Multiplex IHC & Pathology applications

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25-SEP-23

PATHOLOGY SYMPOSIUM

CellCarta

MAPPING PRECISION MEDICINE

AGENDA

1. CellCarta Histopathology
2. Concept & principle of multiplexing
3. CD8 multiplexing examples
   - CD8/panCK
   - CD8/GZMB/FOXP3

CellCarta: global CRO for end-to-end precision medicine

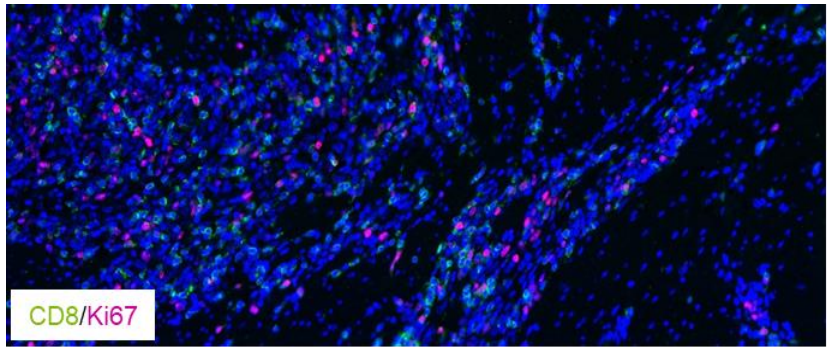
Clinical Operations

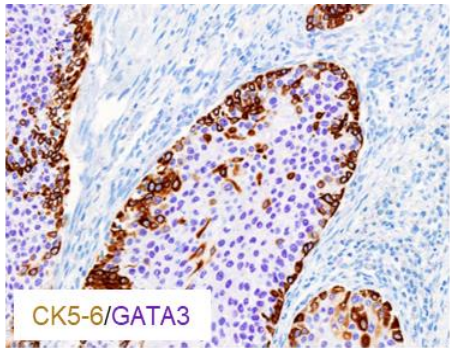
Assay Development

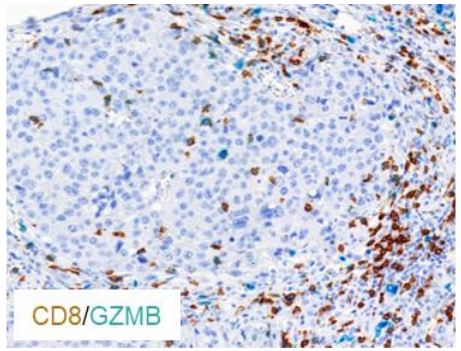
RnD

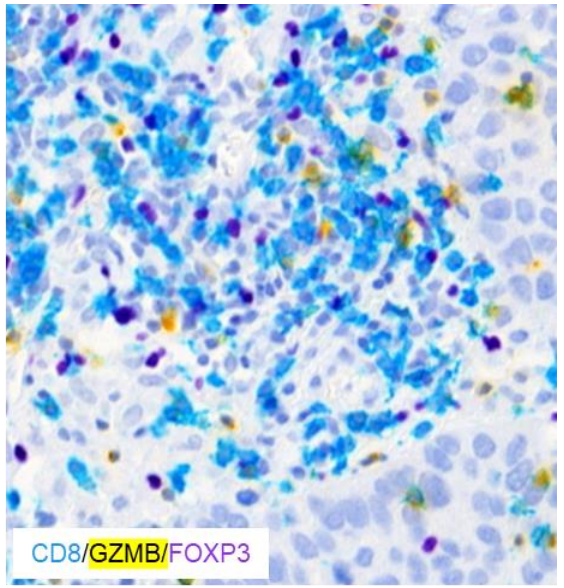
Histopathology

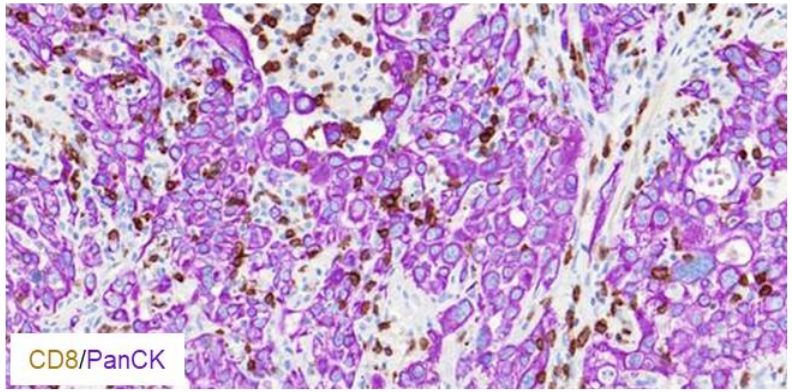
Glimpse into the multiplex portfolio (IHC, mIHC, mIF, RNA ISH, FISH)

