Q&A: Stem-immunity hubs associated with response to immunotherapy

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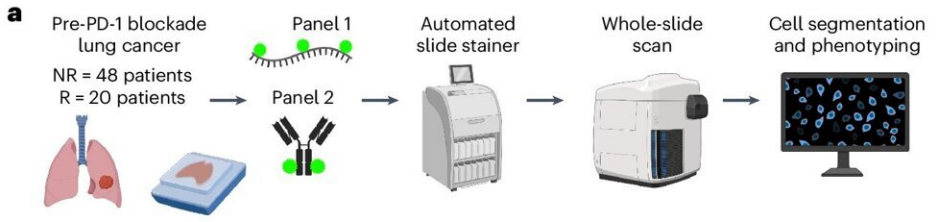
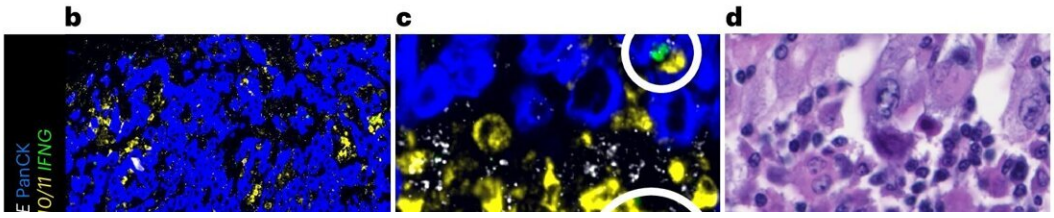


Panel 1: tumor immunity hub		
Target	Type	TSA fluorophore
CXCL13	RNA-ISH	480
CXCL10/11	RNA-ISH	520
		570
CD3E	RNA-ISH	620
IFNG	RNA-ISH	690
PanCK	Ab	780

Panel 2: T cell state			
Target	Type	Staining pattern	TSA fluorophore
CD8	Ab	Membrane	480
Ki67	Ab	Nuclear	520
PD-L 1	Ab	Membrane	570
PD-1	Ab	Membrane	620
TCF7	Ab	Nuclear	690
PanCK	Ab	Membrane	780



Immunity hubs in tumors are associated with positive clinical responses in patients with NSCLC. **a**, Serial sections of pre-PD-1-blockade formalin-fixed paraffin-embedded (FFPE) NSCLC samples were stained with two multiplex fluorescence panels for 68 patients (20 responders and 48 non-responders; created with BioRender.com). Panel 1 uses RNA smFISH/IF to identify

immunity hub components, and panel 2 solely uses immunofluorescence (IF) to determine $CD8^+$ T cell states. Whole-slide images were captured and analyzed by automated cell segmentation and phenotyping. **b**, Representative low-power image of one of the 68 tumors (patient no. 43) stained with the multiplexed RNA smFISH/IF panel. **c**, High-power view from the boxed region in **b** showing an immunity hub. Cells positive for *IFNG* are circled in white. **d**, High-power view of a hematoxylin and eosin (H&E)-stained serial section from an area matched to that in **c**. **e**, Representative image of a tumor section (patient no. 43) stained with the multiplexed RNA-ISH/IF panel shows the focal expression pattern of *CXCL10/CXCL11* (red). **f**, A grid of 50 × 50-μm windows was overlaid on images. *CXCL10/CXCL11*$^+$ windows (red) were identified using *k*-means clustering based on *CXCL10/CXCL11*$^+$ cell count ($k = 2$). **g**, Adjacent *CXCL10/CXCL11*$^+$ windows were aggregated into immunity hubs. Singleton windows were not included as hubs. **h**, Immunity hub area as a fraction of total tumor area in responders (R; complete response and partial response; $n = 20$) versus non-responders (NR; stable disease and progressive disease; $n = 48$). A two-sided Mann–Whitney test *P* value is shown. **I** , Paired comparisons of the density of cellular phenotypes from the RNA-ISH/IF panel within immunity hubs versus total assessed area, colored by response status ($n = 59$; 1 R and 8 NR tumors lacked immunity hubs). **j**, Paired comparisons of density of cellular phenotypes from the IF-only panel within immunity hubs versus total assessed area, colored by response status ($n = 43$; 22 samples had images that could not be co-registered, and 3 samples lacked immunity hubs). Each pair of dots connected by a colored line represents a patient. Horizontal black lines denote the median and 95% confidence interval. Two-sided Wilcoxon matched-pairs signed-rank test Benjamini–Hochberg (BH)-adjusted *P* values are shown. Credit: *Nature Immunology* (2024). DOI: 10.1038/s41590-024-01792-2

