

Orion™: 16-Plex Single-Step Stain & Imaging of Oral Squamous Cell Carcinoma

RareCyte, Inc.

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Precision Biology for Life Sciences

Overview

This is a whole-slide section of an infiltrating squamous cell carcinoma of the tongue. The normal mucosal surface of the tongue is at the bottom of the image and the nodule of invasive cancer is seen in the top half, surrounded by fibrous reaction (figure 1).

Note that at the lower left edge of the section is a brightly stained region. This is fluorescence of a marking pigment used to delineate the border of the tumor resection specimen.

Normal Tongue Mucosa

The mucosa of the tongue contains a stratified squamous epithelium with extensions (rete pegs) that project into the underlying connective tissue (lamina propria) (figure 2).

The basal layer of the epithelium can be identified by the expression of E-Cadherin. Scattered proliferating cells can be identified in this layer by the nuclear expression of the marker Ki67. As the cells differentiate into squamous keratinocytes toward the surface, cytokeratins are prominently expressed.

Between the rete pegs are connective tissue papillae that contain CD31+ capillary endothelial cells in venules, smooth muscle actin (SMA) in arterioles and a collection of immune cells (CD45+, white).

Infiltrating Squamous Cell Carcinoma

The tumor cells recapitulate the organizational structure of the normal epithelium. Most express E-Cadherin similar to basal mucosal epithelium. Yet in some regions, the cells differentiate into squamous cells and express Pan-CK. In contrast to the normal epithelium, the tumor is highly proliferative, with many Ki67+ cells. Scattered T cells (CD3) surround the cancer cells (figure 3).

Host Response to Tumor

The tumor stimulates a host fibrocellular immune response that is demonstrated by a mixture of immune cells at the border of the tumor nodule (E-Cadherin and Pan-CK) (figure 4). Collections of interspersed B cells (CD20), helper T cells (CD4) and cytotoxic T cells (CD8+) are present within an active area of cellular fibrosis (FAP, white).

Submucosal Stroma

In the submucosal region, there are stromal cells that include endothelial cells (CD31), vascular smooth muscle cells (SMA, MYH11), and fibroblasts (FAP, white) (figure 5).

The arteriolar blood vessel walls exhibit a bright orange color due to the co-expression of SMA and MYH11. Vimentin is expressed in many different types of stromal cells.

T Cell Subsets

Multiplexed biomarkers allow the study of a variety of T cell sub-types, including regulatory T cells and activated T cells. This region shows an aggregate of CD4+ and CD8+ T cells adjacent to nests of tumor cells (E-Cadherin, cytokeratin) (figure 6).

Regulatory T cells, typically CD4 T cells, are identified by FOXP3 staining of the nucleus. PD-1 marks activated cells.

Within the aggregate, the checkpoint marker PD-1 is observed on CD4 T cells, some of which are FOXP3+ cells. There are lone CD8 T (upper right) cells that strongly express PD-1 within the tumor nests which may represent exhausted tumor infiltrating lymphocytes.