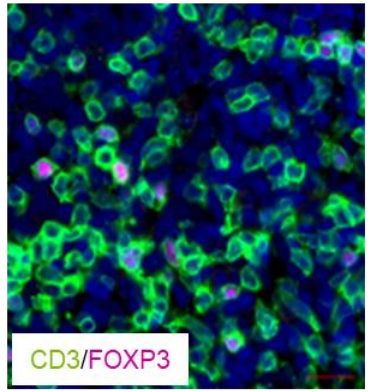


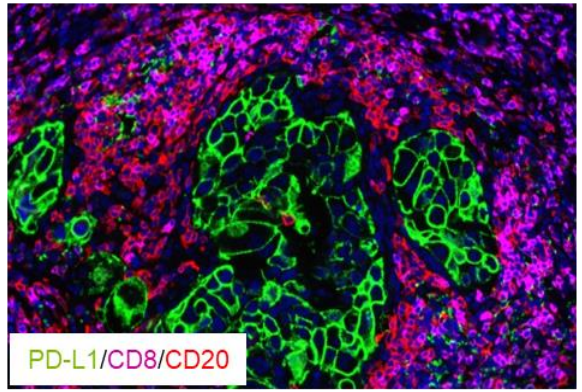


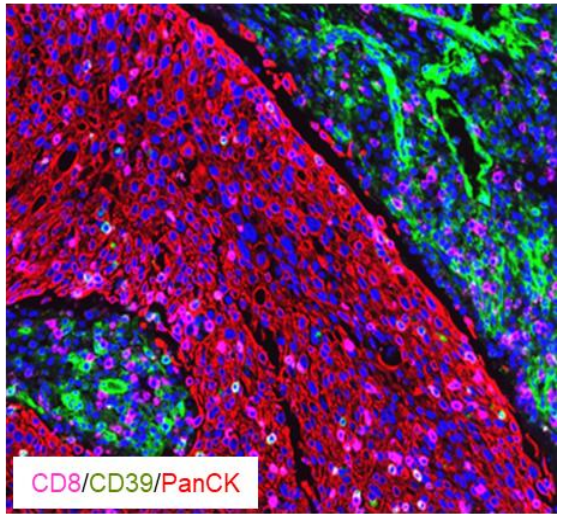


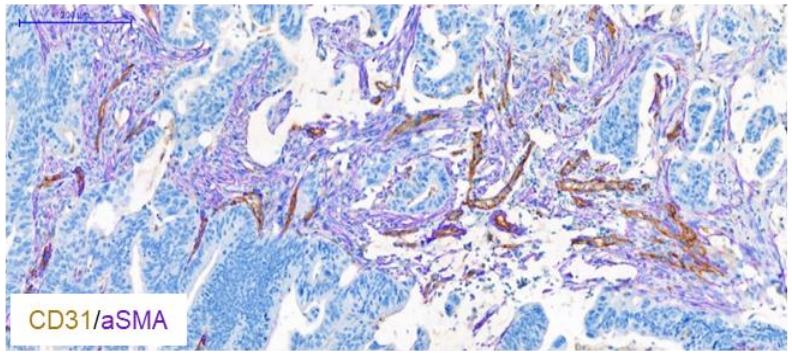


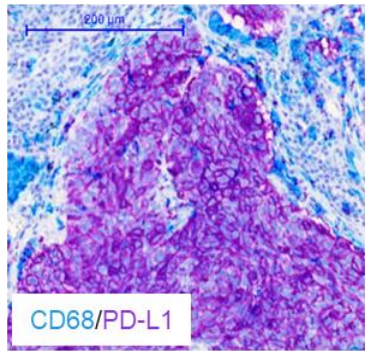


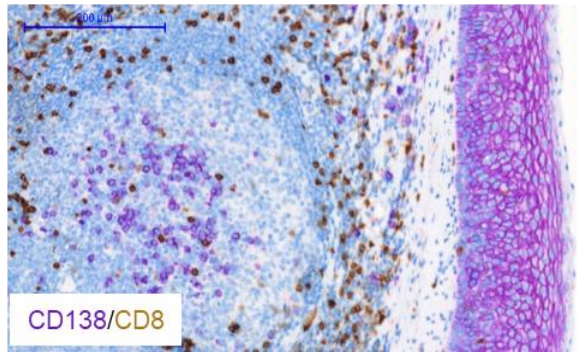












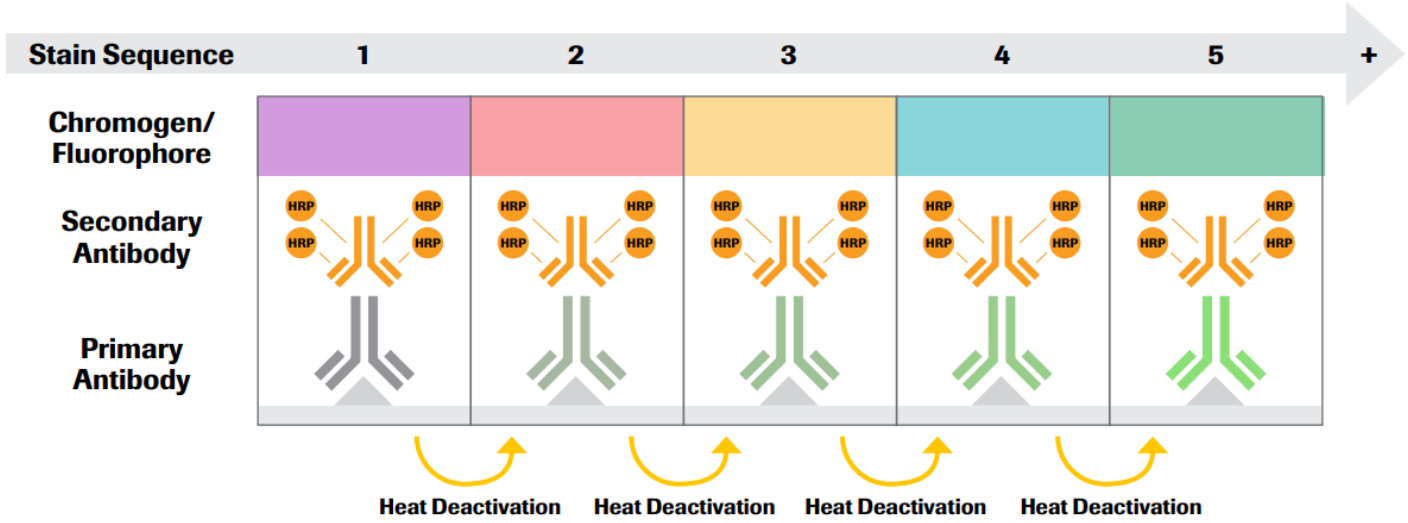
CONCEPT OF MULTIPLEXING

Doing more with less:

Simultaneous detection of multiple markers while preserving precious samples

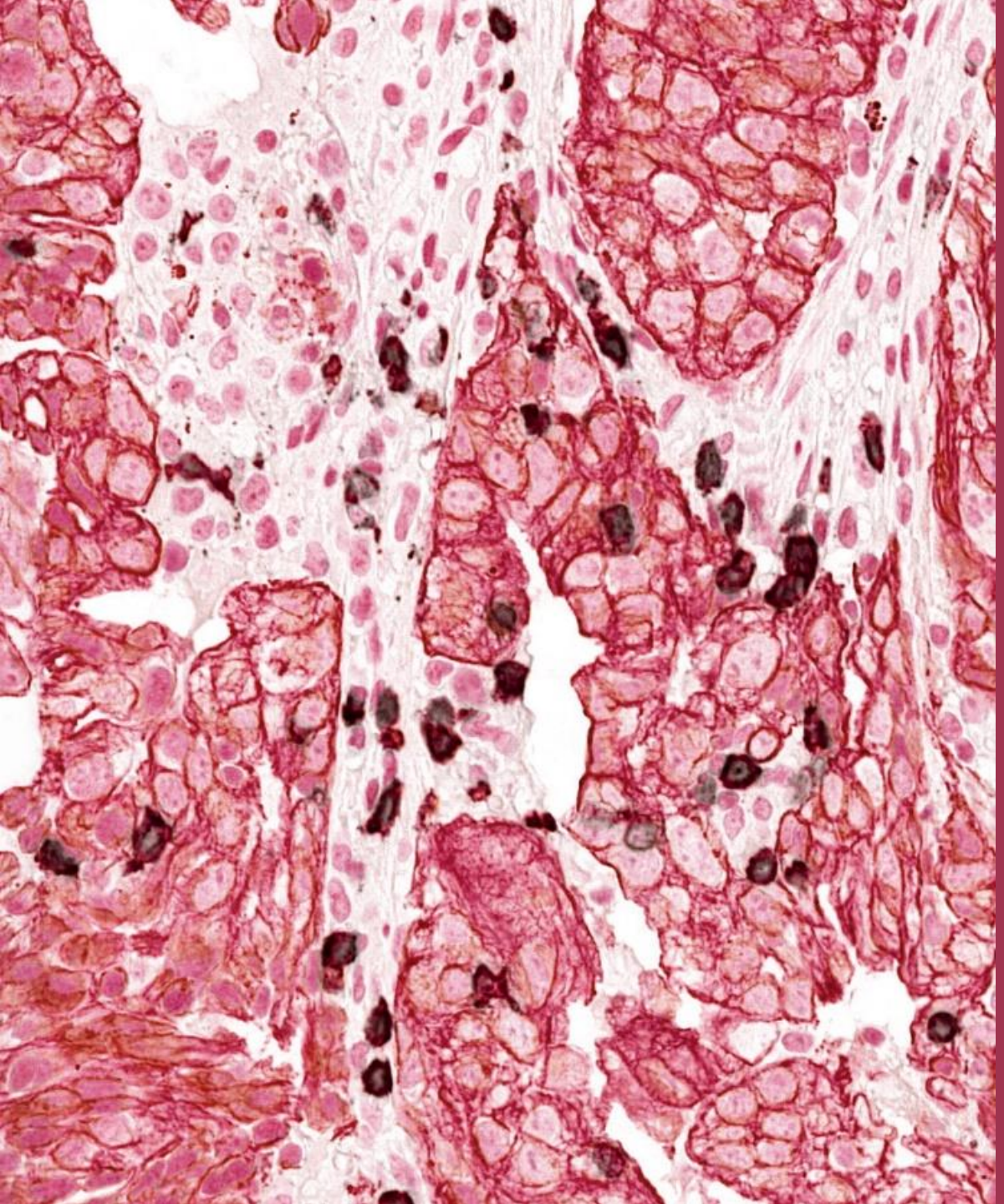
- Spatial localization (identification & interpretation within the morphology context)
- cellular function/activation and
- cell-cell interactions

