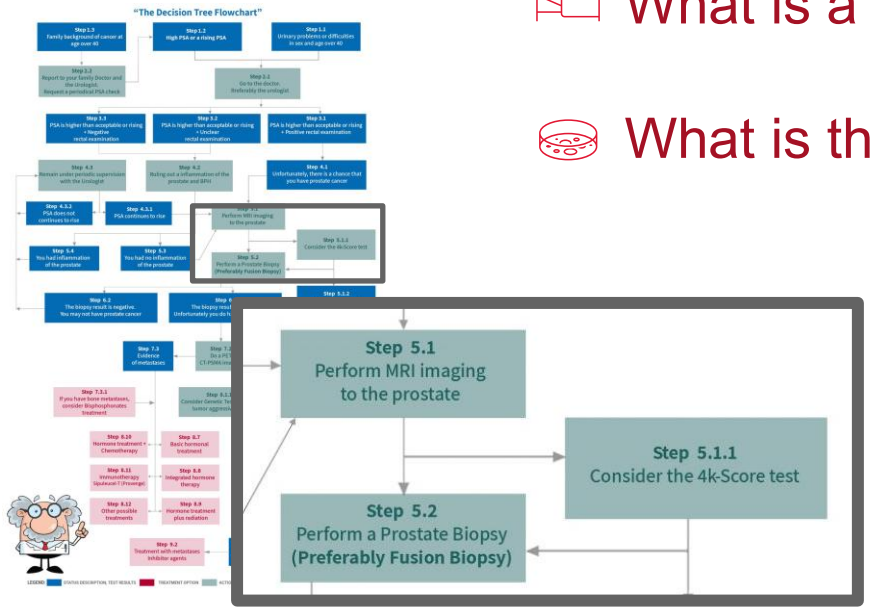
Clinical Biomarkers and Therapy Candidates via Spatial Analysis

Alex Xu

Spatial Biology Congress

UNIVERSITY OF MARYLAND

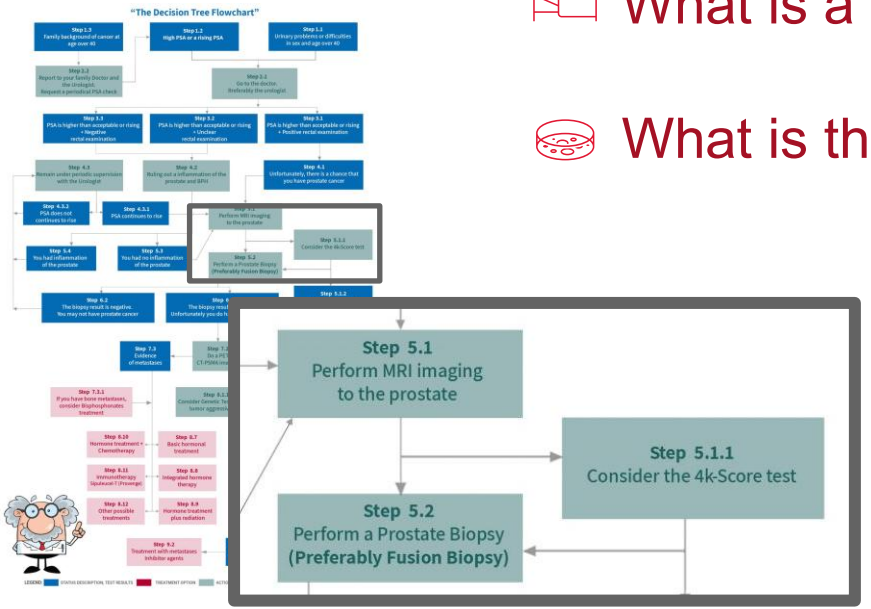
Clinical Decision Making and Motivations



- What is a patient's diagnosis (risk)?
- What is the molecular basis for the disease?

UNIVERSITY OF MARYLAND

Clinical Decision Making and Motivations



What is a patient's diagnosis (risk)?

What is the molecular basis for the disease?

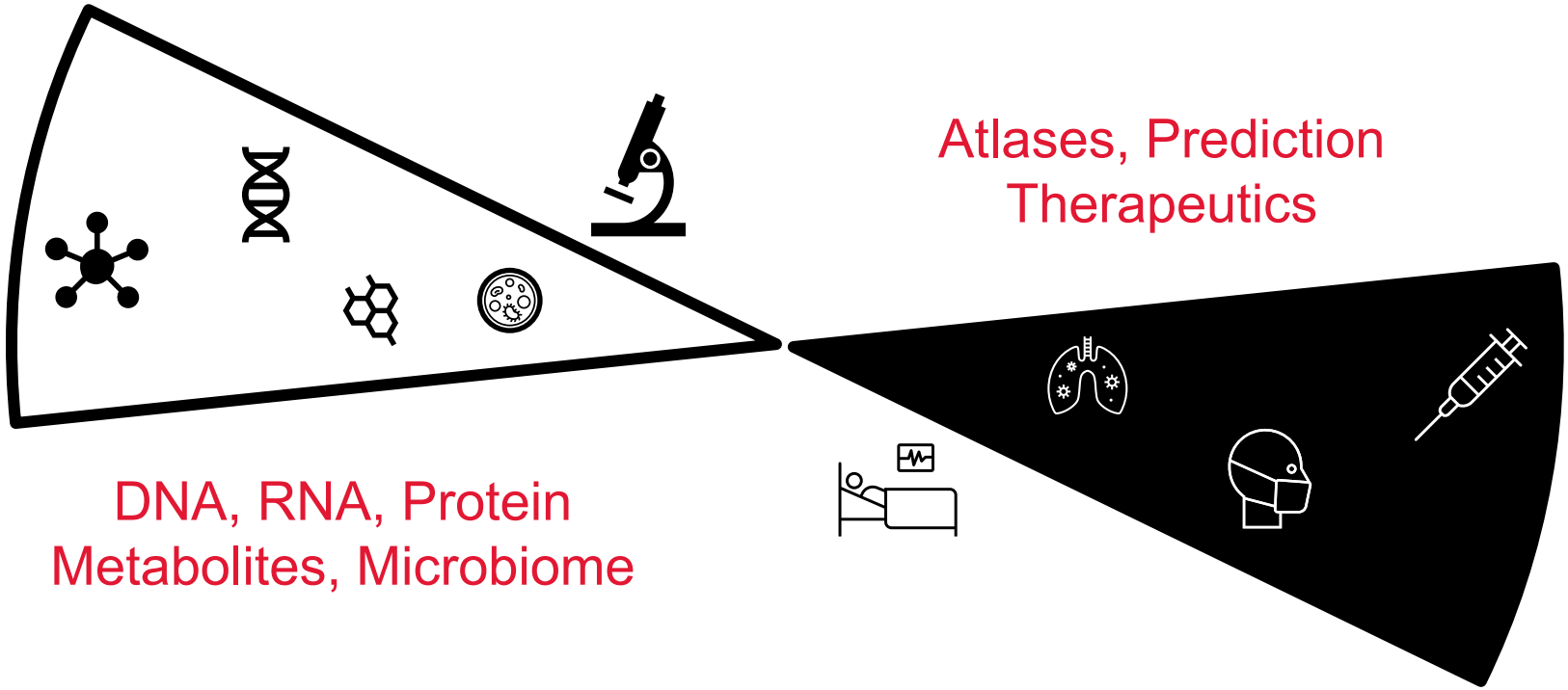
Is there a concise measurement that captures risk or biology?

Empirical Hypothesis-Driven

a.k.a. a Biomarker?

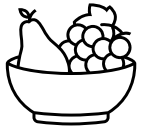
UNIVERSITY OF MARYLAND

Spatial Biology Methods and Applications



UNIVERSITY OF MARYLAND

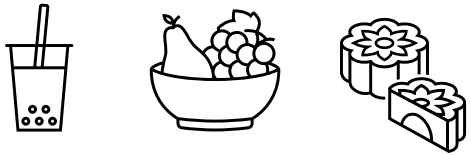
A Simple Analogy for Biomarkers



Do I want to eat this fruit?

A Simple Analogy for Biomarkers

Smoothie-salad-fruit cake analogy

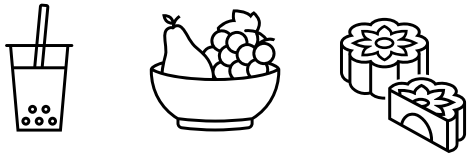


Bulk mixed fruits
Single mixed fruits
Spatial mixed fruits

Do I want to eat this fruit?

A Simple Analogy for Biomarkers

Smoothie-salad-fruit cake analogy



Bulk mixed fruits
Single mixed fruits
Spatial mixed fruits

Do I want to eat this fruit?

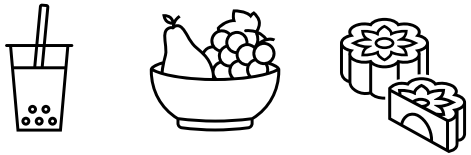
Gooey cake analogy

Pineapple-y cherries?
Cake-y cherries?

How does spatial change the flavor of fruit?

A Simple Analogy for Biomarkers

Smoothie-salad-fruit cake analogy



Bulk mixed fruits
Single mixed fruits
Spatial mixed fruits

Do I want to eat this fruit?

Gooey cake analogy

Pineapple-y cherries?
Cake-y cherries?

How does spatial change the flavor of fruit?

Unpublished data

UNIVERSITY OF MARYLAND

Acknowledgment

Since 2020

Spatial Molecular Profiling Shared Resource

We empower researchers with tools to study protein and gene activity and offer collaborative grant opportunities to advance spatial research.

Send a Message

Akil Merchant

Director, Spatial Molecular Profiling Shared Resource

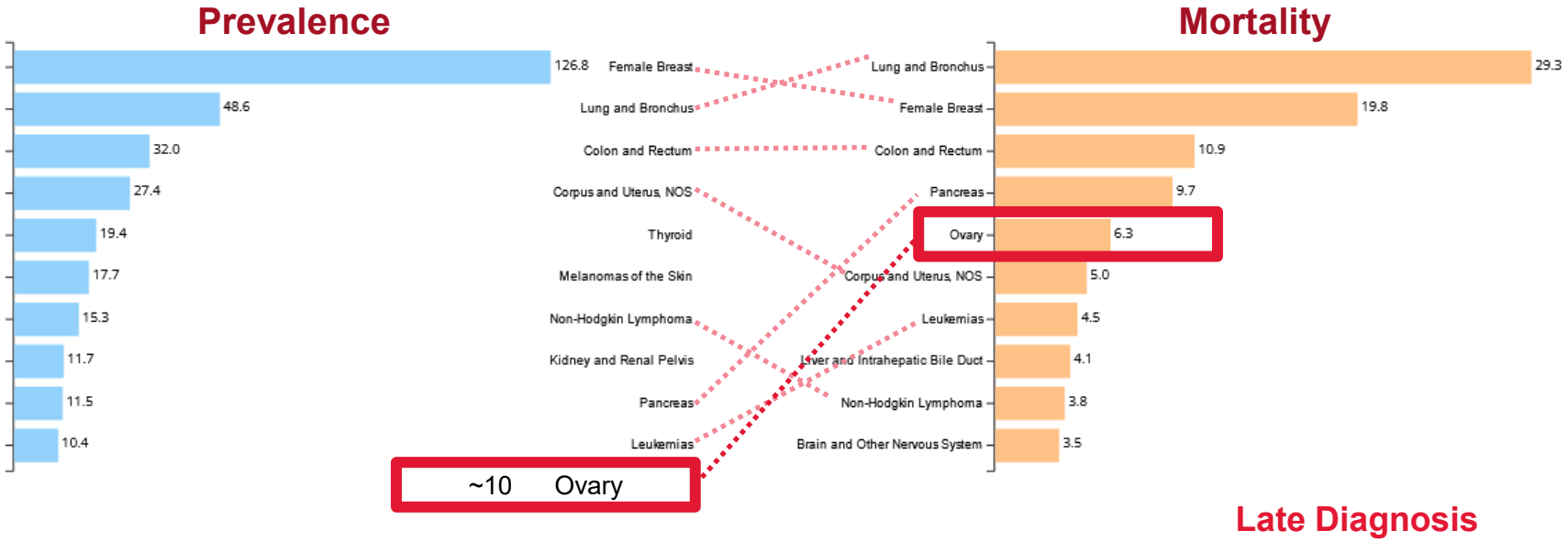
View Profile

Send a Message

UNIVERSITY OF MARYLAND

Ovarian Cancer Burden and Severity

Ovarian cancer is a leading cause of cancer death in women



Late Diagnosis

UNIVERSITY OF MARYLAND

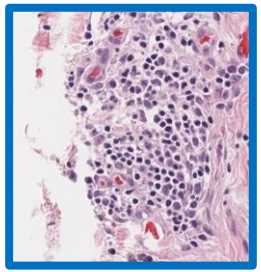
https://www.cdc.gov/cancer/ovarian/

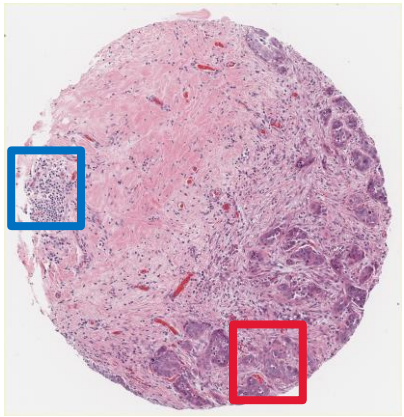
Tumor Tissue Composition is Linked to Outcomes

Tumor, stroma, and immune cells

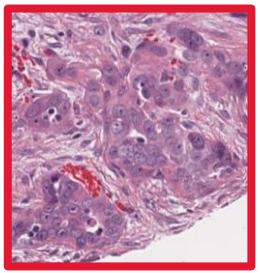
Tumor Tissue Composition is Linked to Outcomes