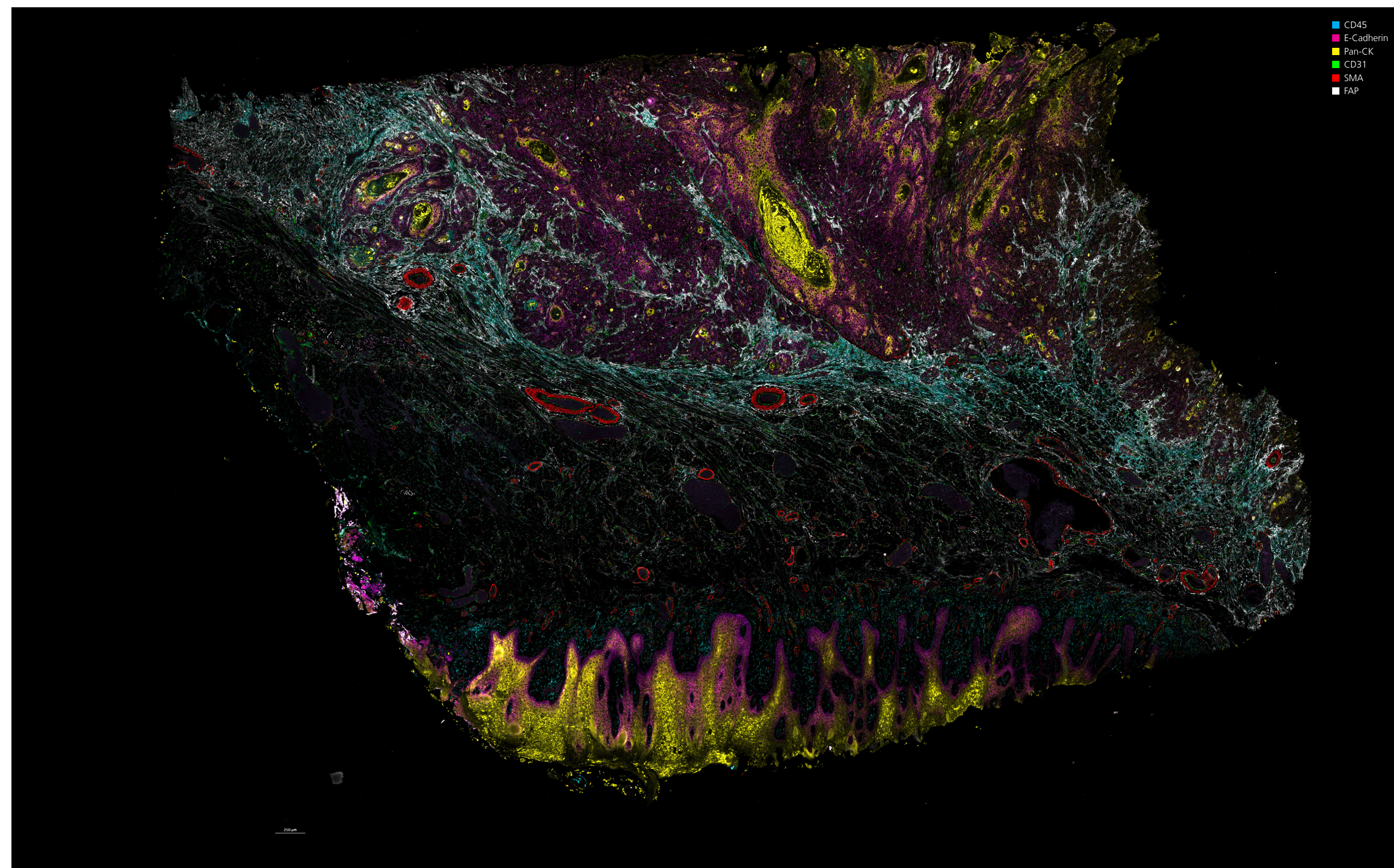


Fig 1. Whole-slide tissue section of an infiltrating squamous cell carcinoma of the tongue stained with a 16-plex biomarker panel and imaged in one scan on Orion.

Summary of Orion Workflow



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

Orion Squamous Cell Carcinoma Sample Information

- The FFPE squamous cell section was stained with a 16-plex immunofluorescence (IF) panel in one staining round followed by whole slide imaging with the Orion instrument in one imaging round.
- Tissue autofluorescence was imaged and isolated as an additional fluorescence channel

