescent, grey) are surrounded by T cells (magenta), Pan-CK (cyan) highlights neoplastic glands, and macrophages are shown to co-express CD163 and FOLR2 (orange/yellow) (figure 2).

Neoplastic Glands

Neoplastic glands adjacent to a necrotic area (center grey region) are shown to be infiltrated by histiocytes (yellow). Many of the metastatic epithelial cancer cells are proliferating (magenta) (figure 3).

Immune Infiltrate

The immune infiltrate consists of T cell subsets (CD4, cyan and CD8a, magenta) and macrophages co-expressing CD163 and FOLR2 (orange/yellow) (figure 4).

Co-Expression of PD-1 and PD-L1

Comparison in the same region demonstrates CD4 positive cells co-expressing PD-1 and FOLR2 positive macrophages co-expressing PD-L1 (figure 4).

Triple Positive Macrophages

Triple positive macrophages are clearly visible in the FOLR2, CD68, and CD163 biomarker channels (figure 5).

MARKER LIST

Hoechst
Albumin
CD31
CD4
CD68
CD3e
CD8a
CD163
CD20
FOLR2
Ki-67
Pan-CK
PD-1
PD-L1
AF

Summary of Orion Workflow

PREPARE
Standard microscope slide and tissue section

STAIN
Single staining process, standard IF protocols

IMAGE
Highly multiplexed imaging in one scan

ANALYZE
OME-TIFF output for viewing and quantification

Tissue Staining and Scanning Protocol

- Mount sections on glass slides
- De-paraffinize and perform antigen retrieval
- Quench autofluorescence
- Stain slides with panel of ArgoFluor™ conjugated antibodies
- Coverslip with ArgoFluor Mounting Medium and cure overnight
- Image whole slides at 20X magnification using Orion instrument
- Process to ome.TIFF and analyze
- De-coverslip in aqueous solution
- Perform H&E staining and scanning on same section

Liver Tissue Sample Information

The FFPE liver section was stained using a 14-plex immunofluorescence (IF) panel in one staining round, followed by whole-slide imaging with the Orion instrument. All of the markers were quantitated in one imaging round, along with tissue autofluorescence, which was isolated as an additional channel to provide additional tissue morphology information. Following IF scanning, the slide was stripped and stained with H&E. Brightfield imaging was carried out on the Orion instrument. By utilizing multimodal imaging on the same sample, the traditional H&E pathology analysis was complimented by phenotyping of the cells of the tumor microenvironment.