Tumor, stroma, and immune cells





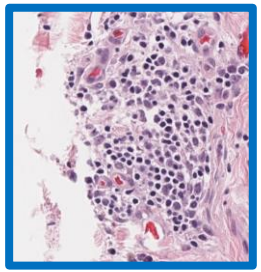
Immune Cells

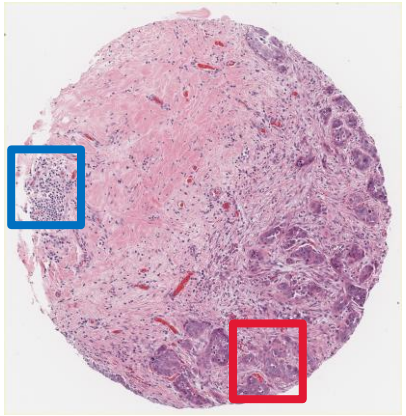


Tumor + Fibroblasts

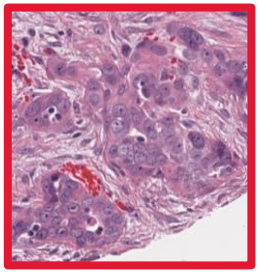
Tumor Tissue Composition is Linked to Outcomes

Tumor, stroma, and immune cells



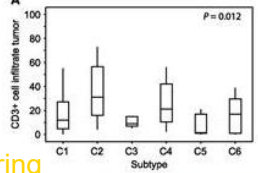


Immune Cells

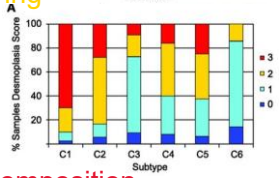


Tumor + Fibroblasts

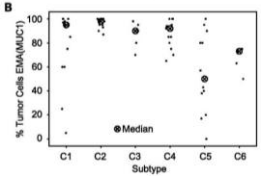
Immune infiltrate



Stromal scoring



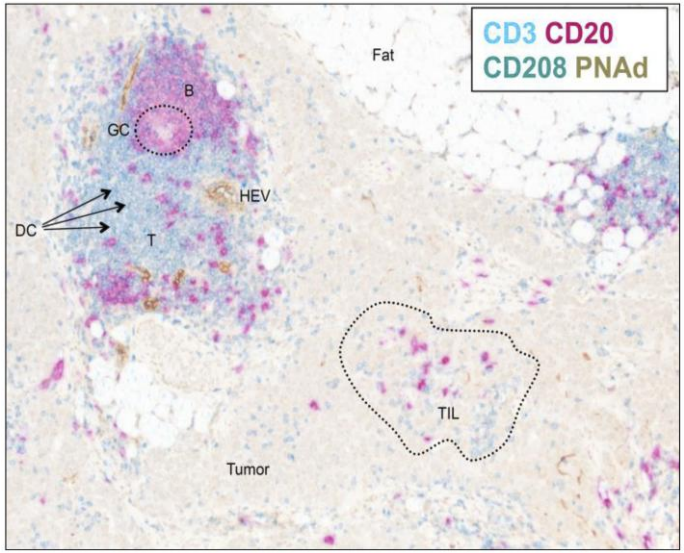
Tumor cell composition

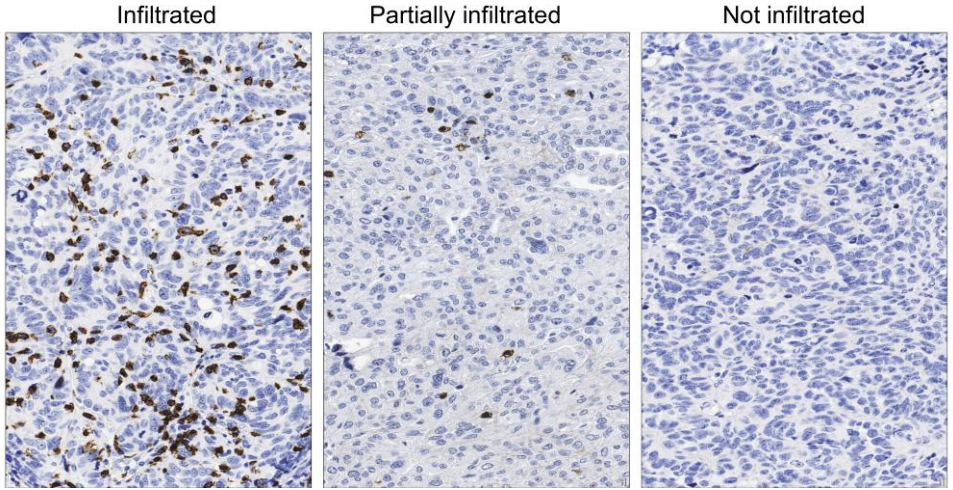


UNIVERSITY OF MARYLAND

Measuring the Tumor Microenvironment is Imprecise

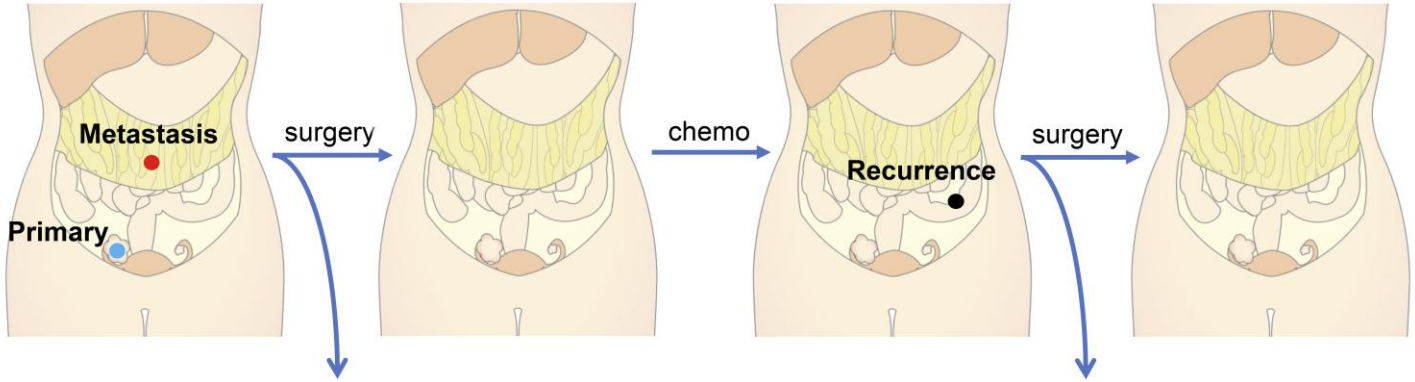
Clinical descriptors are semi-quantitative