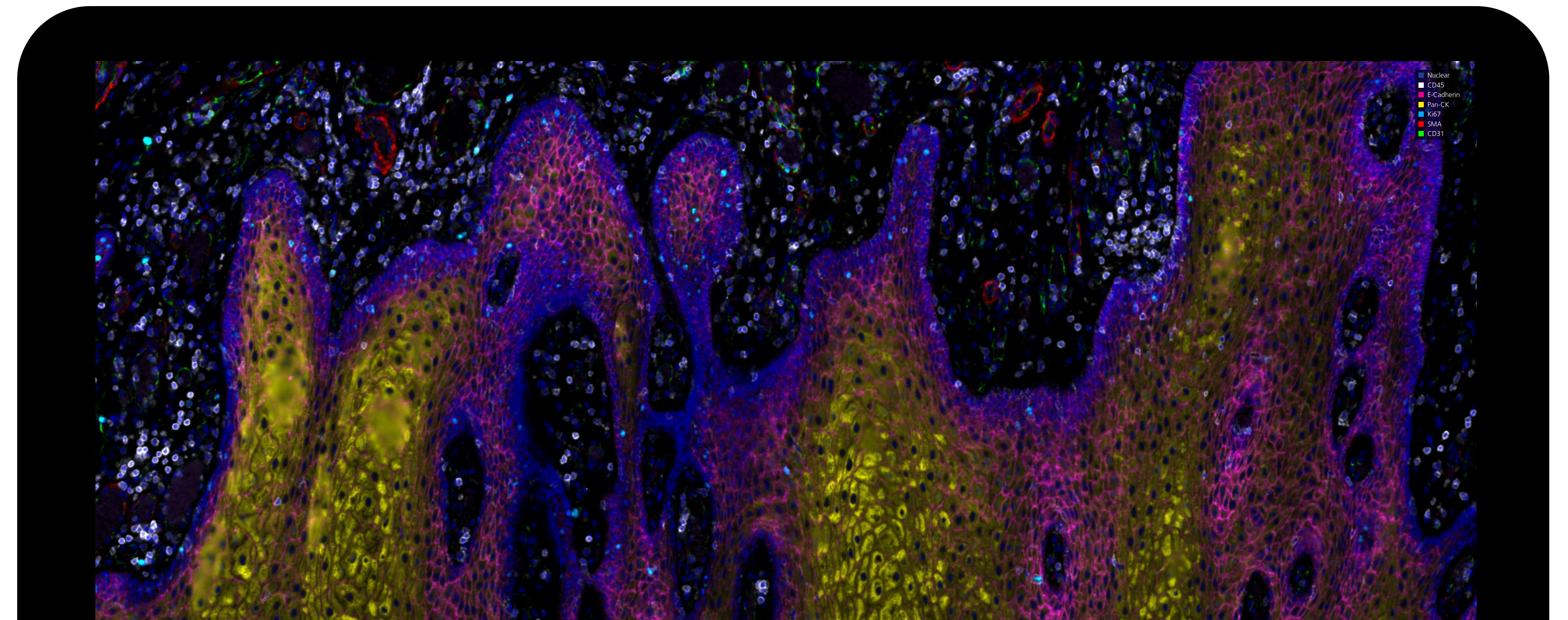


Fig 2. The mucosa of the tongue contains a stratified squamous epithelium with extensions (rete pegs) that project into the underlying connective tissue (lamina propria). The basal layer of the epithelium can be identified by the expression of E-Cadherin (magenta). Scattered proliferating cells are identified in this layer by the nuclear expression of Ki67 (dark blue). Between the rete pegs are connective tissue papillae that contain CD31+ (green) capillary endothelial cells, smooth muscle actin (red) in arterioles, and immune cells (CD45+, white).