ORION BENCHTOP FOOTPRINT

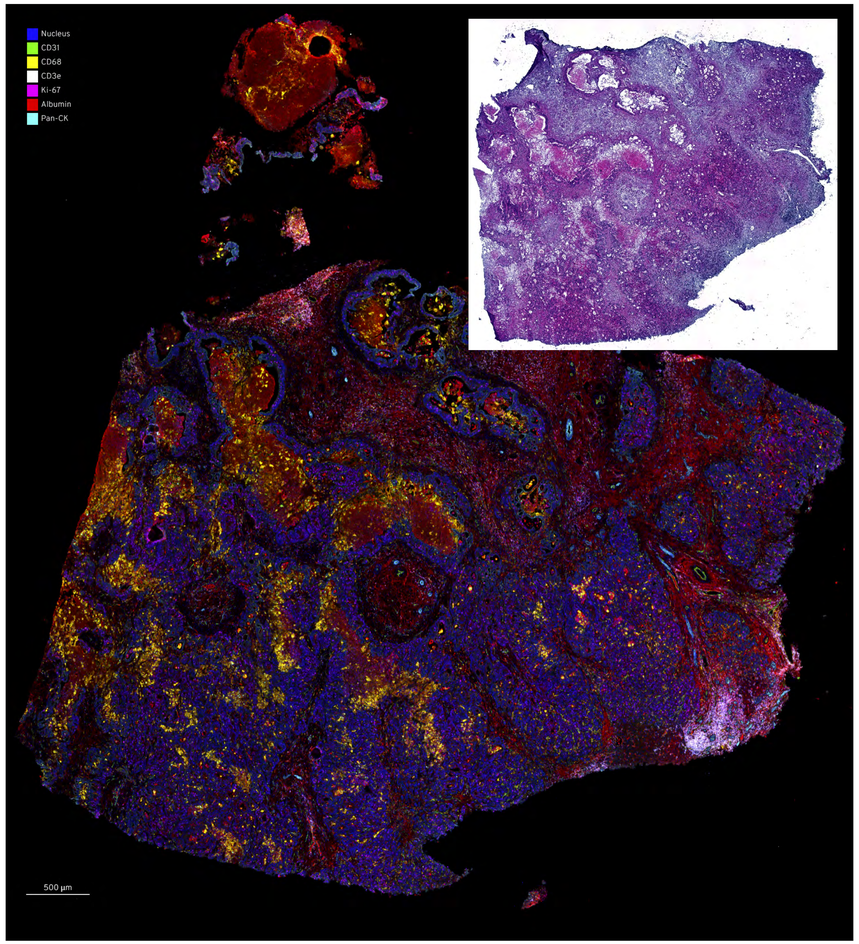

Fig 1a. Whole slide liver section stained with a 14-plex panel and imaged on Orion shows extensive infiltration by metastatic moderately-differentiated adenocarcinoma that is morphologically consistent with primary tumor from the colon.

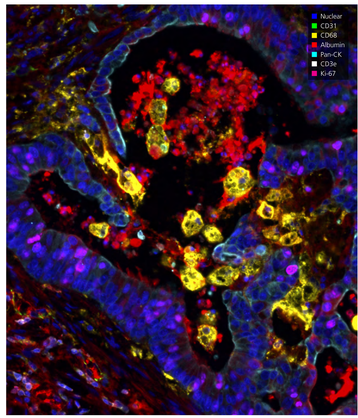

Fig 1b. Neoplastic gland (cyan) with histiocytes (yellow) scavenging necrotic material.

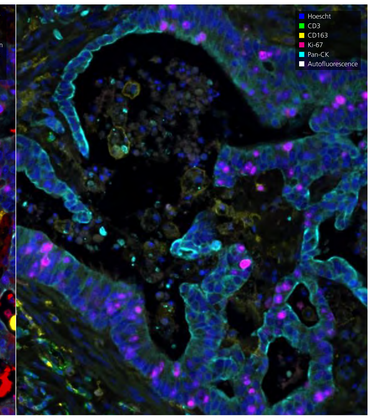

Fig 1c. Many of the metastatic epithelial cancer cells are proliferating (magenta).

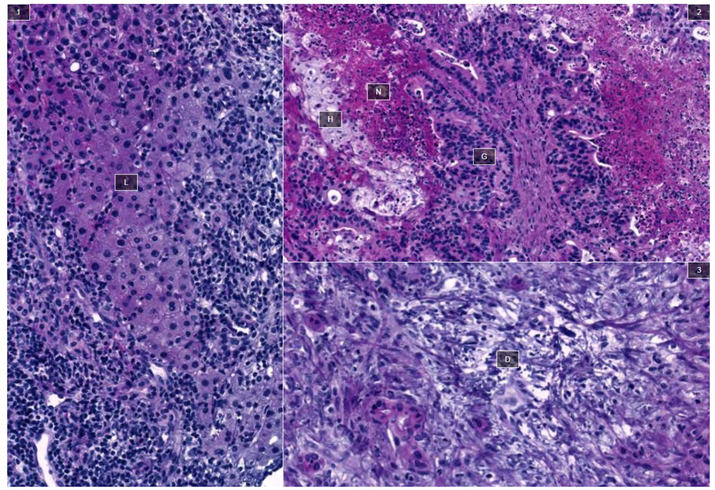

Fig 1d. 1: Normal liver cells (L) surrounded by lymphocytic infiltrate. 2: Neoplastic glands (G) surrounded by necrotic areas (N) infiltrated by histiocytes (H). 3: Desmoplastic stroma (D) rich in histiocytes, lymphocytes, and capillaries.

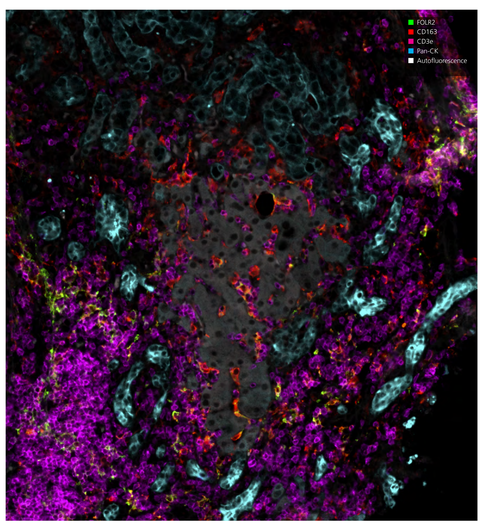

Fig 2. Hepatocytes (Autofluorescent, grey) surrounded by T cells.