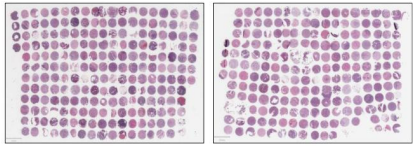
UNIVERSITY OF MARYLAND

Recurrent Ovarian Cancer Cohort



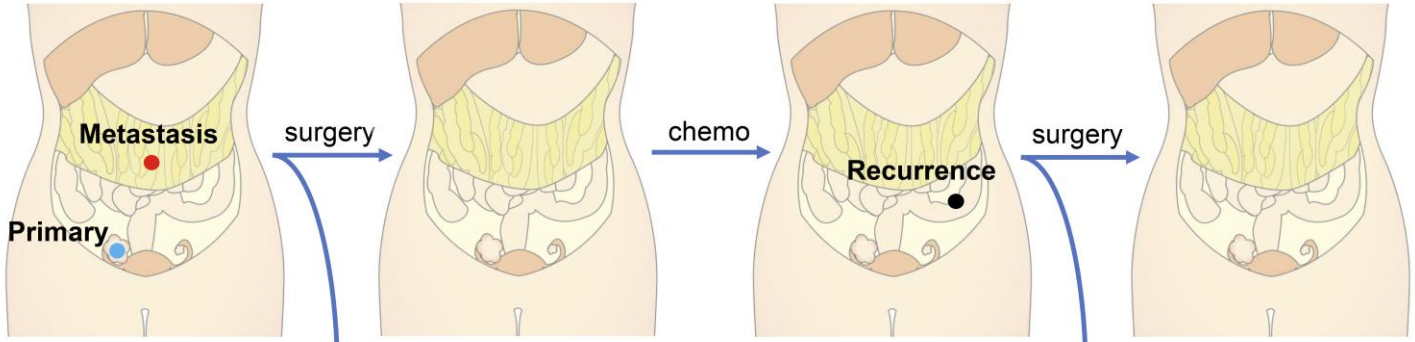
N = 42

Tissue Microarray

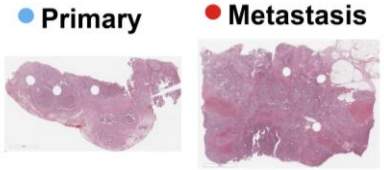


UNIVERSITY OF MARYLAND

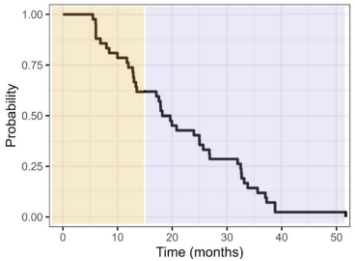
Recurrent Ovarian Cancer Cohort

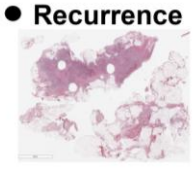


N = 42

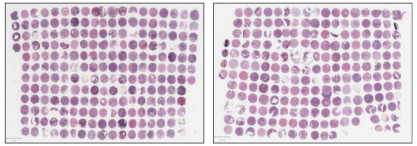


Early vs. Late Relapse





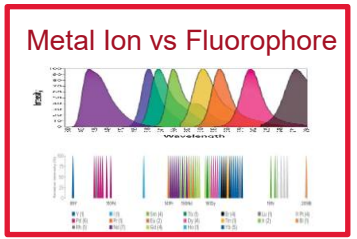
Tissue Microarray

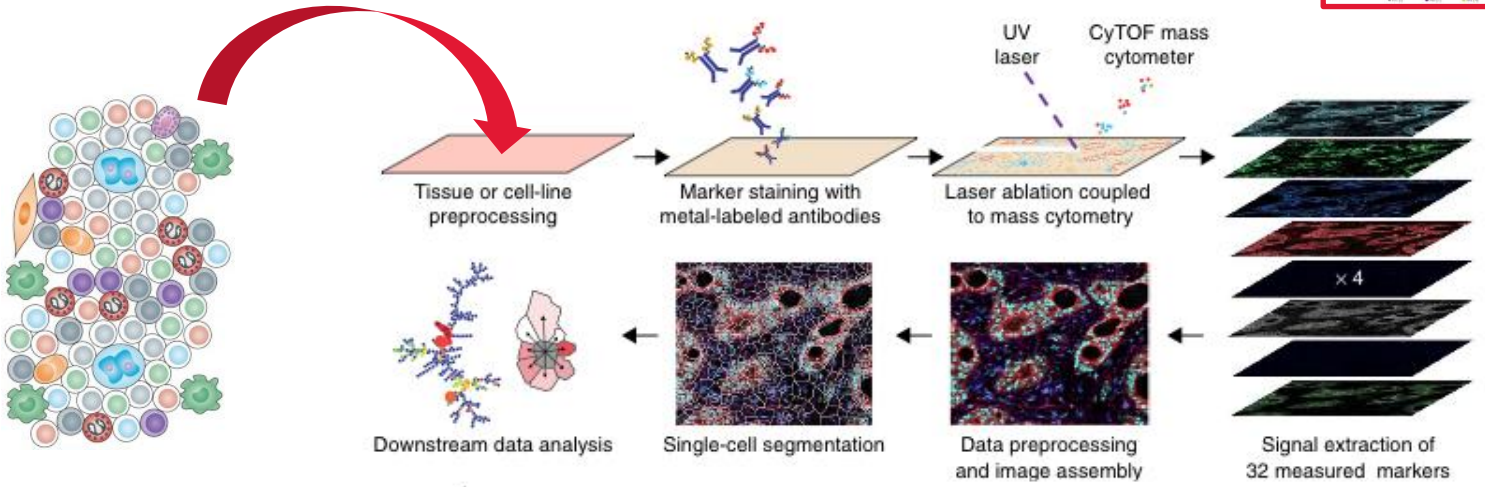


UNIVERSITY OF MARYLAND

Highly Multiplexed Spatial Analysis of TME

Imaging Mass Cytometry profiles ~40 proteins at 1 µm resolution



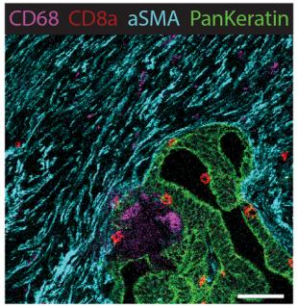


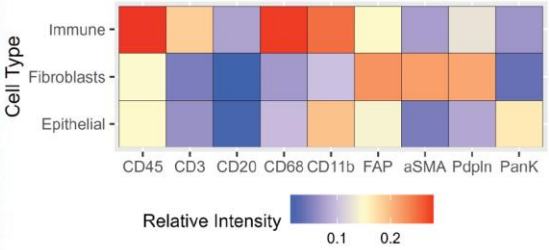
UNIVERSITY OF MARYLAND

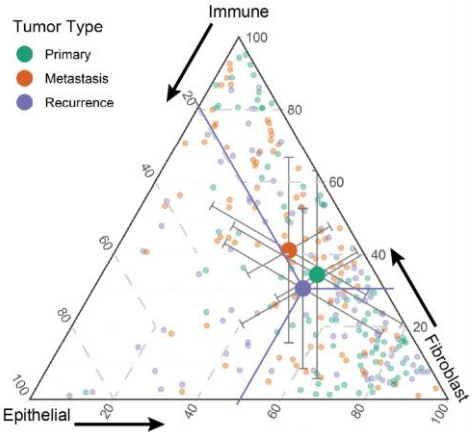
Giesen et al., Nat. Methods, 2014

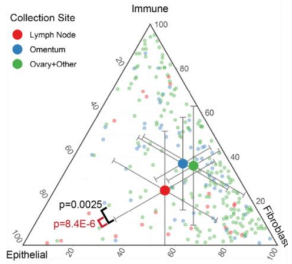
Tumor Heterogeneity

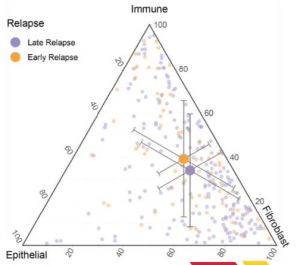
Tumor + Stroma + Immune Composition











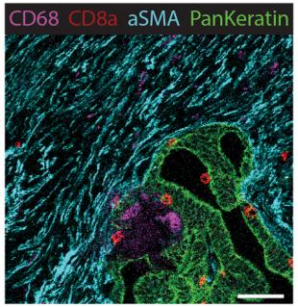
No significant differences

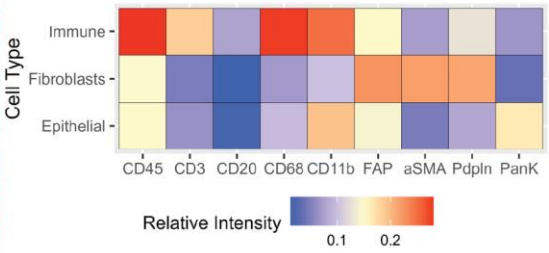
Observed more immune in recurrence

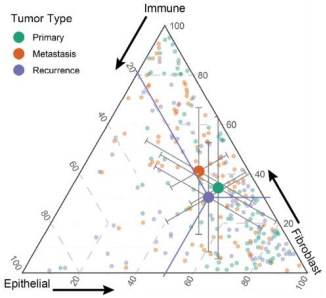
UNIVERSITY OF MARYLAND

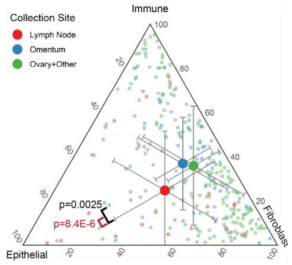
Tumor Heterogeneity

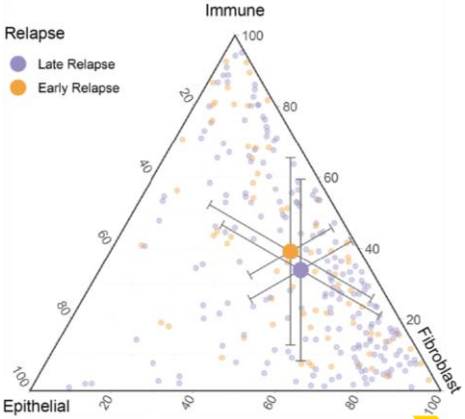
Tumor + Stroma + Immune Composition











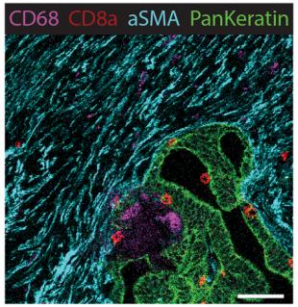
No significant differences

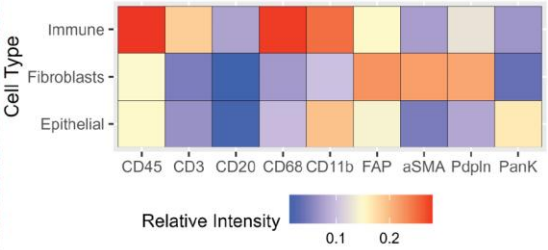
Observed less tumor in early relapse

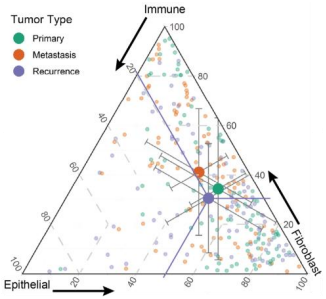
UNIVERSITY OF MARYLAND

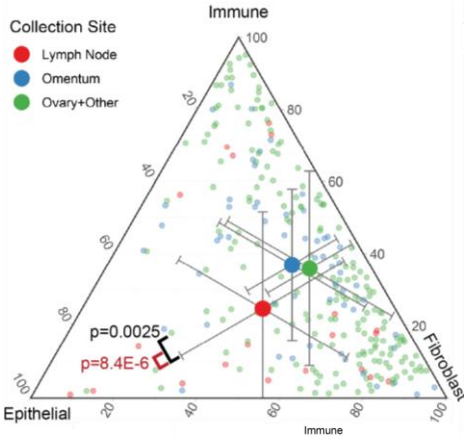
Tumor Heterogeneity

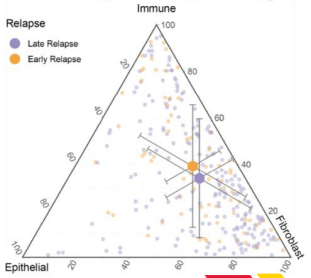
Tumor + Stroma + Immune Composition









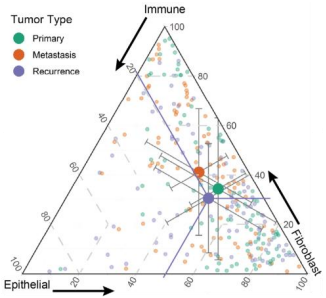


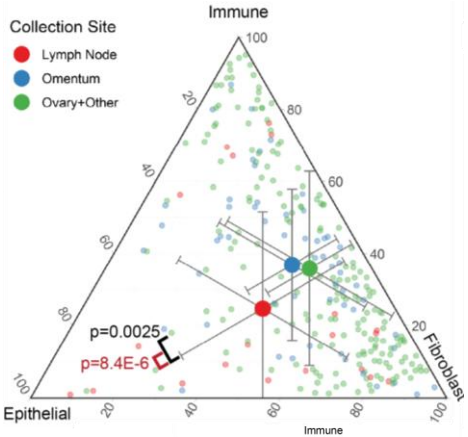
Lymph node metastasis/recurrence

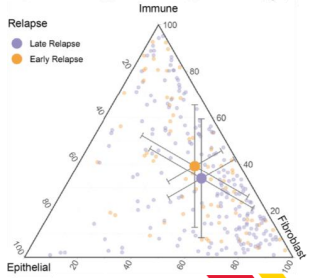
UNIVERSITY OF MARYLAND

Tumor Heterogeneity

Tumor + Stroma + Immune Composition







Lymph node metastasis/recurrence

UNIVERSITY OF MARYLAND

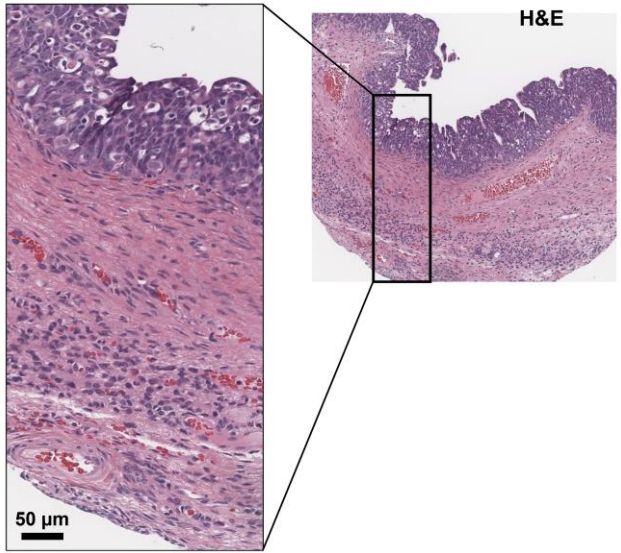
Tumor Heterogeneity

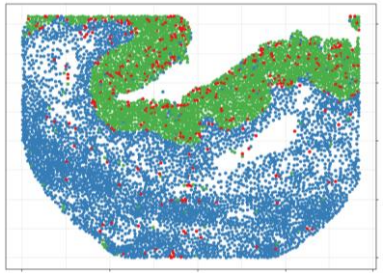
Tumor + Stroma + Immune Composition

Spatial vs bulk

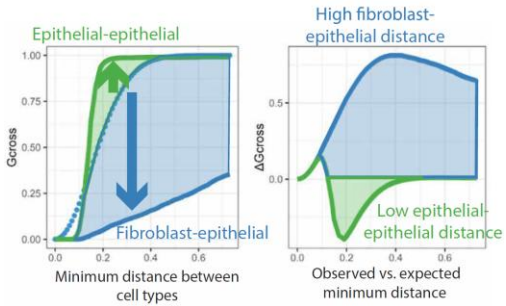
Immune/Stroma/Tumor Distributions Describe the TME