PRINCIPLE OF MULTIPLEXING



Use of automated staining platforms and detection kits lead to straightforward & robust assays

- Sequential staining provides flexibility to combine markers
- Automated denaturing prevents cross reactivity
- Open or ready-to-use detection allows assay customization



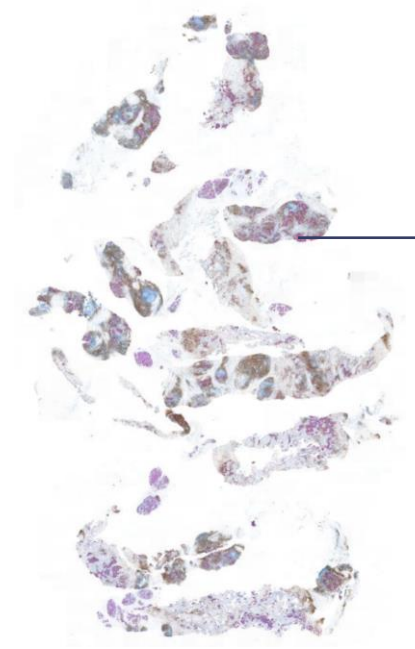
CellCarta

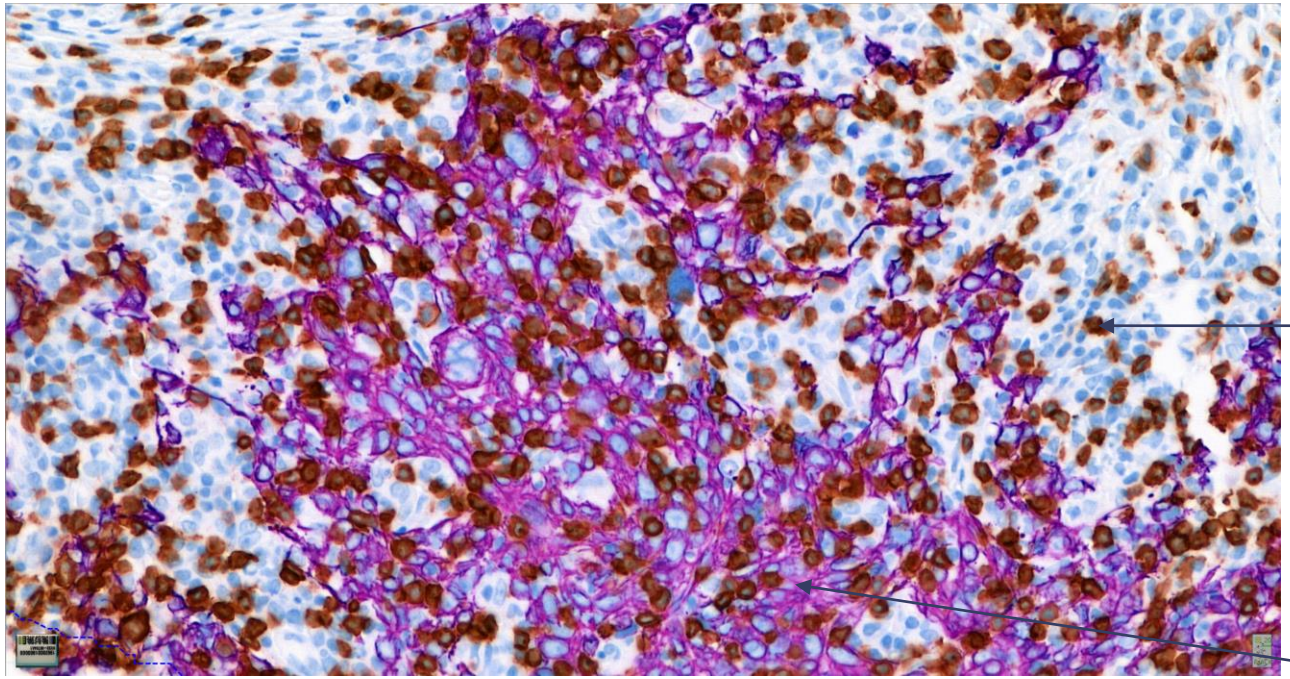
CD8/panCK

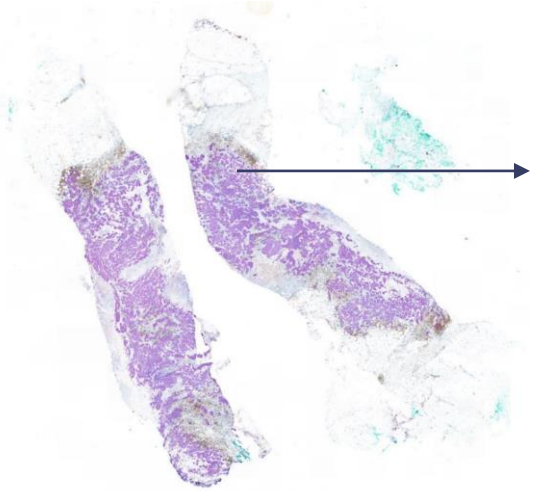
Numerous studies have described the relationship between the presence and spatial distribution of TILs and treatment benefit