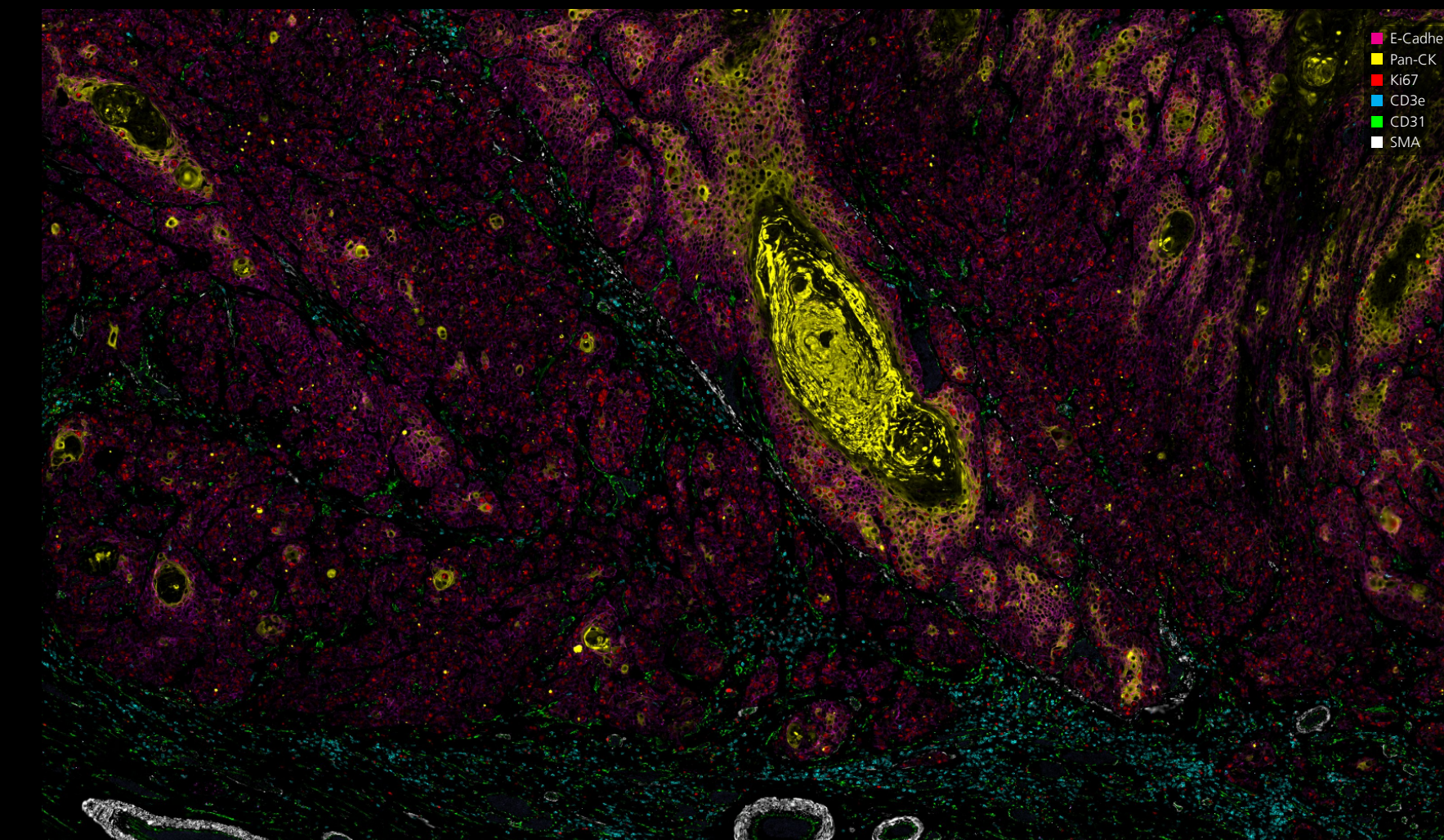


Fig 3. Tumor cells recapitulate the organizational structure of the normal epithelium. Most express E-Cadherin (magenta) similar to basal mucosal epithelium, yet some cells differentiate into squamous cells and express Pan-CK (yellow). The tumor is highly proliferative seen by Ki67+ (red) cells with scattered T cells (CD3, blue) surrounding the cancer cells.