Quantifying cell-cell contacts – bulk Gcross

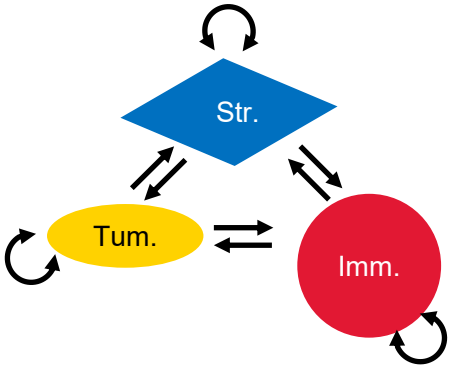




Cell Type
- Immune
- Fibroblasts
- Epithelial

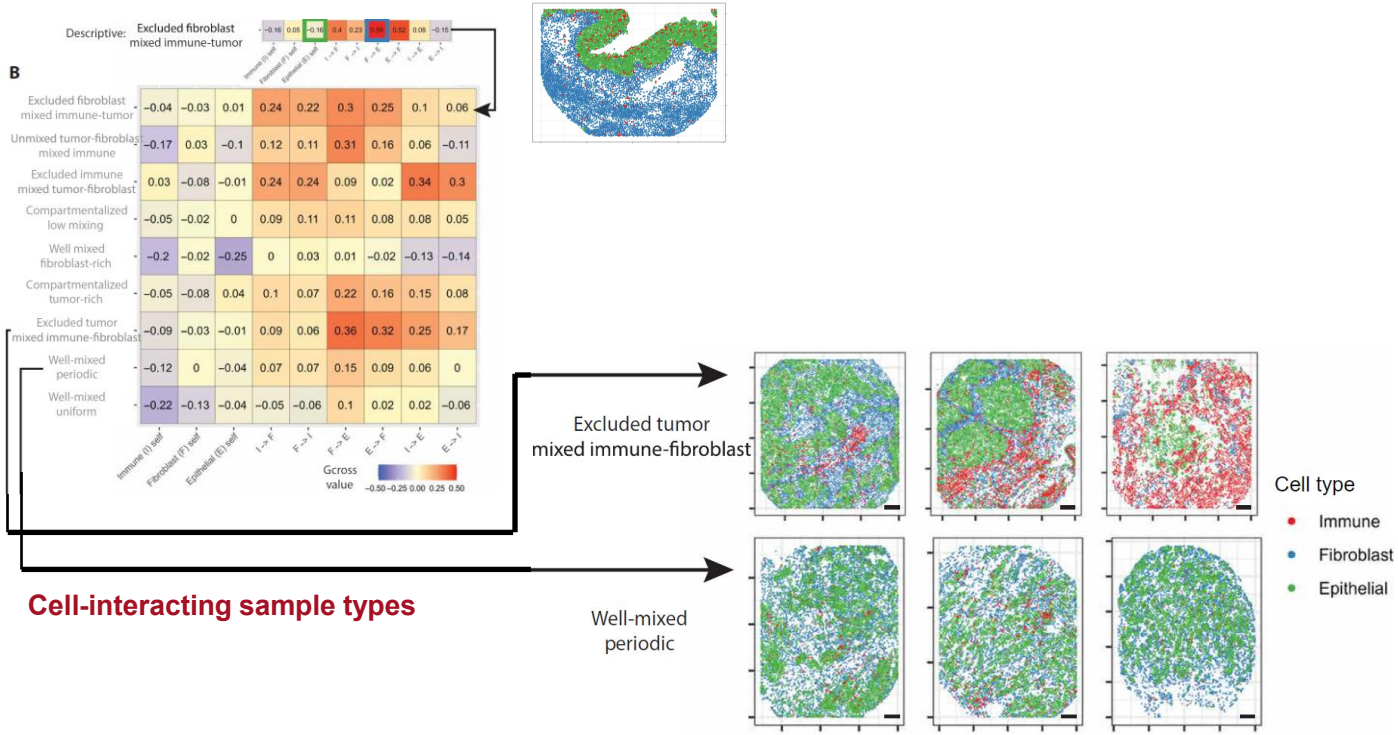


For each sample: Interaction score between cell types

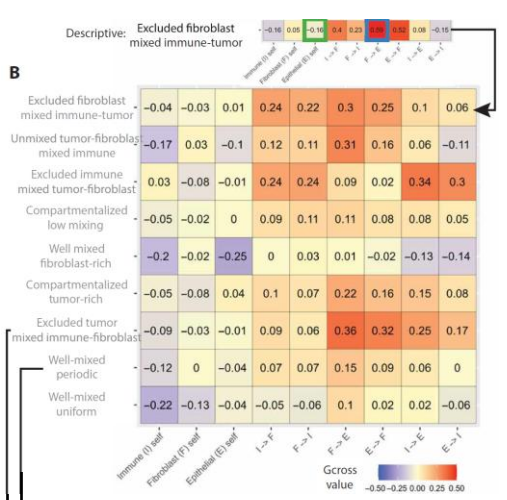


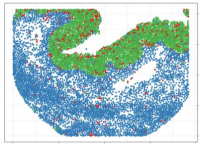
UNIVERSITY OF MARYLAND

Distribution Carries More Information

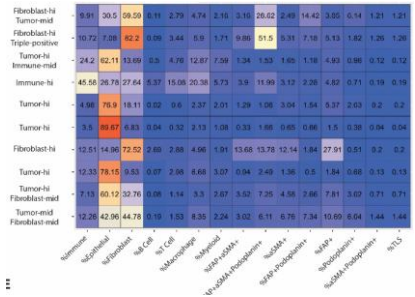


Distribution Carries More Information



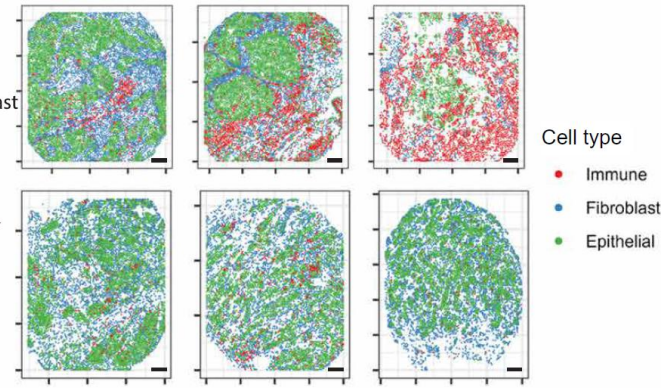


Cell-proportion sample types



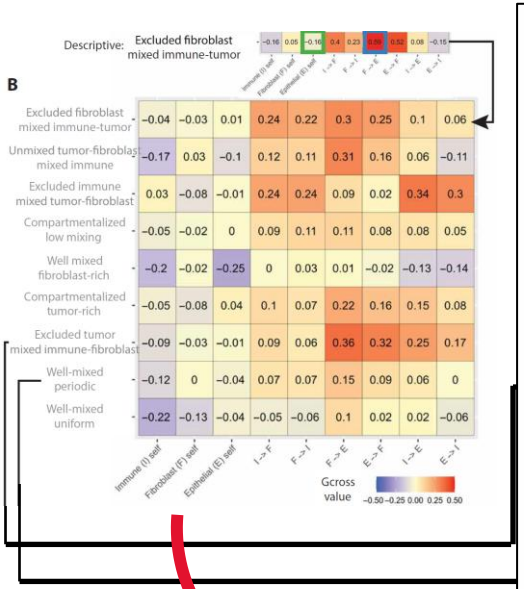
Excluded tumor mixed immune-fibroblast

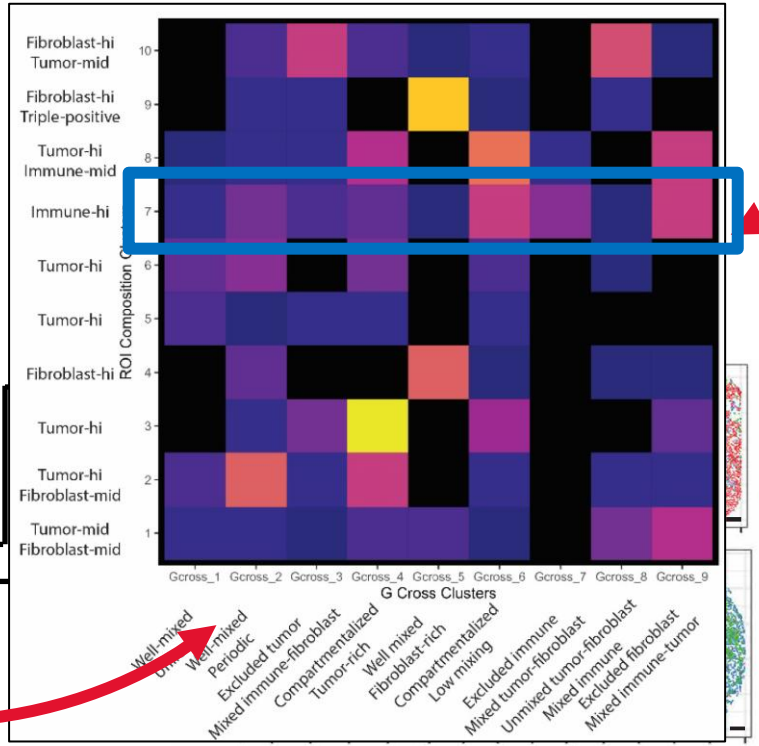
Well-mixed periodic

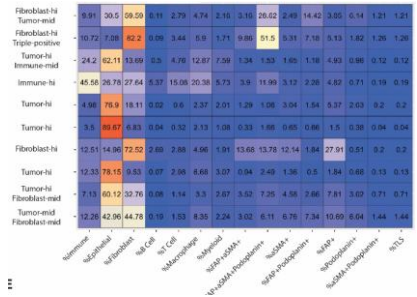


Cell-interacting sample types

Distribution Carries More Information







Cell-proportion sample types

Cell-interacting sample types

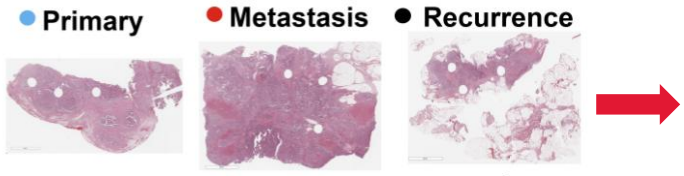
Cell type
- Immune
- Fibroblast
- Epithelial

No significant associations with early relapse

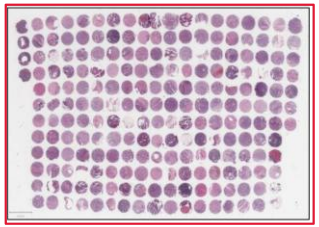
UNIVERSITY OF MARYLAND

Tumor Microarrays are Curated

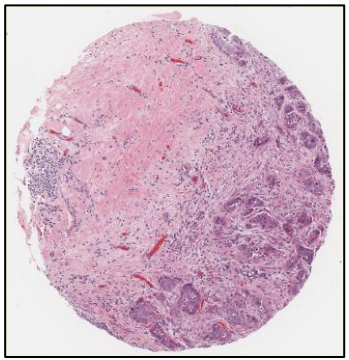
Representative Regions Selected



Representative Regions



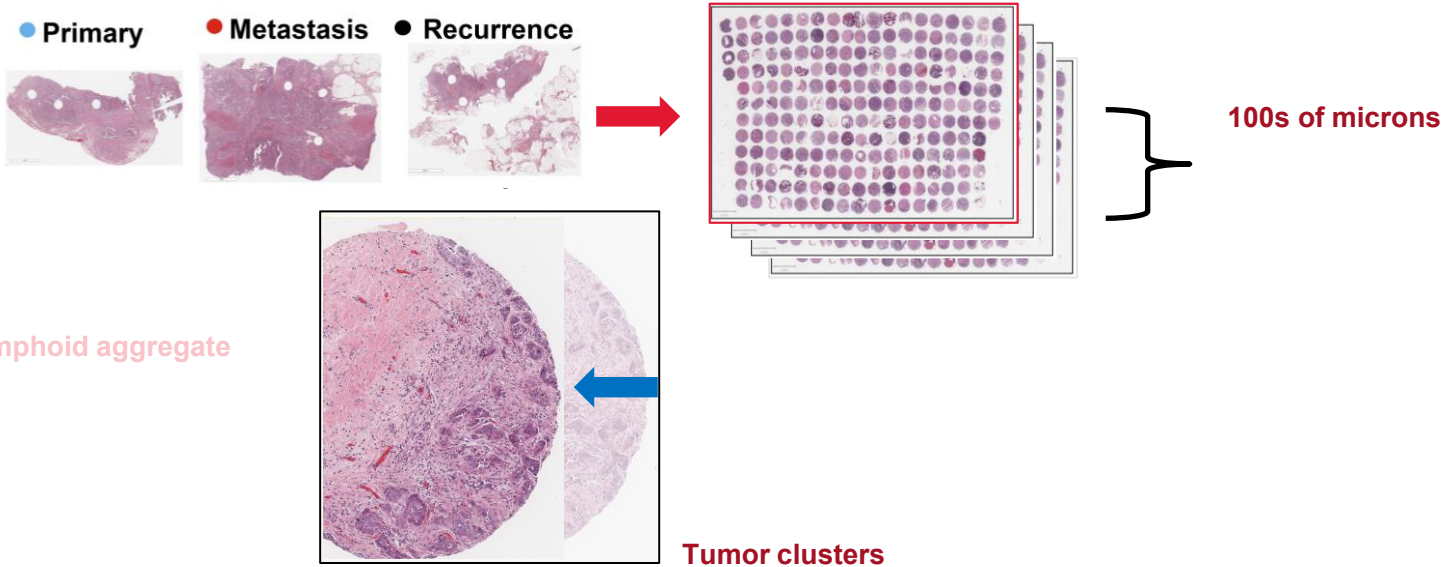
Lymphoid aggregate



Tumor clusters

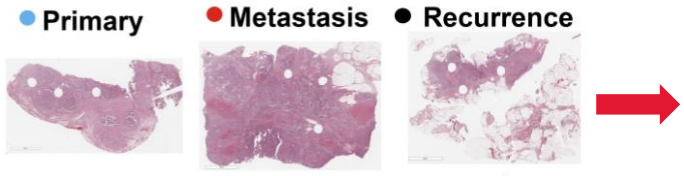
Tumor Microarrays are Curated

Representative Regions Selected

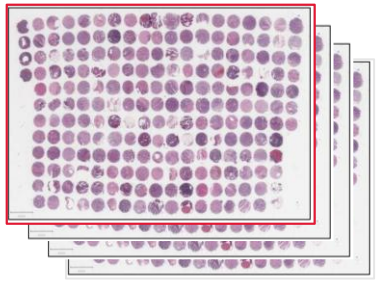


Tumor Microarrays are Curated but not Static

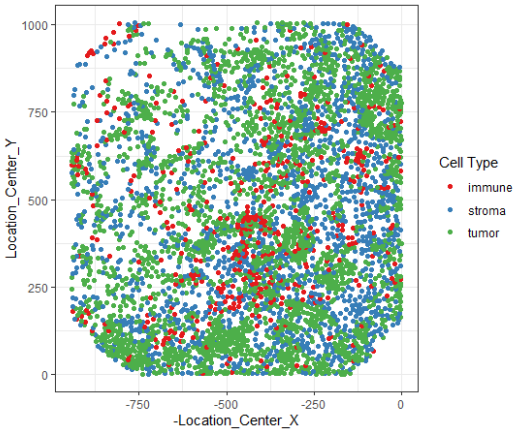
Representative Regions Selected



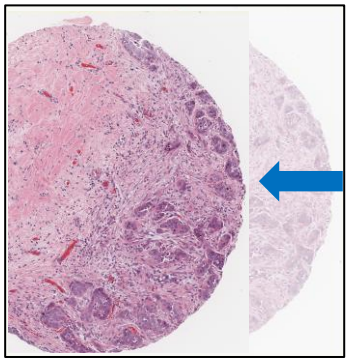
Representative Regions



100s of microns



Semi-Representative



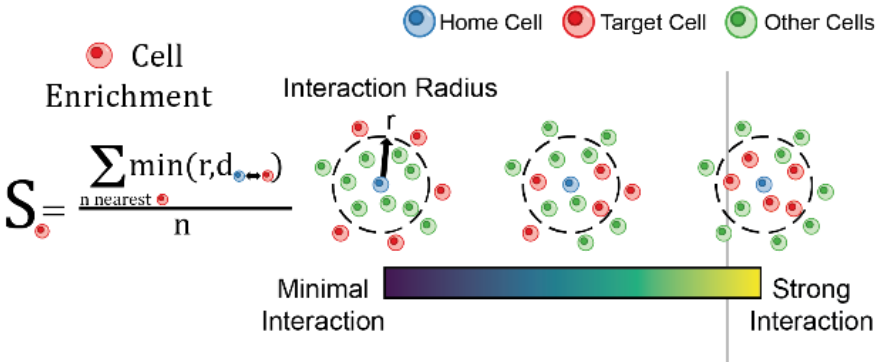
Lymphoid aggregate

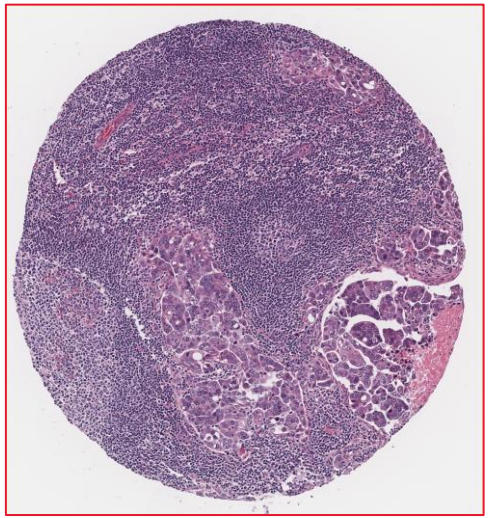
Tumor clusters

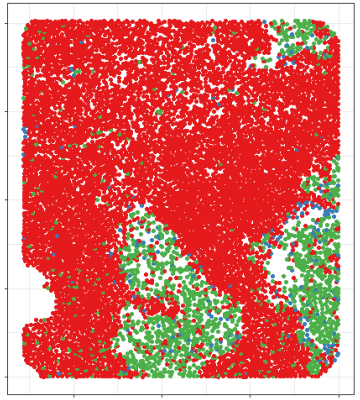
UNIVERSITY OF MARYLAND

Cell-Type Specific Spatial Enrichments Describe the TME

Spatial enrichment score → local microenvironment



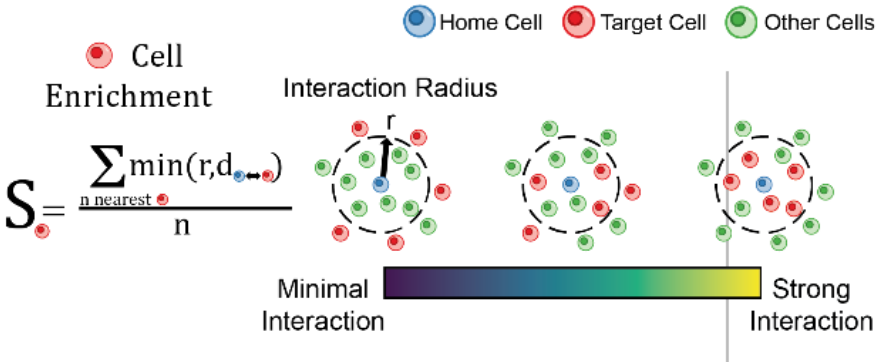


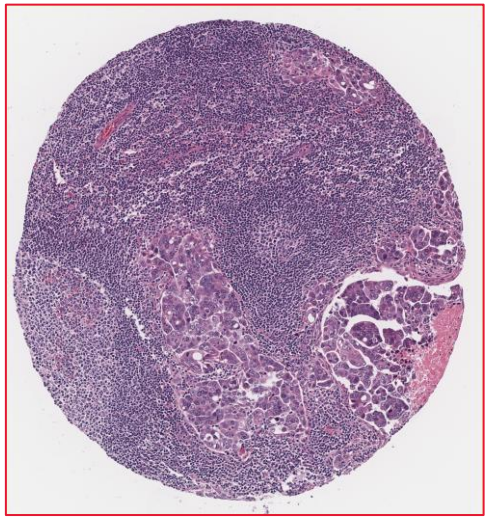


Immune-surrounded tumor

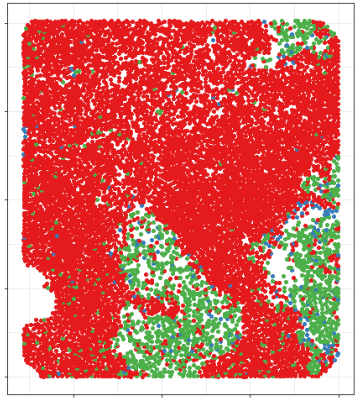
Cell-Type Specific Spatial Enrichments Describe the TME