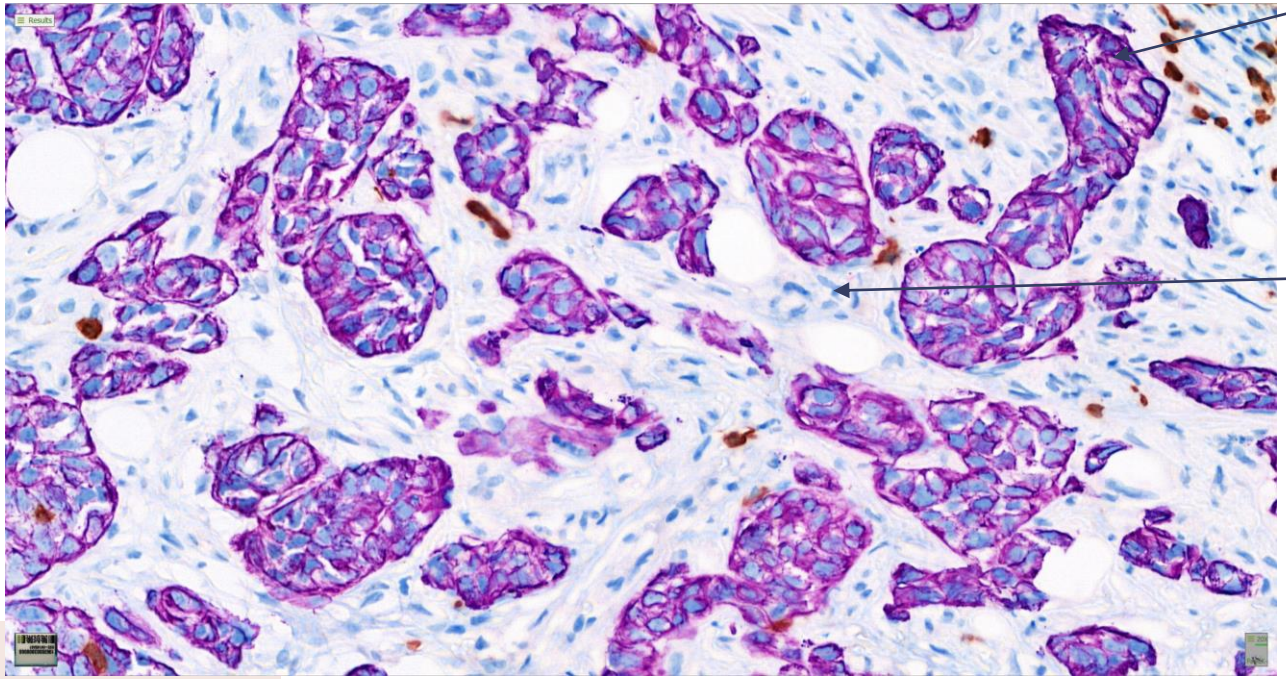
- highlights the role of CTL biomarkers

CD8/panCK









CD8+ lymphocytes stain brown with DAB

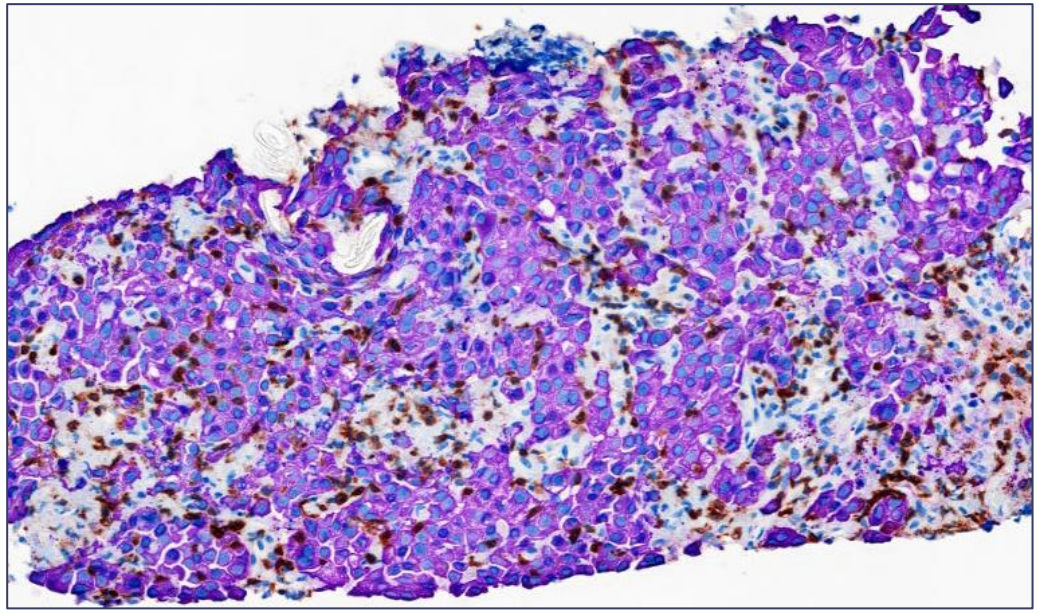
Tumor cell cytokeratin stains purple with DISC chromogen

Hematoxylin counterstain

SCORING

Image analysis

Single Section Topological Analysis (SSTA)



Pathologist scoring

Density Proportion Score (DPS)

IMAGE ANALYSIS WORKFLOW

H&E EVALUATION BY PATHOLOGIST

→

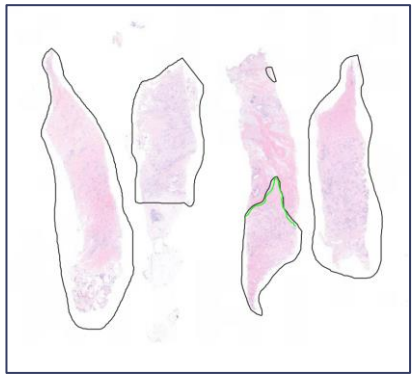
ANNOTATION OF TUMOR AND INVASIVE MARGIN ON H&E IMAGE BY PATHOLOGIST

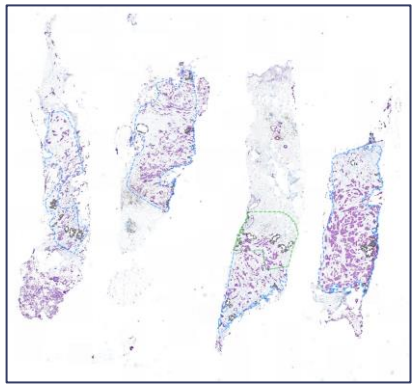
→

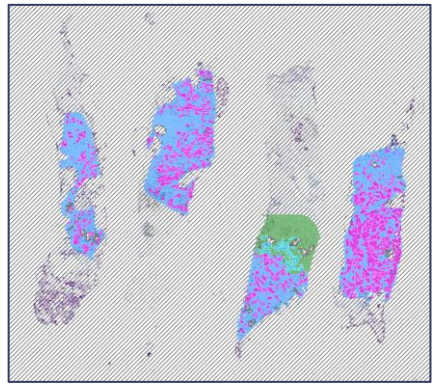
DETAILED ANNOTATION OF ROIS ON IHC IMAGE (EXCLUSION OF ARTIFACTS)