Jonathan Chen, MD, Ph.D., an investigator in the Department of Pathology at Massachusetts General Hospital, and Nir Hacohen, Ph.D., director of the Center of Cancer Immunology at Massachusetts General Hospital, are co-authors of a recently published study in *Nature Immunology*, Human Lung Cancer Harbors Spatially-organized Stem-immunity Hubs Associated with Response to Immunotherapy.

Here, they discuss their findings.

What question were you investigating?

Multicellular networks are critical in mediating immune responses. How do immune cells organize within tumors to effectively eliminate malignant cells?

We recently reported the discovery of a network of immune cells found in colorectal cancer. We termed these networks "immunity hubs," and they are characterized as foci of activated T cells abutting tumors and immune myeloid cells expressing T cell-attracting molecules.

This finding suggests the existence of a positive feedback loop in which activated T cells drive further T cell recruitment by local cells in the tumor.

We reasoned that immunity hubs might be predictive of response to immunotherapy because they were enriched in a class of colorectal tumors known to have a higher rate of response to PD-1 blockade immunotherapy.

However, while our study of colorectal samples includes this class of tumors, the patients in our study were not treated with immunotherapy.

In this new study, we sought to answer whether immunity hubs are indeed predictive of response to standard-of-care immunotherapy.

We studied non-small cell lung cancer (NSCLC), which is commonly treated with PD-1 blockade immunotherapy and is the leading cause of cancer death worldwide.

What methods or approach did you use?

We first used a focused approach to image key transcripts and proteins of the hubs to detect immunity hubs in a cohort of 68 lung cancer patients, allowing us to discover a new variant of the immunity hub, which we termed the "stem-immunity hub."

We then developed a less biased approach to image 479 transcripts that represent many cell types and gene programs in the tumor microenvironment to better understand the composition of these hubs and their interactions with other cells in the tumor.

This high-resolution spatial approach allowed us to discover important cell-cell interactions that may regulate the formation and function of these hubs.

What did you find?

We found that patients without immunity hubs in their tumors prior to PD-1 blockade therapy had poor outcomes relative to patients with hubs.

Critically, we discovered the stem-immunity hub, a subtype of immunity hub that is strongly associated with favorable immunotherapy response.

Stem-immunity hubs were enriched for a type of T cell remarkable for its ability to divide and invigorate the antitumor immune response after PD-1 blockade. So, the finding of these stem-immunity hubs represents a new way to think about how the immune response is organized in human cancer.

What are the implications?

The most obvious implication of our work is that we can use the presence of the immunity hub, particularly the stem-immunity hub subclass, as a biomarker to predict response to immunotherapy.

The current standard biomarker for prediction of immunotherapy response is PD-L1 antibody staining, but it can be inaccurate and difficult to use.

We are currently testing a simple two marker tissue stain that reflects immunity and can be assessed by pathologists using a standard workflow.

We think the immunity hub may be how the immune system organizes to fight tumors.

Therefore, we aim to develop therapeutics based on findings in this study to augment immunity hubs and support the anti-tumor immune response.

What are the next steps?

As stated above, we are testing a simple two-color slide stain for stem-immunity hubs that may be clinically useful for guiding treatment decisions.

Additionally, we hypothesize that stem-immunity hubs may be sites where stem-like T cells are nurtured and could serve as platforms for launching a durable anti-tumor response.

We are currently testing the role of stem-immunity hubs in mouse models to determine if they induce greater and more durable anti-tumor immune response.

More information: Jonathan H. Chen et al, Human lung cancer

harbors spatially organized stem-immunity hubs associated with response to immunotherapy, *Nature Immunology* (2024). DOI: 10.1038/s41590-024-01792-2

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