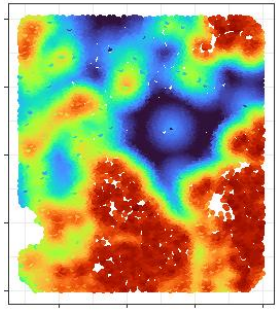
Spatial enrichment score → local microenvironment





Tumor enrichment

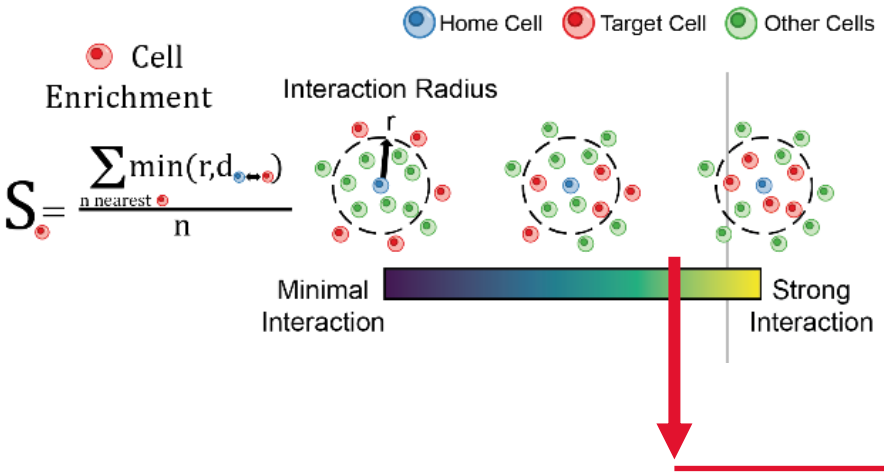


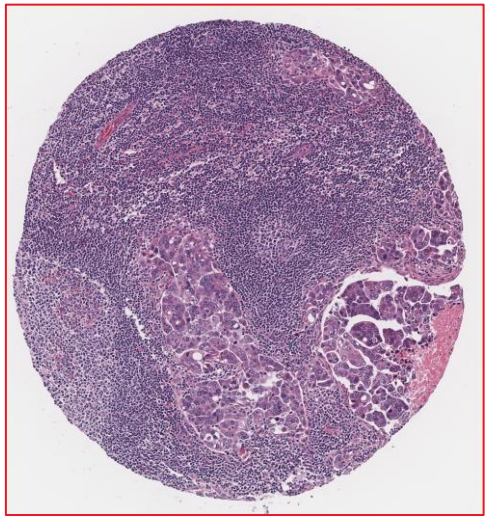


UNIVERSITY OF MARYLAND

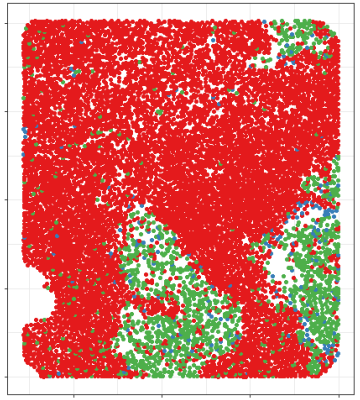
Cell-Type Specific Spatial Enrichments Describe the TME

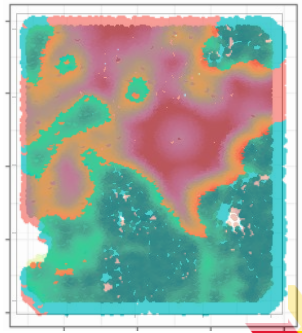
Spatial enrichment score → local microenvironment





Tumor Relevant Regions Isolated

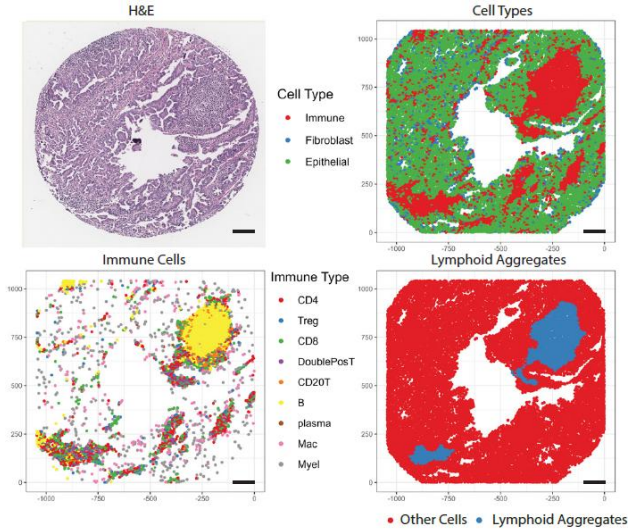




UNIVERSITY OF MARYLAND

Spatial Enrichment Analysis

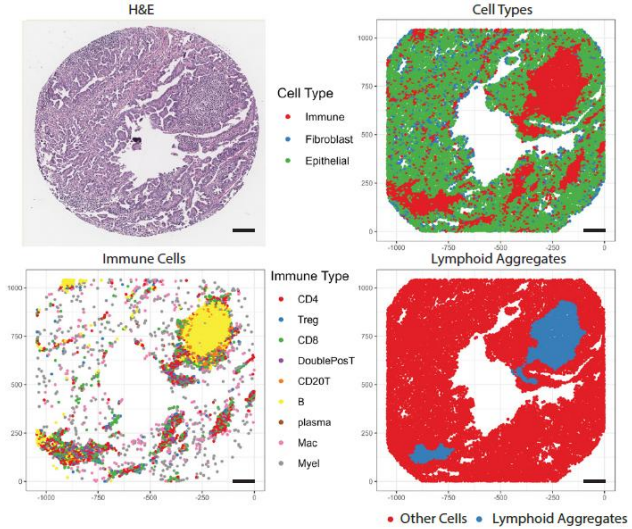
Lymphoid Aggregates



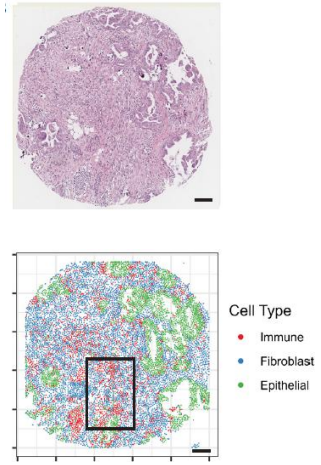
UNIVERSITY OF MARYLAND

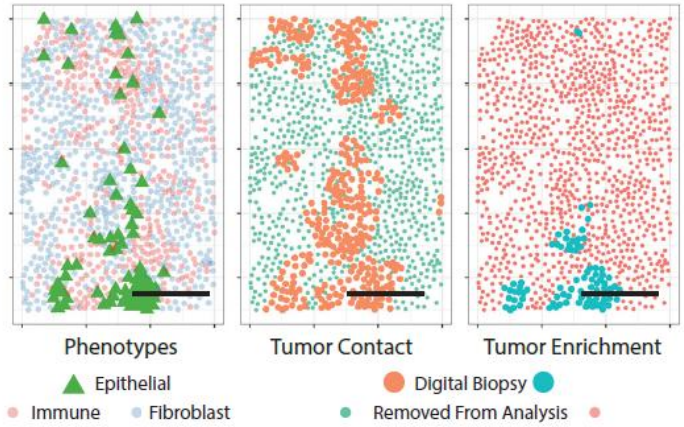
Spatial Enrichment Analysis

Lymphoid Aggregates



Context-specific niches (Digital biopsy)



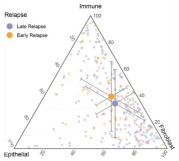


UNIVERSITY OF MARYLAND

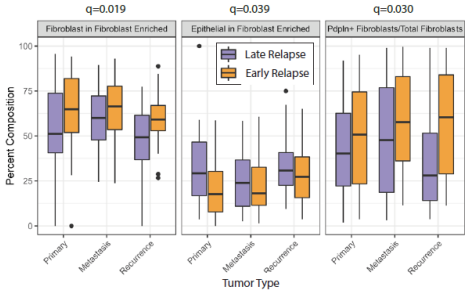
Spatial Predictors of Early Relapse

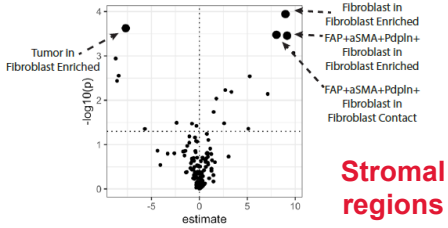
Statistical Associations between early/late relapsers

Spatially-restricted cell proportions



No significance





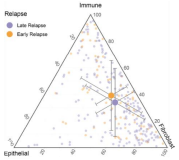
Stromal regions

UNIVERSITY OF MARYLAND

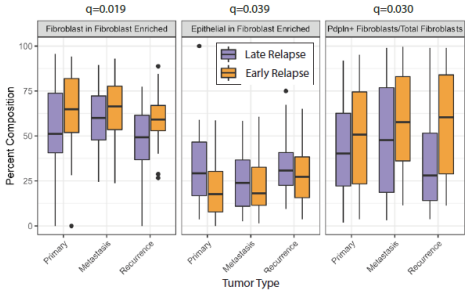
Spatial Predictors of Early Relapse

Statistical Associations between early/late relapsers

Spatially-restricted cell proportions

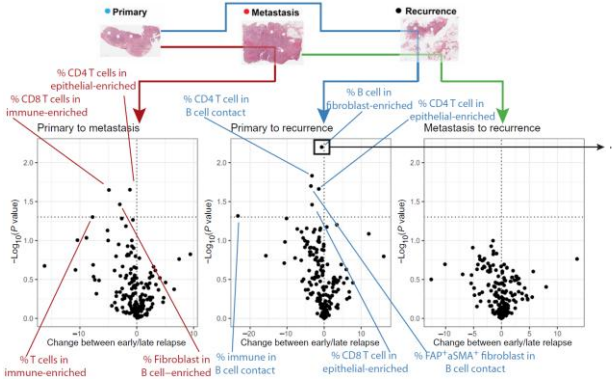


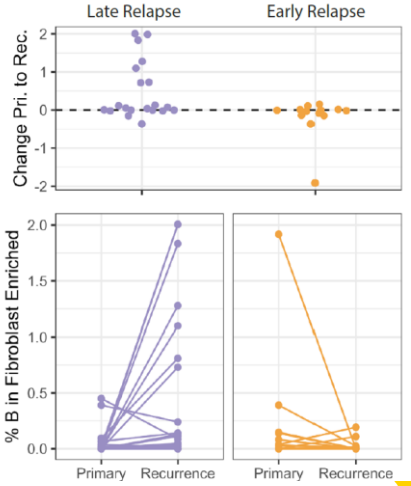
No significance



Stromal regions

Spatio-temporal associations with early relapse



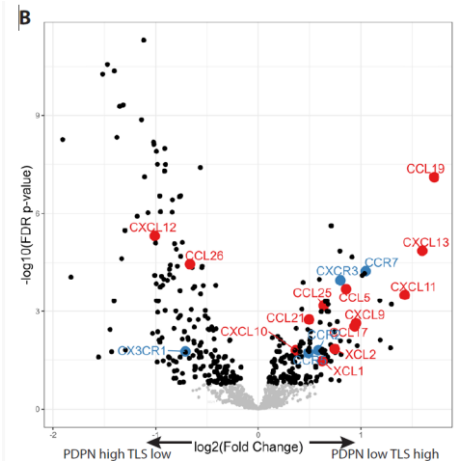


UNIVERSITY OF MARYLAND

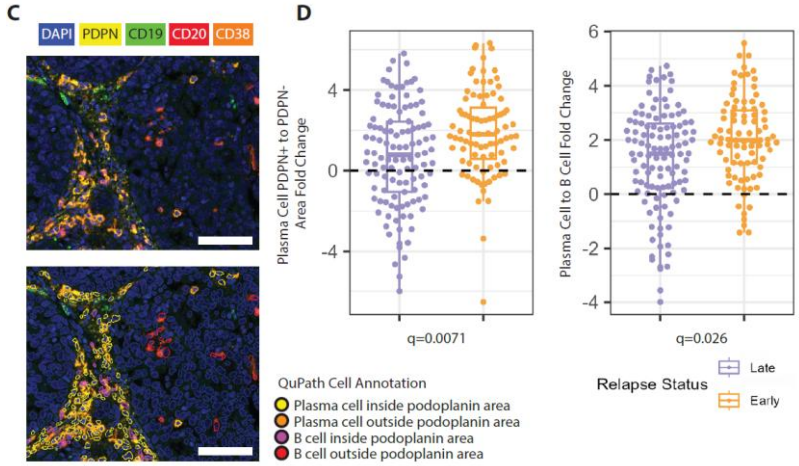
Follow ups

Alternative omics follow up to spatially significant B cell changes

Visium gene expression



Multiplex IF



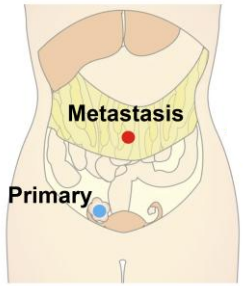
UNIVERSITY OF MARYLAND

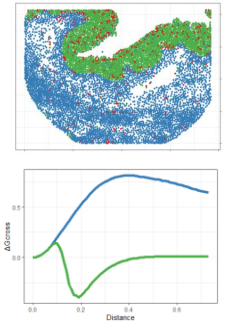
Conclusions

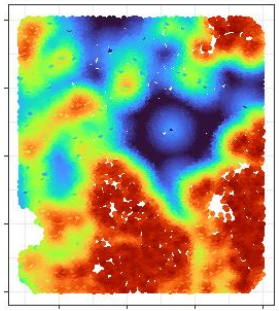
42 patients with matched samples over tumor progression

Qualitative eye-test vs quantitative

Digitally isolating specific sample regions reveals significant patterns







Other things:
- Predictors of early relapse
- IMC/H&E Concordance
- Unifying hypothesis for B/plasma cells