→

RUNNING TEST SPECIFIC ANALYSIS & QUALITY CHECK



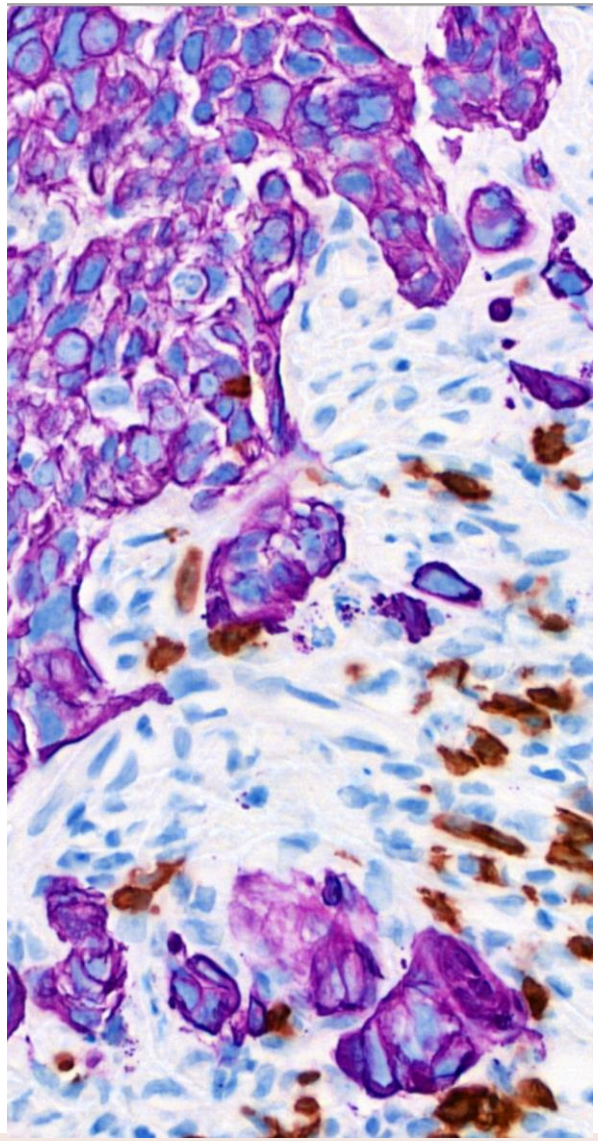




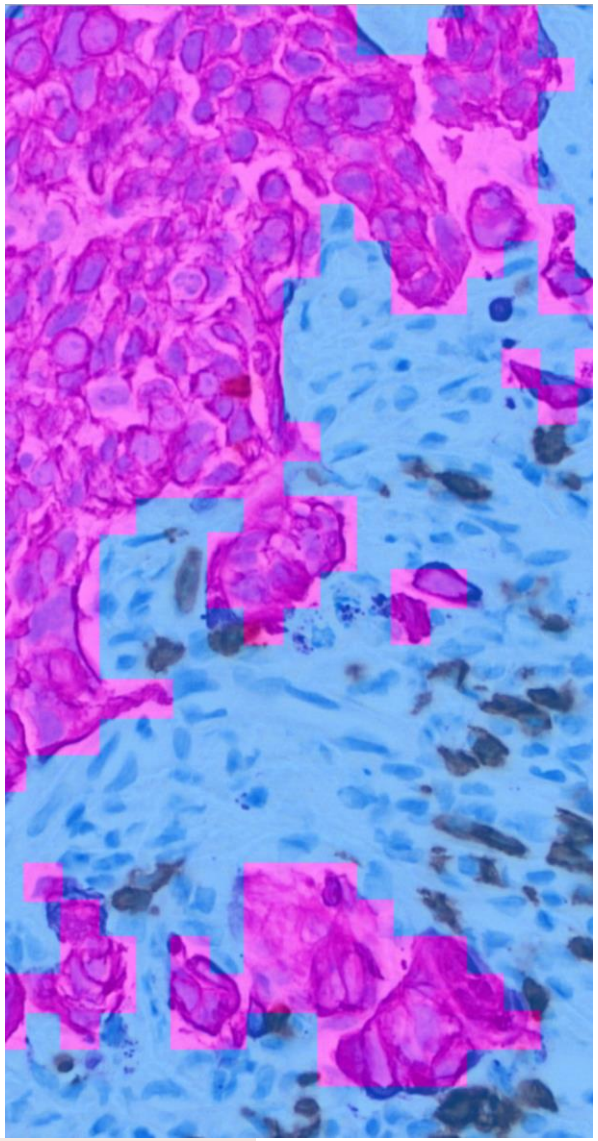
PanCK-CD8

Image analysis

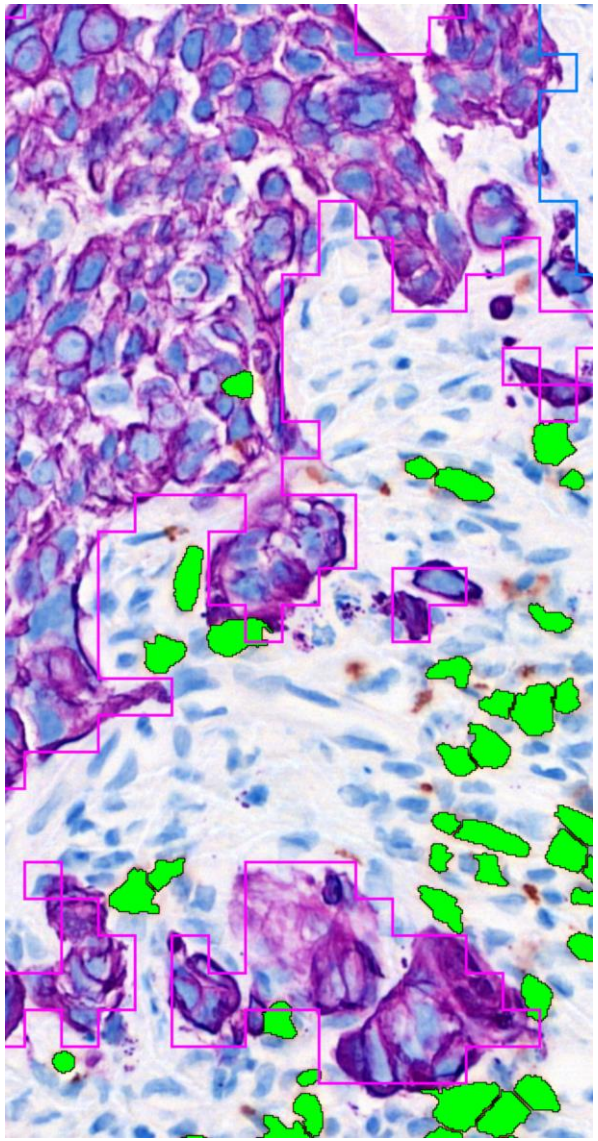
PanCK - image



PanCK-CD8 – mask



PanCK-CD8 – CD8 detection



Reportables:
- CD8 area relative to
- CD8 density within
- …

Central tumor
Invasive Margin
Tumor stroma
Carcinoma cell compartment

PATHOLOGIST (DENSITY PROPORTION SCORING) WORKFLOW

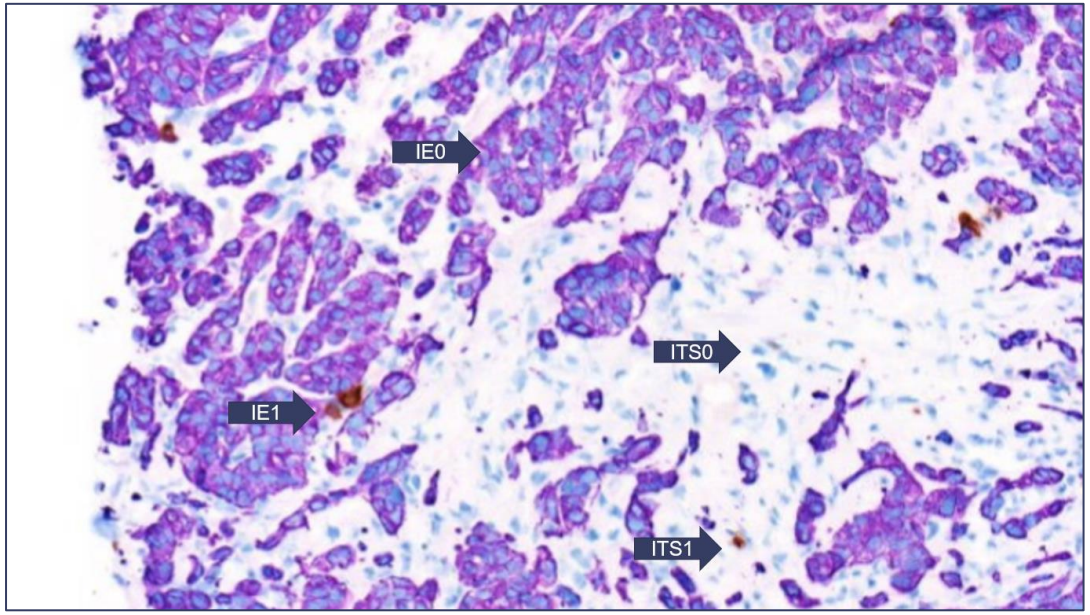
SURVEY SLIDE AT 10X → **TUMOR STROMA (ITS)** → **TUMOR NESTS (IE)** → **PHENOTYPE**