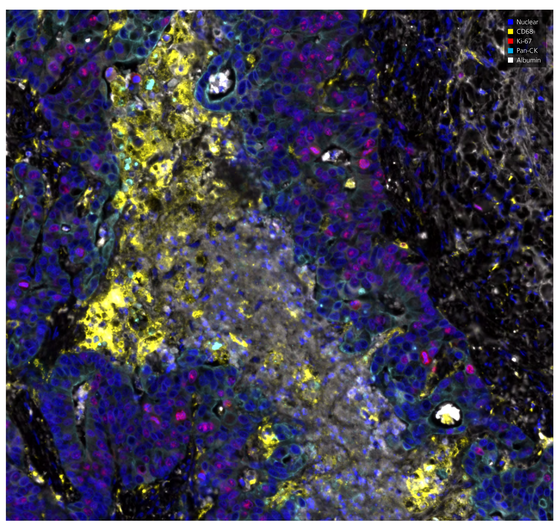

Fig 3. Neoplastic glands adjacent to a necrotic area (center grey region) infiltrated by histiocytes.

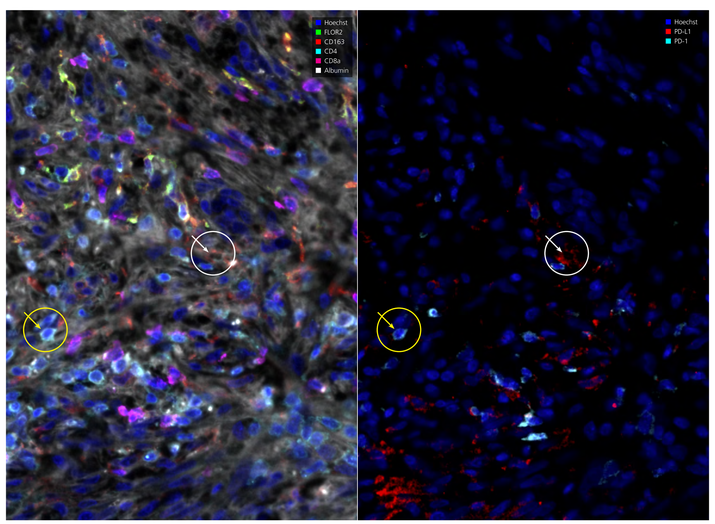

Fig 4. CD4+ T cell co-expressing PD-1 (yellow arrow). FOLR2+ macrophage co-expressing PD-L1 (white arrow).

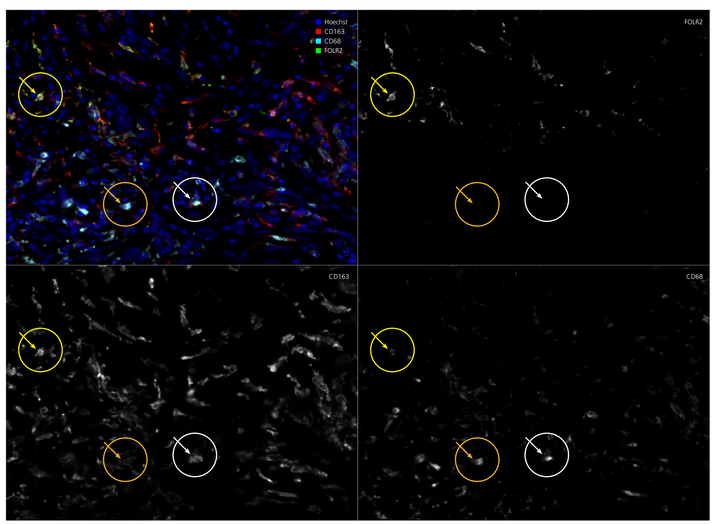

Fig 5. Triple positive (FOLR2, CD68, CD163) macrophage (yellow arrow). Double positive (FOLR2 negative) macrophage (white arrow). CD68 single positive macrophage (orange arrow).