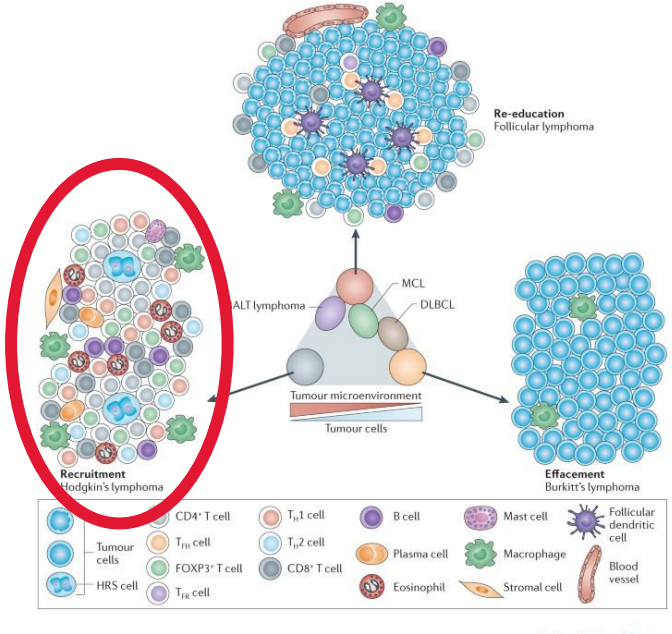
Xu et al., **Science Advances, 2024**

UNIVERSITY OF MARYLAND

The Hodgkins Lymphoma (HL) Tumor Microenvironment (TME) is immune-rich

Immune-rich tumor microenvironments (TME) of B-cell Lymphomas

Hodgkin's: Rare CD30+ tumor cells



Nature Reviews | Cancer

UNIVERSITY OF MARYLAND

Scott and Gascoyne, Nat. Rev. Cancer, 2014 Steidl et al, NEJM, 2010

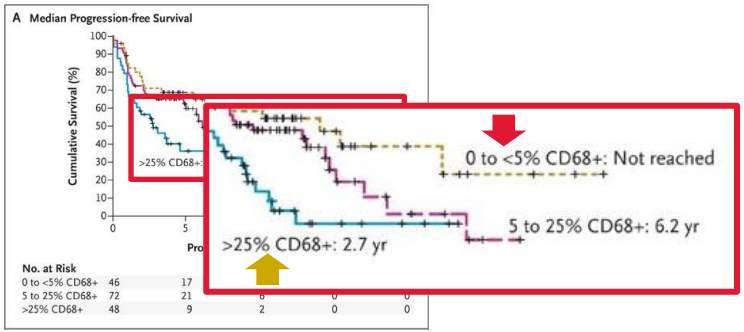
TME Elements are associated with clinical outcomes

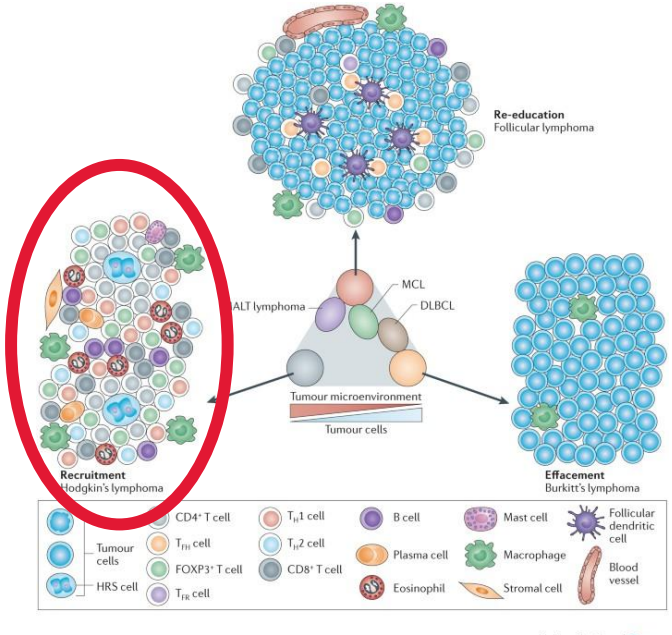
Immune-rich tumor microenvironments (TME) of B-cell Lymphomas

Hodgkin's: Rare CD30+ tumor cells

Stratifying patients for prognosis, outcomes

HL: CD68+ macrophages, etc.



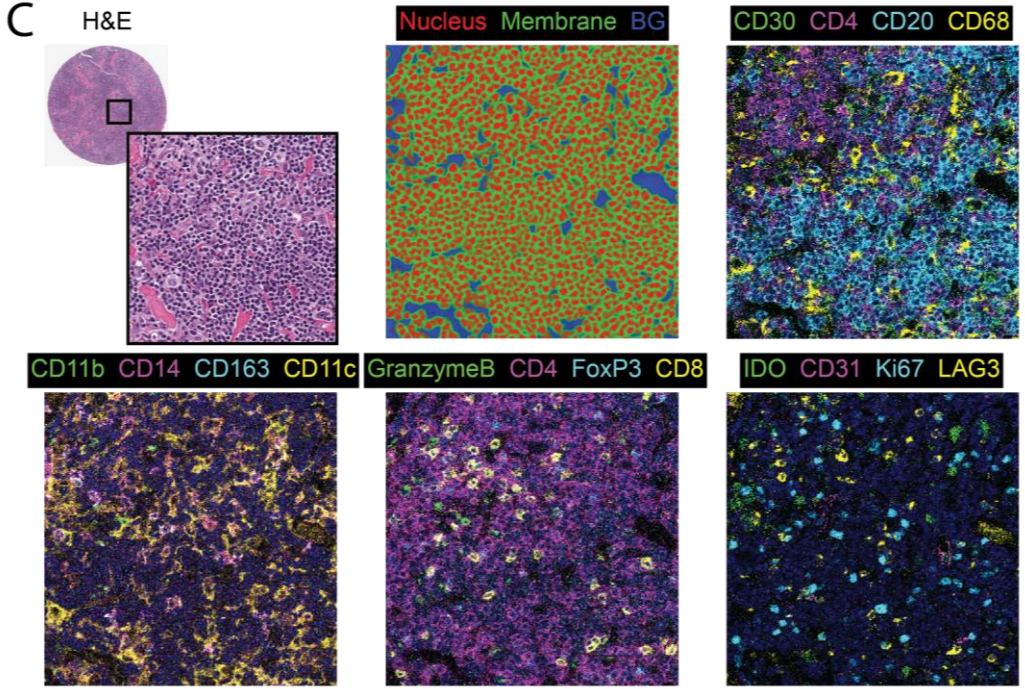


Scott and Gascoyne, Nat. Rev. Cancer, 2014 Steidl et al, NEJM, 2010

UNIVERSITY OF MARYLAND

HL TME is densely populated with immune cells

Segmentation
Sparse tumor cells (CD30)
Myeloid/Lymphoid infiltrate

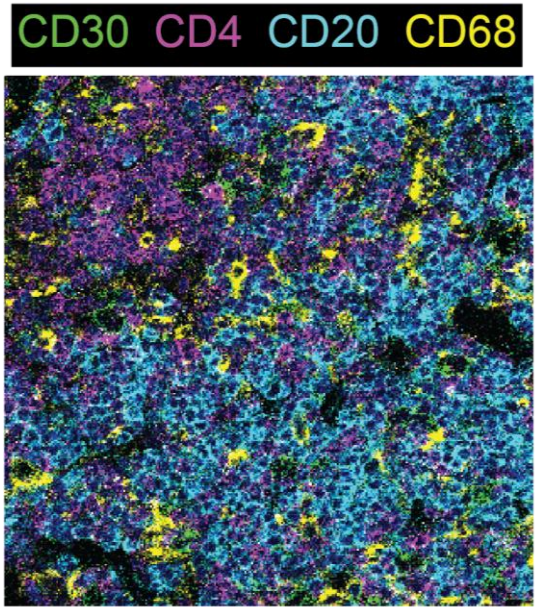


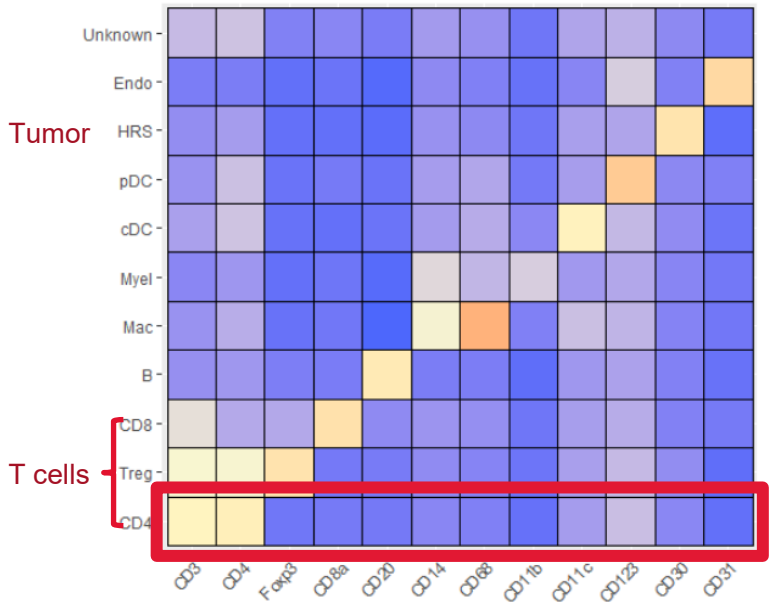
UNIVERSITY OF MARYLAND

Phenotyping identifies 10 major cell types

Sparse tumor cells (CD30), immune rich – T cells (CD4, CD8), Macrophages (CD68)

10 major phenotypes



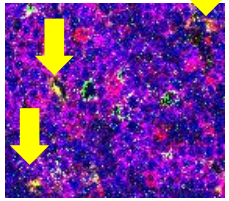


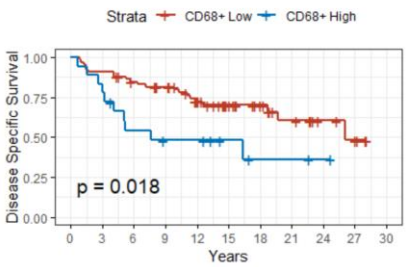
UNIVERSITY OF MARYLAND

Biomarkers of Hodgkin's Lymphoma

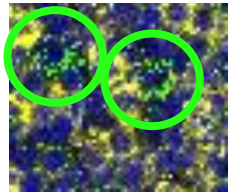
IMC-derived biomarkers in Diagnostic Samples

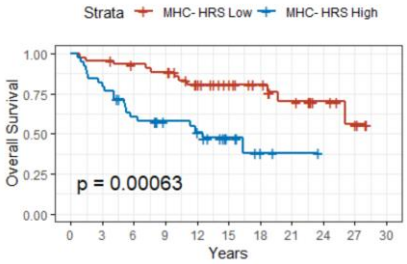
%CD68 Macrophage

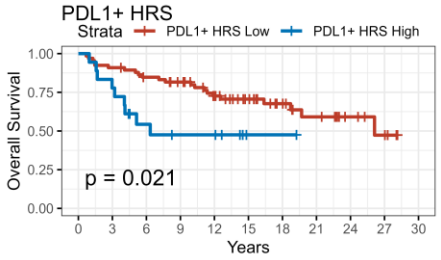


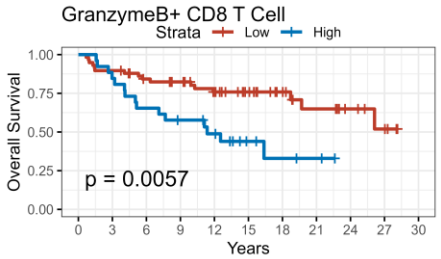


HRS tumor <MHCI than neighbors





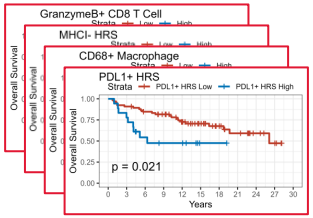


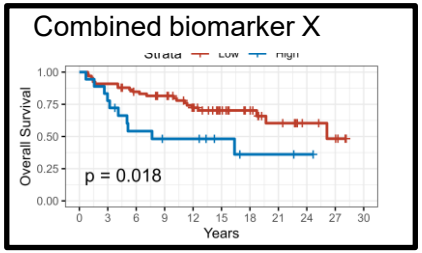


Spatial Biomarkers as a Universal Metric

UNIVERSITY OF MARYLAND

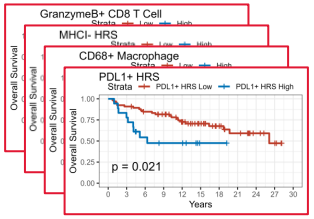
Translating IMC to actionable biomarkers

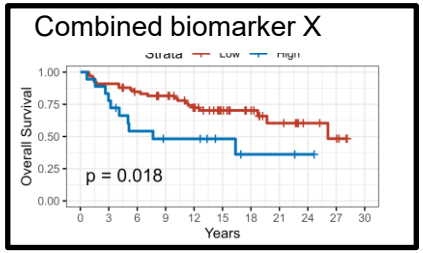




UNIVERSITY OF MARYLAND

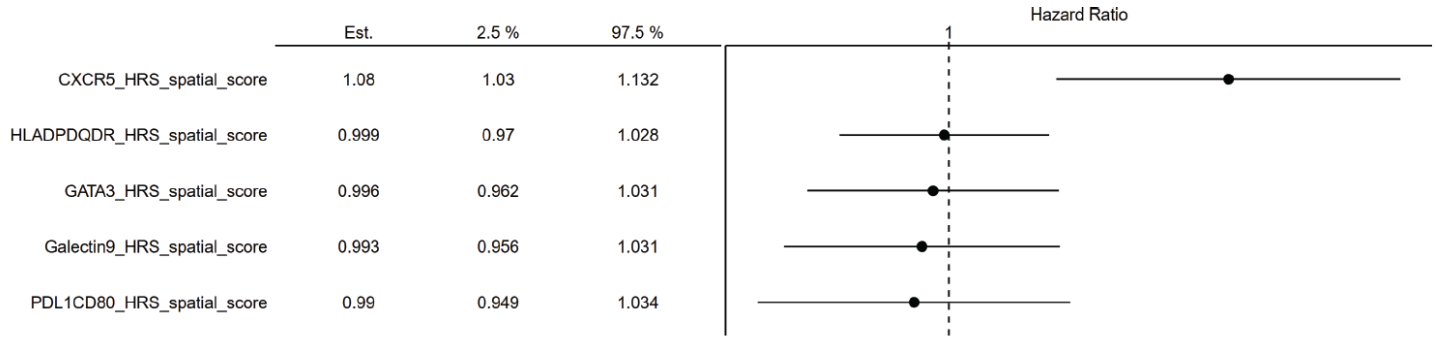
Translating IMC to actionable biomarkers