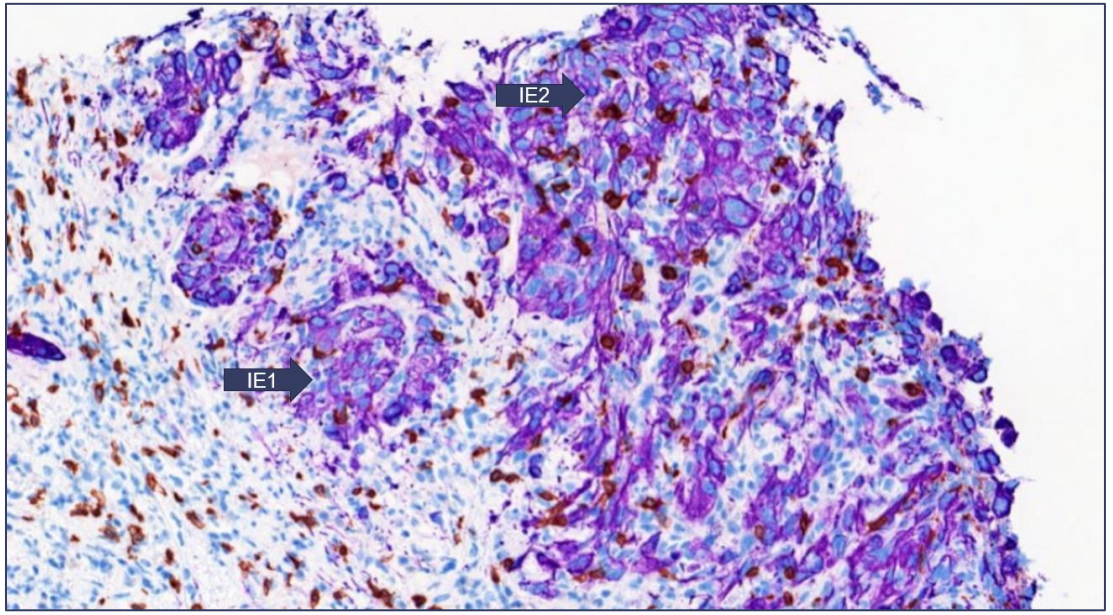
- Determine the general distribution of tumor cell nests, cell strands and stroma
- Score the % Immune Cells (IC) in each density bin (absent, low, moderate, high)
- Score the % IC in each density bin (absent, low, moderate, high)
- Combine scores to determine the overall immune phenotype (Desert – Excluded – Inflamed)