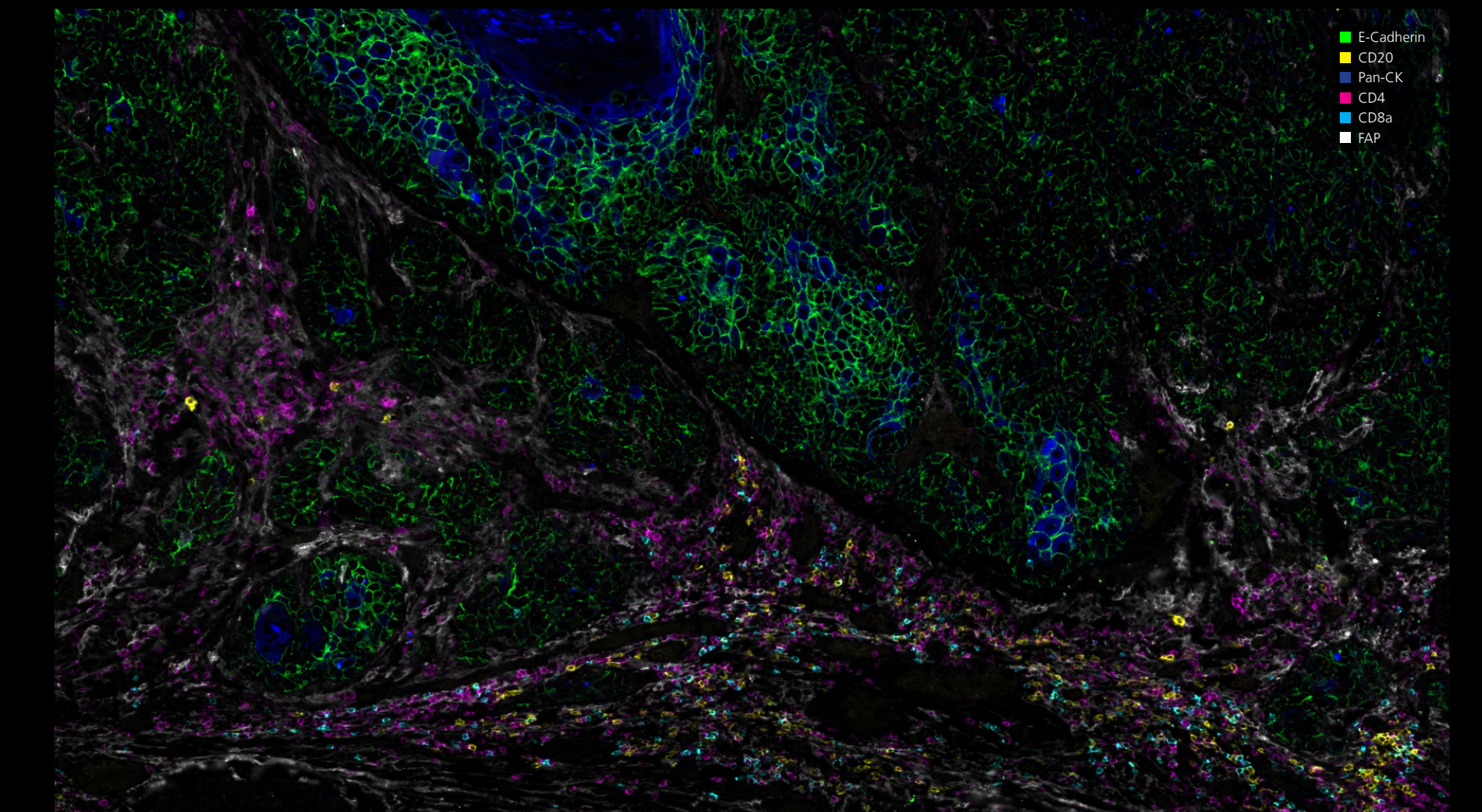


Fig 4. The tumor stimulates a host fibrocellular immune response that is demonstrated by a mixture of immune cells at the border of the tumor nodule (E-Cadherin in green and Pan-CK in dark blue). Interspersed B cells (CD20, yellow), helper T cells (CD4, magenta) and cytotoxic T cells (CD8+, light blue) are present within an active area of cellular fibrosis (FAP, white).