Dimensional reduction of IMC terms identifies significant predictors (LASSO)

Establish a score for each patient based on spatial metrics and a high/low cutoff (XGBoost)

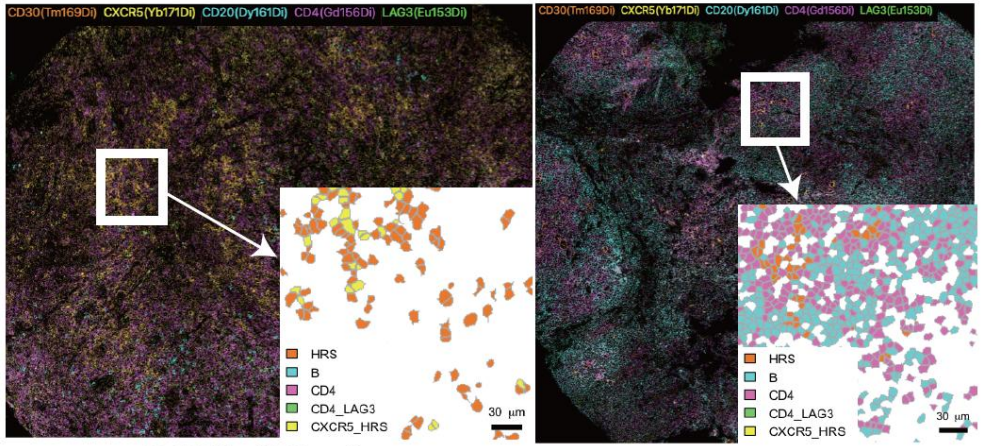


UNIVERSITY OF MARYLAND

Multi-color IF of reduced IMC panel

6 marker IF vs 35 marker IMC
>1.6M combinations

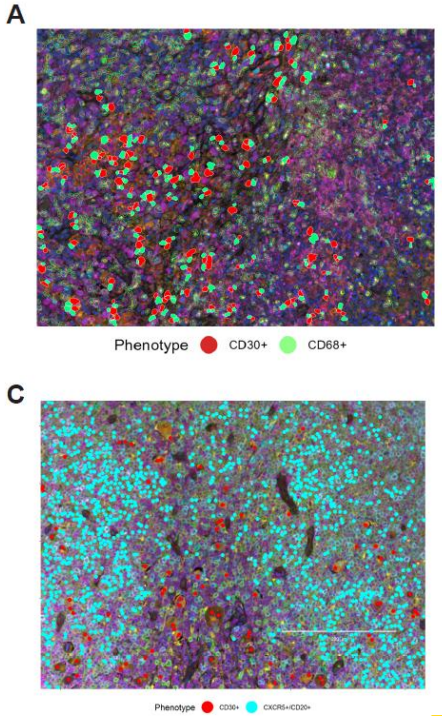
IMC



CXCR5+ HRS cells

CXCR5- HRS cells

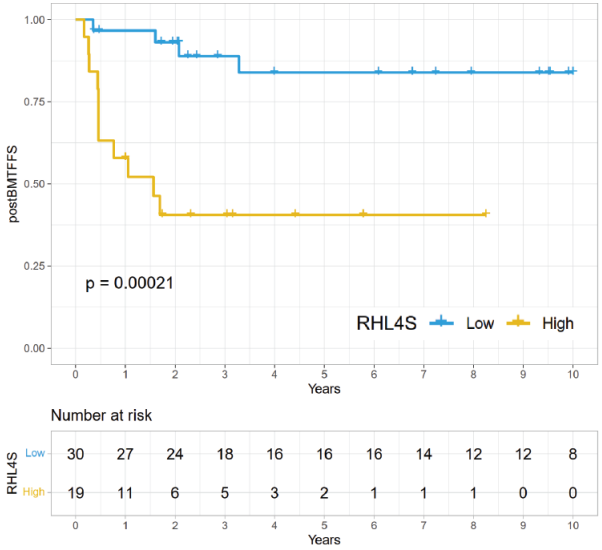
IF



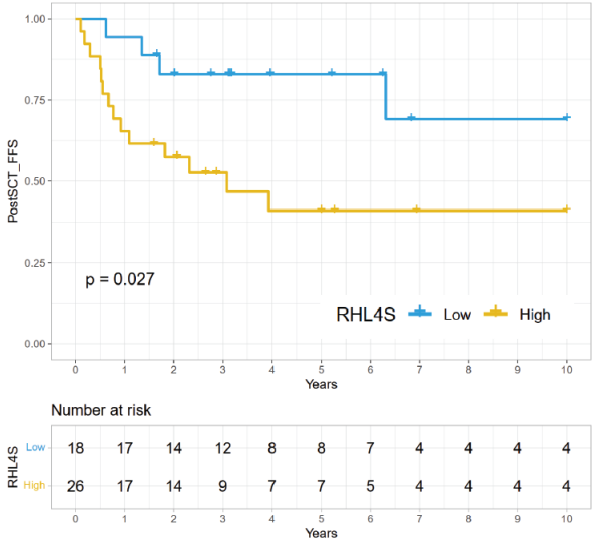
UNIVERSITY OF MARYLAND

Multi-color IF of reduced IMC panel

Survival in IMC Cohort w/ IMC



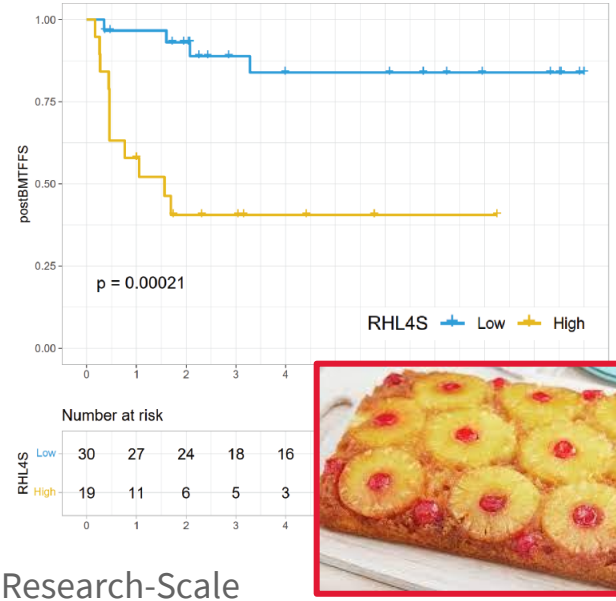
Survival in Validation Cohort w/ mIF



UNIVERSITY OF MARYLAND

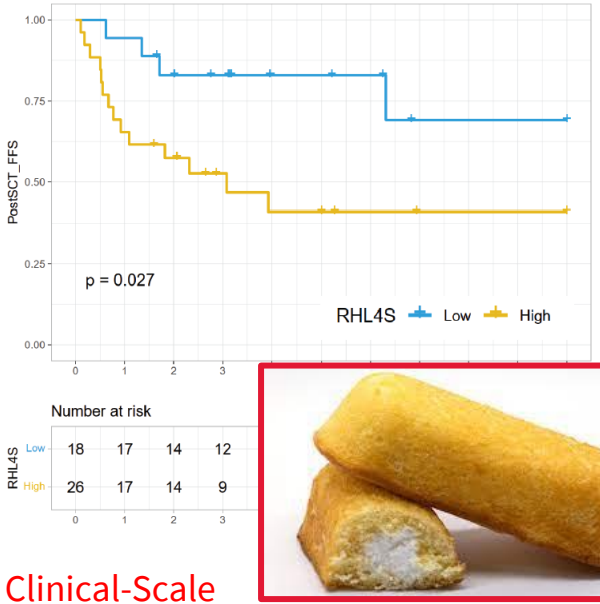
Multi-color IF of reduced IMC panel

Survival in IMC Cohort w/ IMC



Research-Scale

Survival in Validation Cohort w/ mIF



Clinical-Scale

UNIVERSITY OF MARYLAND

Conclusions

95 longitudinal Hodgkin's lymphoma patient samples

The gooey cake fruit – a localized spatial pattern metric predicts outcomes

Translating IMC biomarkers into an mIF assay

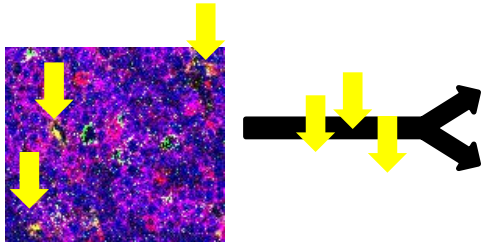
Aoki*, Jiang*, **Xu*** et al., **JCO 2024**

Other things:
- Deep phenotyping
- Spatial clusters/niches
- Temporal changes per patient
- Spatial/protein biomarker search
- Propose biomarker candidates