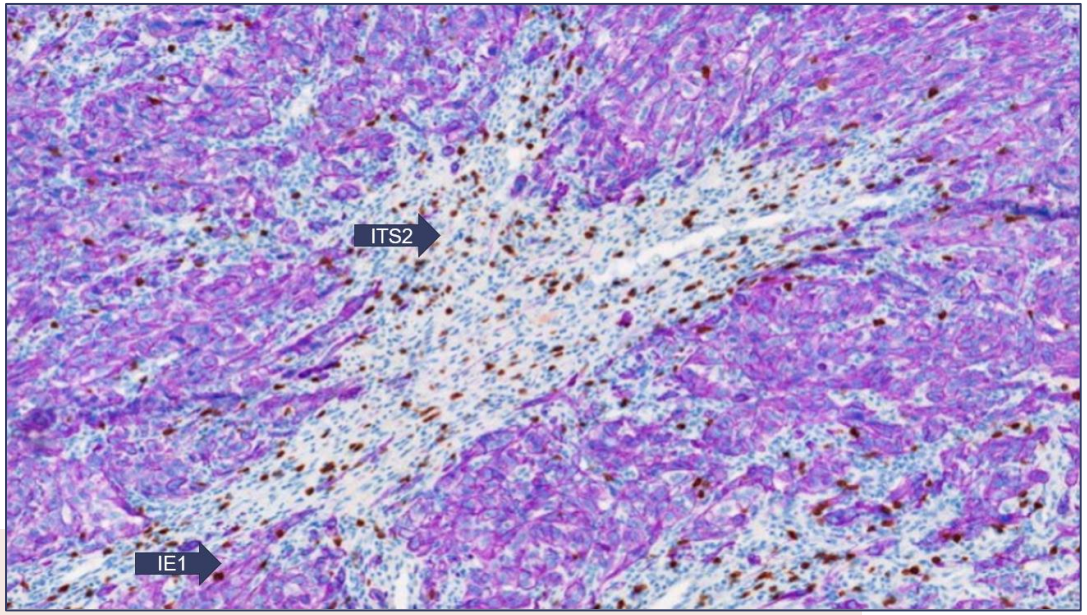
DENSITY PROPORTION SCORE

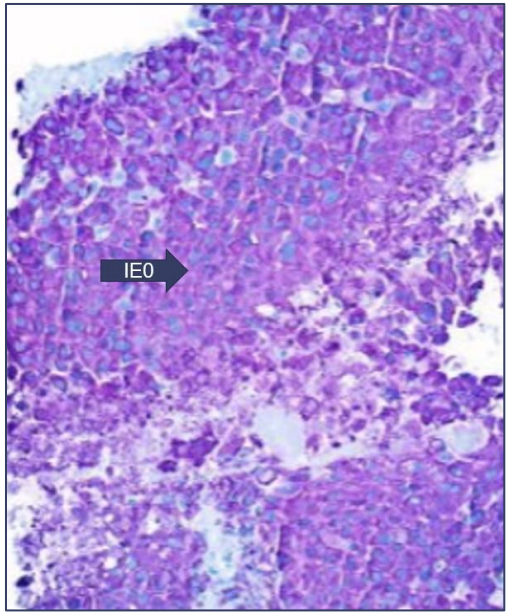
PanCK-CD8

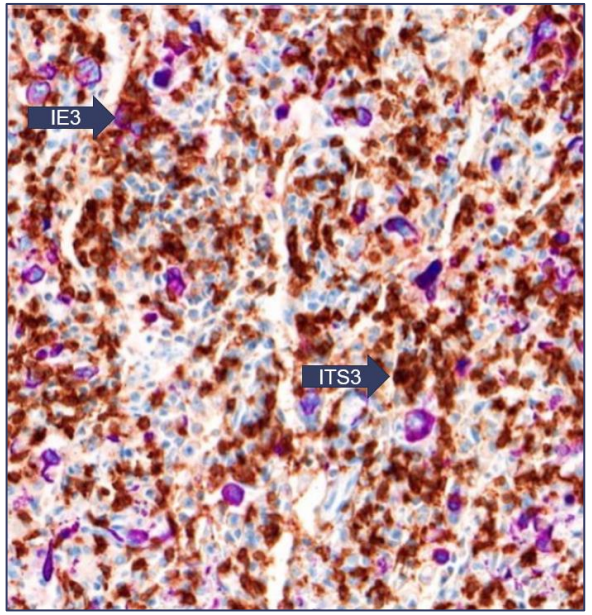
Pathologist - DPS

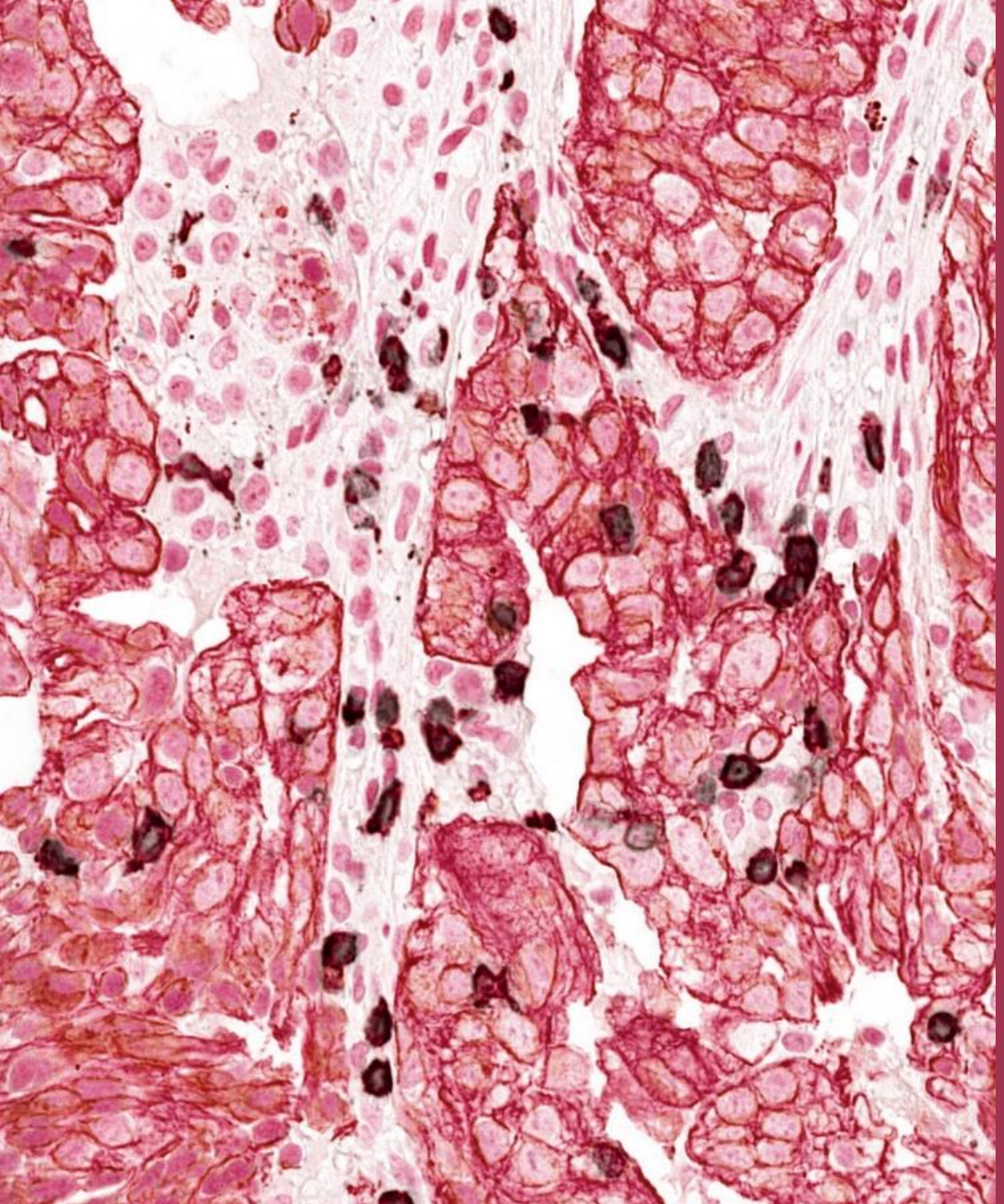












CellCarta

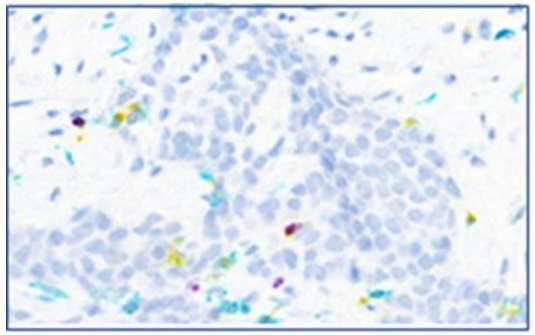
CD8-GZMB-FOXP3

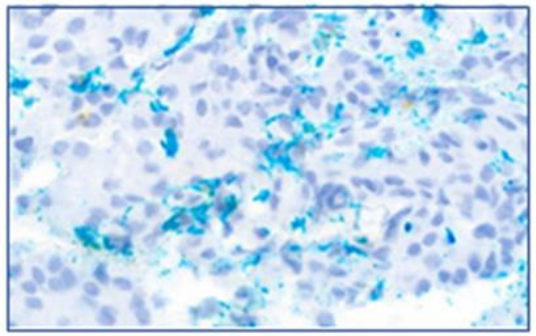
By combining CD8 with the cytolytic GZMB, the density and proportion of the activated CTLs can be assessed

FOXP3+ Tregs are immune suppressive cells, and their abundance/distribution in tumor infiltrates can be associated with unfavorable clinical prognosis

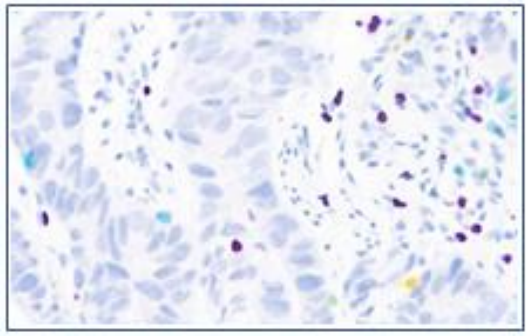
CD8-GZMB-FOXP3

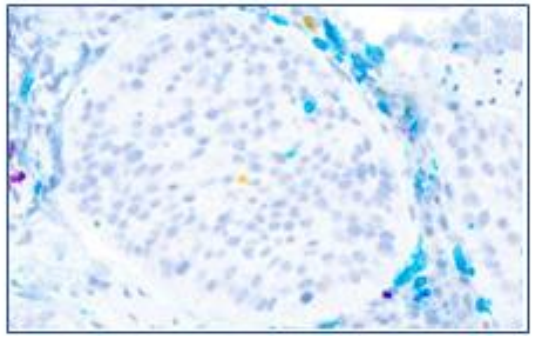
EPITHELIAL NESTS (LOW VS HIGH)

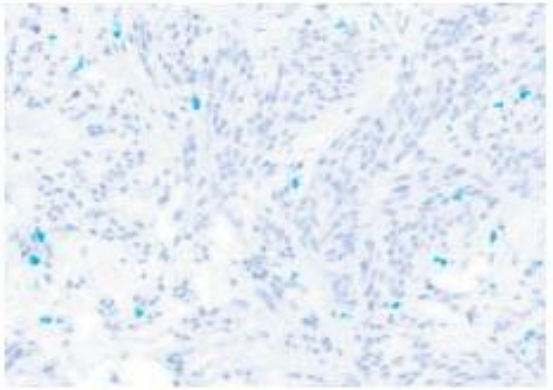
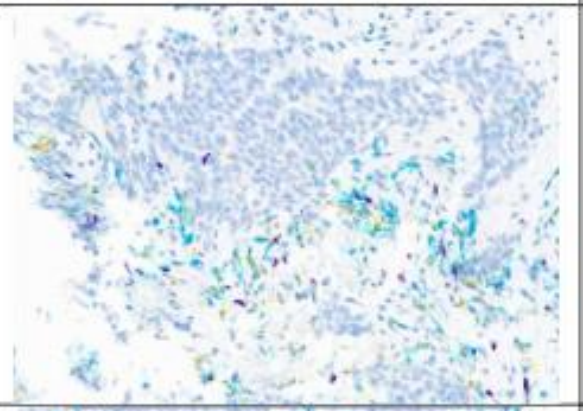
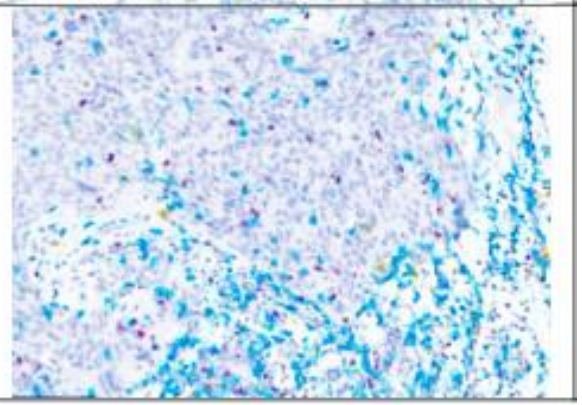