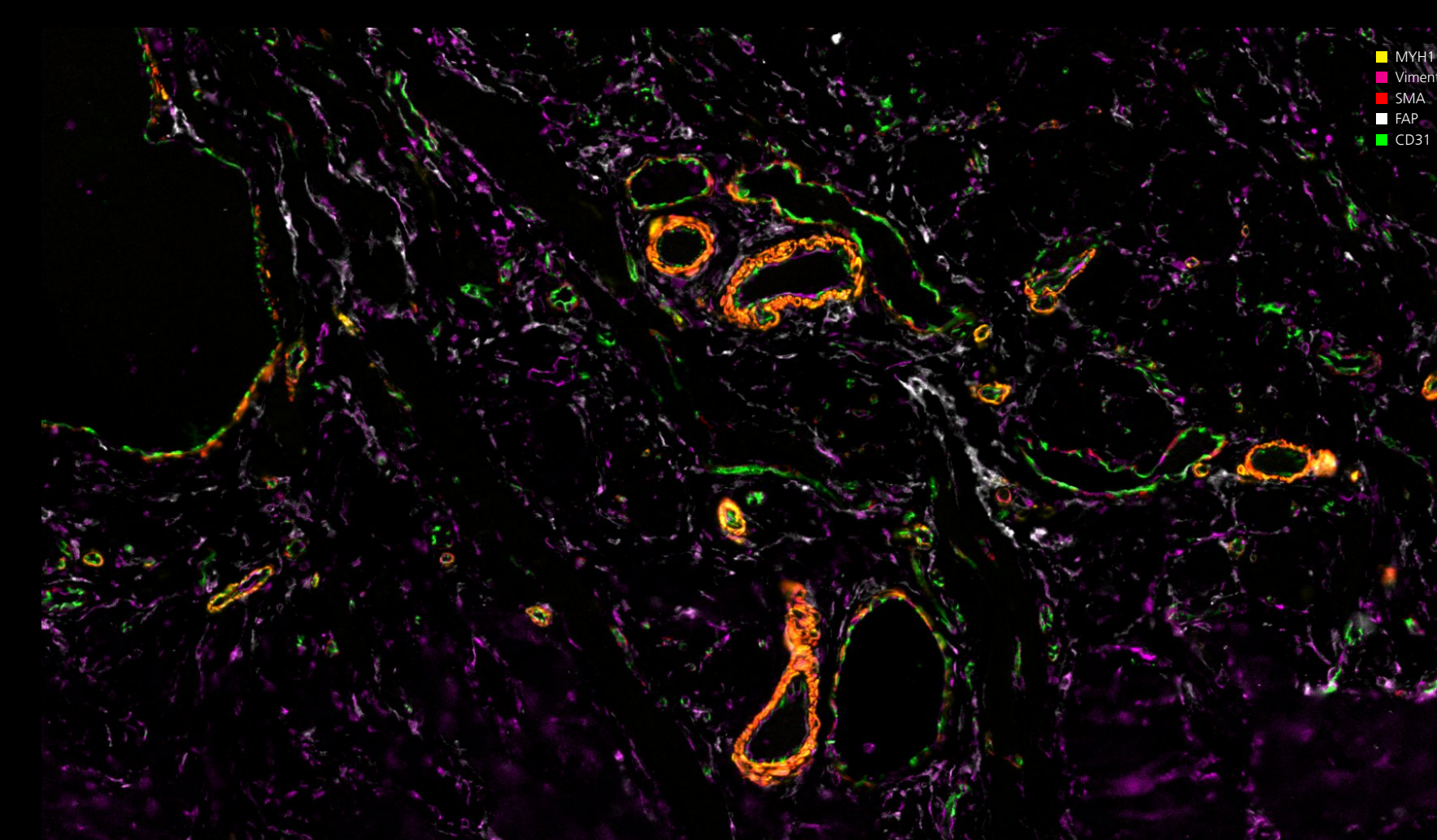


Fig 5. A submucosal region showing stromal cells that include endothelial cells (CD31, green), vascular smooth muscle cells (SMA, MYH11 in yellow), and fibroblasts (FAP, white). The arteriolar blood vessel walls exhibit a bright orange color due to the co-expression of SMA and MYH11.