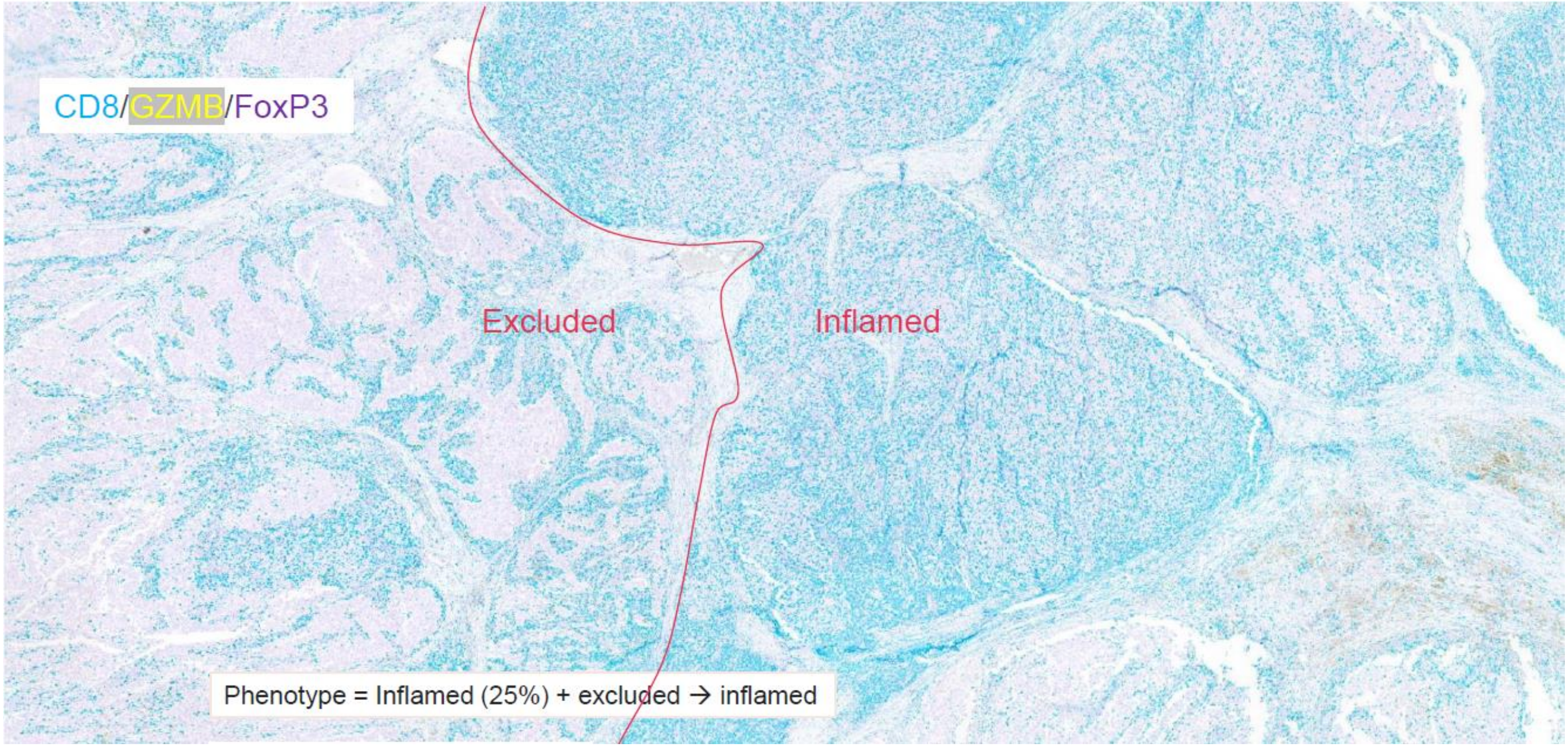
STROMA (LOW VS HIGH)





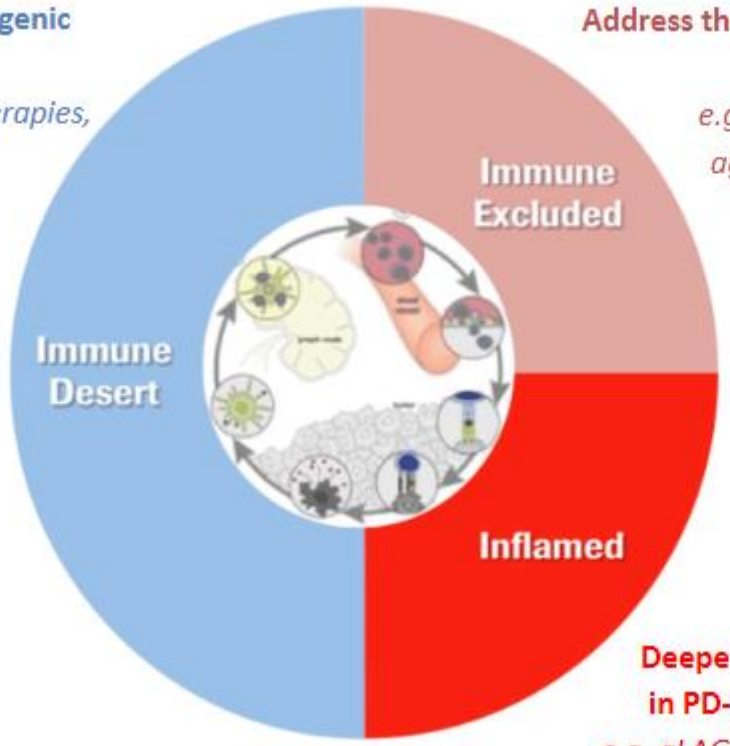
Phenotype	Image
Desert	
Excluded	
Inflamed	

CD8-GZMB-FOXP3



APPLICATIONS

The IC phenotype is dynamic and will change as a tumor develops and in response to therapy.
It is, therefore, a useful parameter to support treatment strategies at different stages of carcinogenesis.



Agents that promote immunogenic tumor cell death
e.g. chemotherapy, targeted therapies, vaccines

Address the barriers to T cell entry into the tumor
e.g. aVEGF, chemokine agonists/antagonists

Deepen responses in PD-L1+ tumors
e.g. aLAG-3, IDOi, aTIGIT

CONCLUDING THOUGHTS

- As the transition from chemotherapy to targeted and immune therapies continues to develop, investigators will utilize more complex clinical trial designs to predict response or resistance to therapy regimens
- Multi-parametric approaches are needed to identify patients most likely to respond to ICI treatment
  - combination of targeted and immune therapies
  - combination of different ICI drugs
- Focus on TME characterization and analysis of the interaction between tumor and effector IC and between effector cells and APCs
  - Spatial relationships and distributions
  - Expression profiles (suggest activation or suppression/exhaustion of effector cells or may hint at mechanisms of resistance)
- **Important to develop robust tissue-based multiplex technologies that can be adopted for routine clinical practice** and – if necessary – successfully shepherded through regulatory approval.