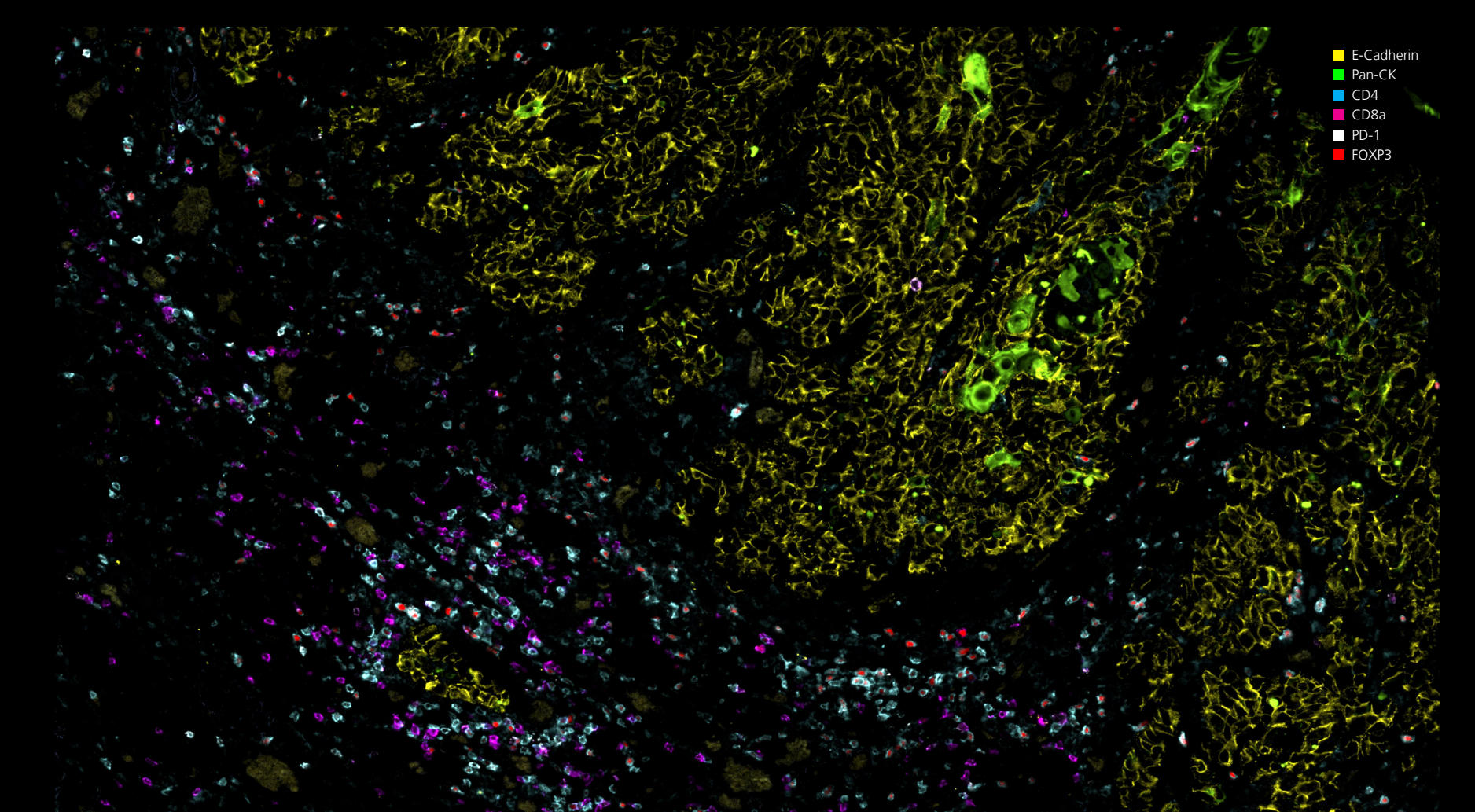


Fig 6. An aggregate of CD4+ (light blue) and CD8+ (magenta) T cells adjacent to nests of tumor cells marked by E-Cadherin (yellow). Regulatory T cells identified by FOXP3 (red) staining of the nucleus and PD-1 (white) marks activated cells.

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