UNIVERSITY OF MARYLAND

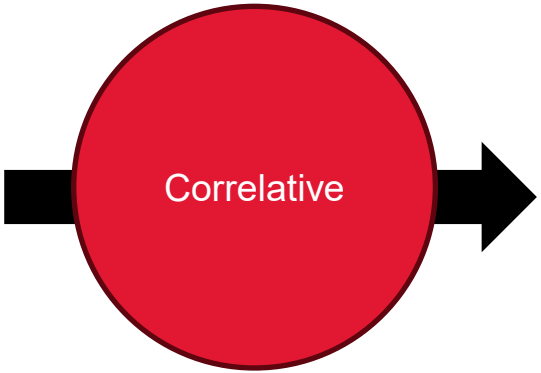
Spatial data for therapy

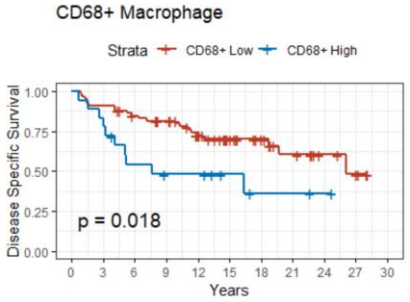
Prognostics vs predictions



Measurement/biomarker

Strata





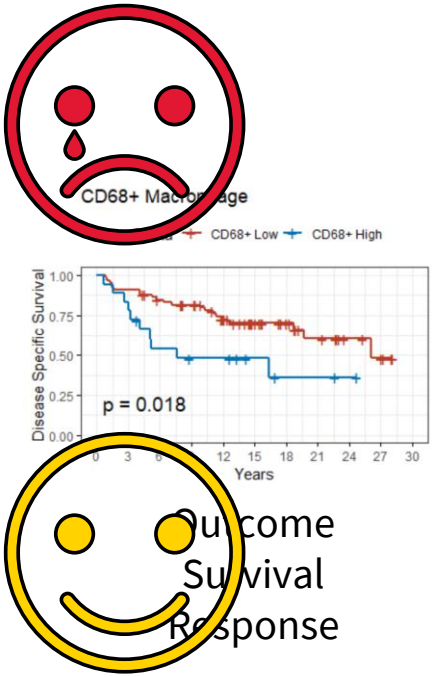
Outcome
Survival
Response

Spatial data for therapy

Pro

Measu

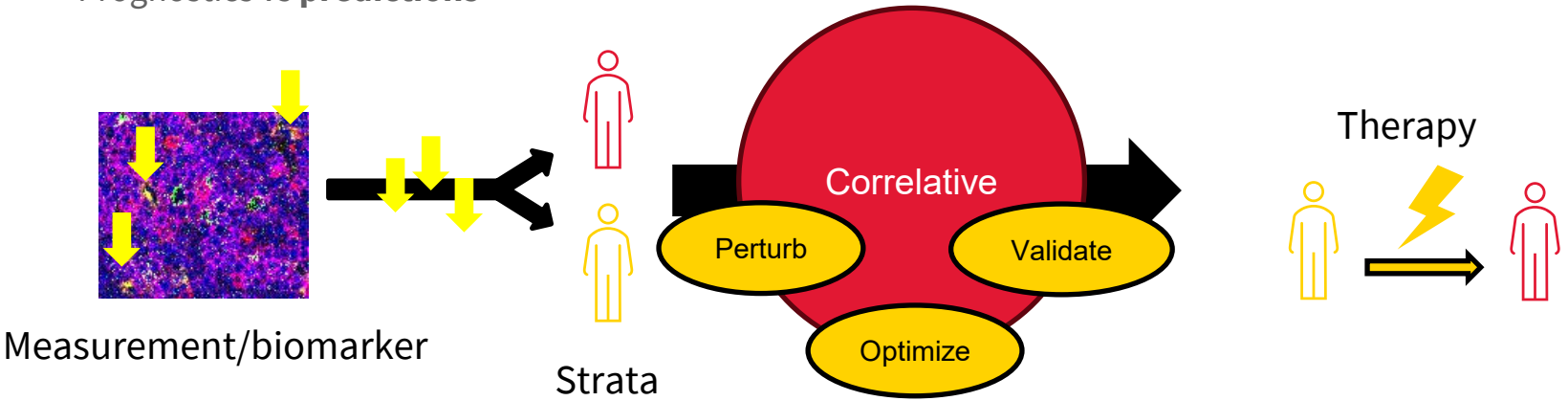
Correlative



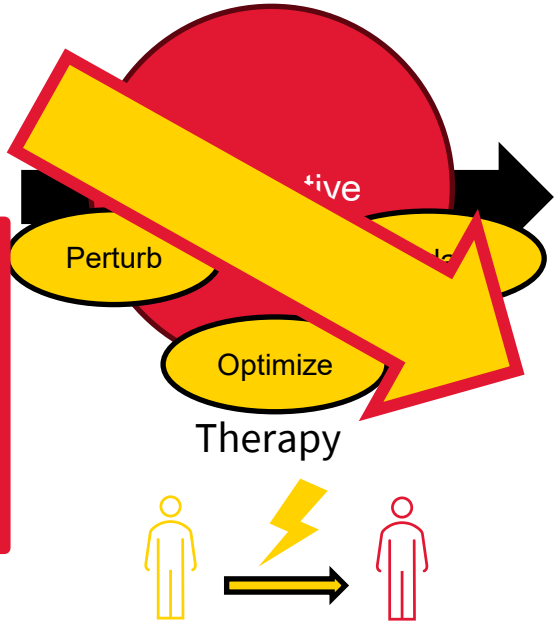
Outcome
Survival
Response

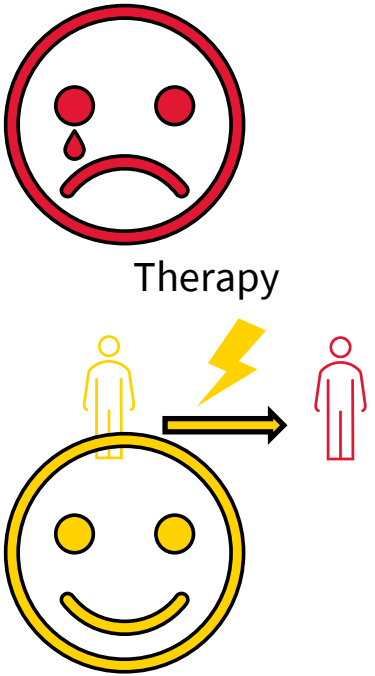
Spatial data for therapy

Prognostics vs **predictions**



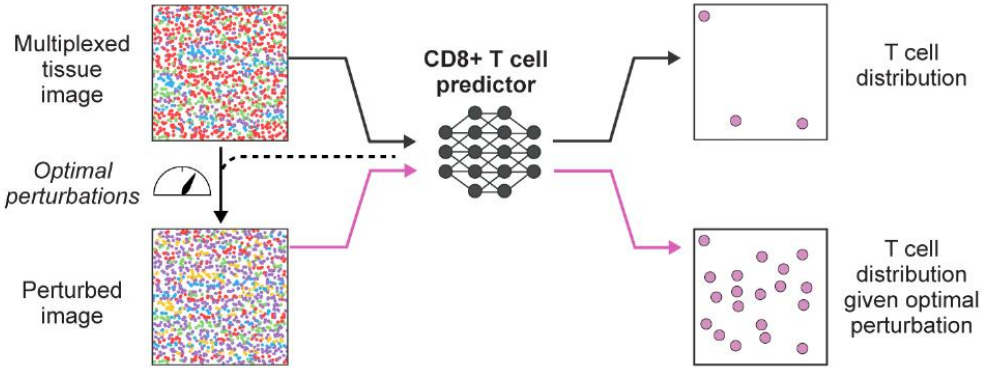
Spatial data for therapy



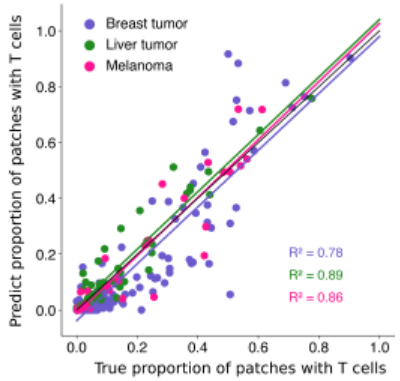


Prediction classifier for spatial features

Predict T cell infiltration in images



Accuracy

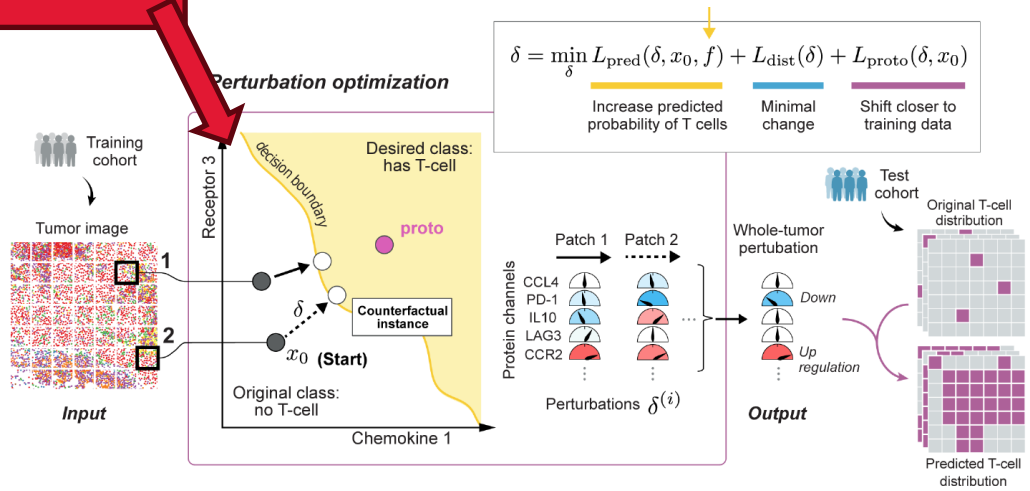


UNIVERSITY OF MARYLAND

Perturbation testing

Predict perturbations constrained by real observations

In silico perturbation



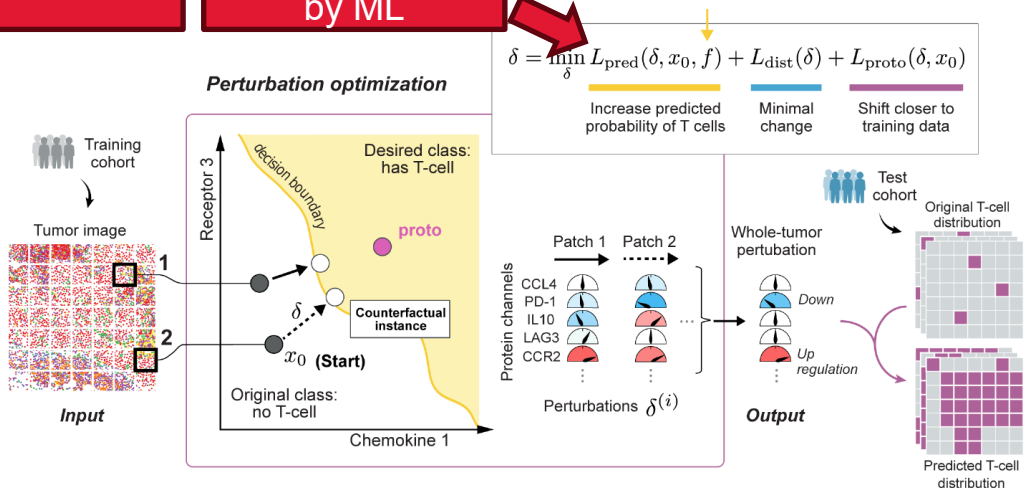
UNIVERSITY OF MARYLAND

Perturbation optimization

Predict perturbations constrained by real observations

In silico perturbation

Predictive spatial features by ML



UNIVERSITY OF MARYLAND

Perturbation optimization

Predict perturbations constrained by real observations

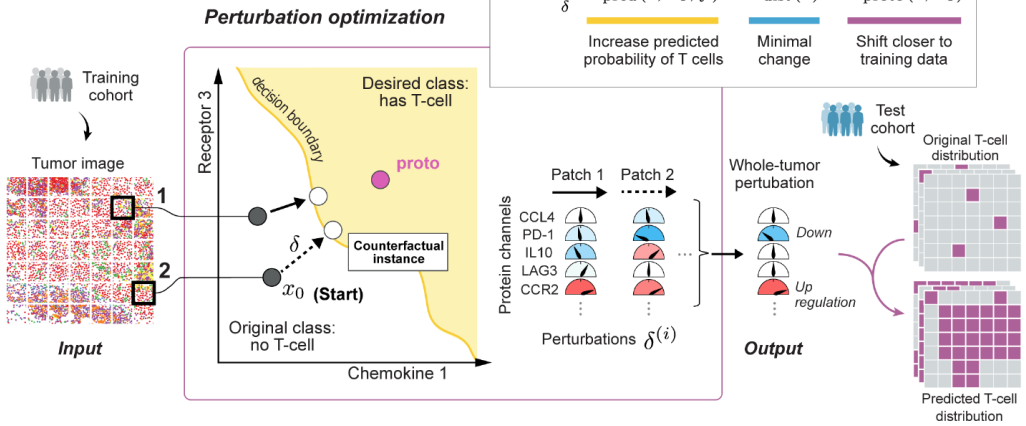
In silico perturbation

Predictive spatial features by ML

Realistic perturbation size

$$\delta = \min_{\delta} L_{\text{pred}}(\delta, x_0, f) + L_{\text{dist}}(\delta) + L_{\text{proto}}(\delta, x_0)$$

Increase predicted probability of T cells | Minimal change | Shift closer to training data

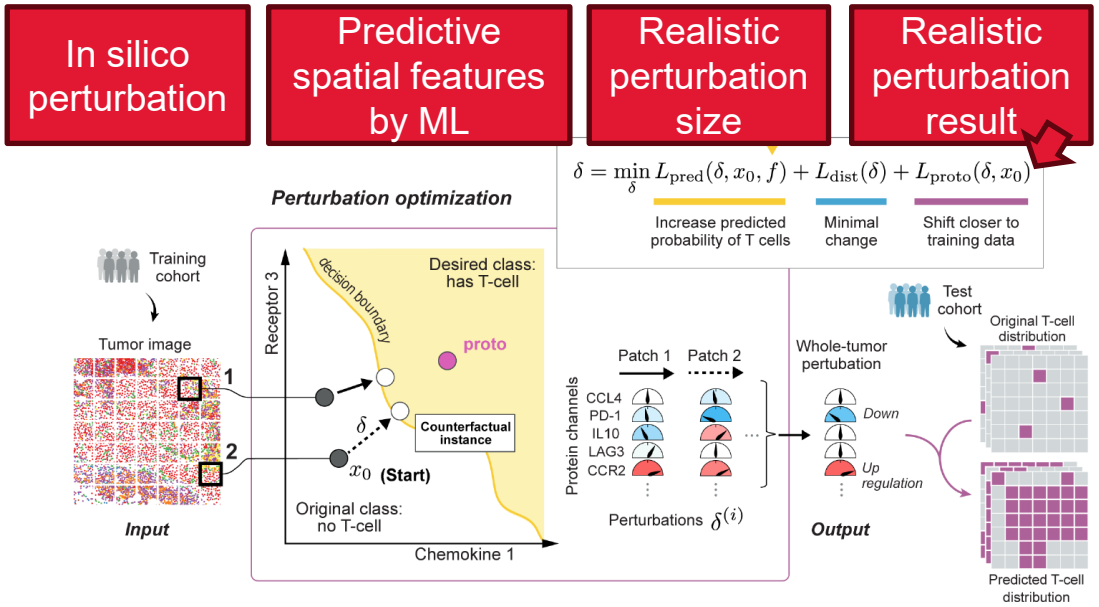


UNIVERSITY OF MARYLAND

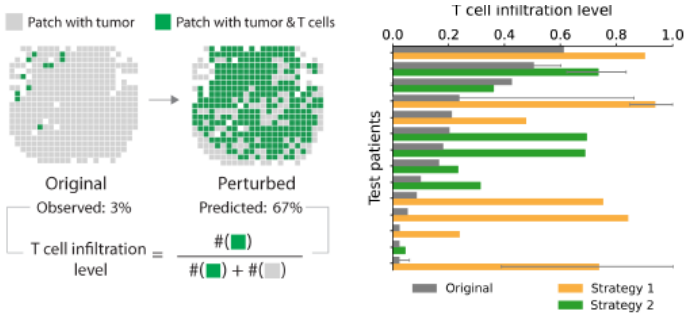
Perturbation optimization

Predict perturbations constrained by real observations

In silico perturbation	Predictive spatial features by ML	Realistic perturbation size	Realistic perturbation result



Increase T cell infiltration



UNIVERSITY OF MARYLAND

Melanoma Test Case

Melanoma + chemokine data set

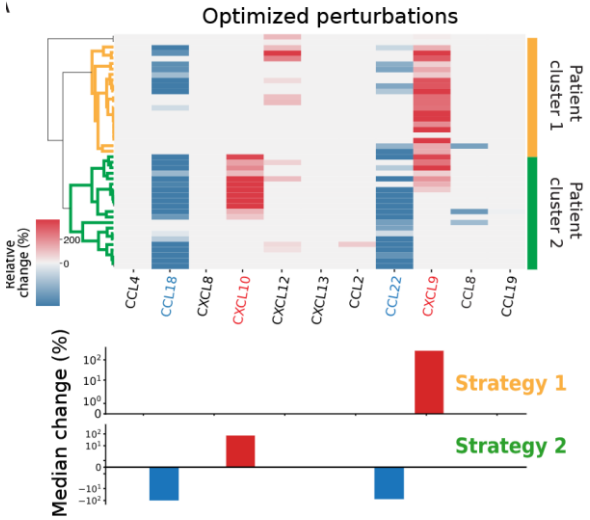
Proposed perturbations

Per patient

Averaged over all patient patches

Multiplexed imaging mass cytometry of the chemokine milieus in melanoma characterizes features of the re-sponse to immunotherapy

TOBIAS HOCH, DANIEL SCHULZ, NILS ELING, JULIA MARTÍNEZ GÓMEZ, MITCHELL P. LEVESQUE, AND BERND BODENMILLER Authors Info & Affiliations



UNIVERSITY OF MARYLAND

Melanoma Test Case

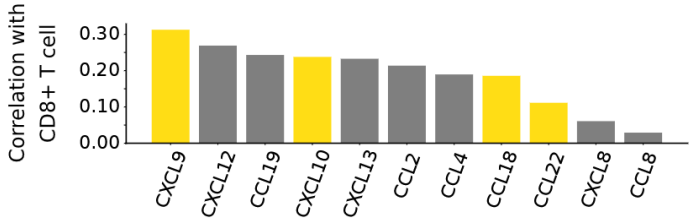
Melanoma + chemokine data set

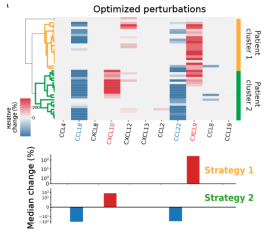
Not necessarily the most heavily correlated

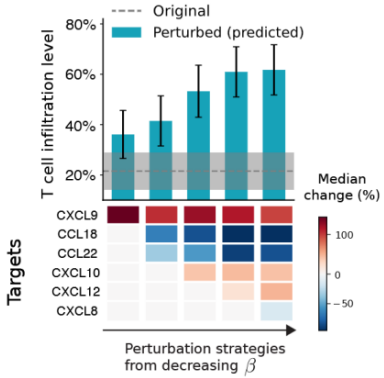
Combinatorial candidates

Multiplexed imaging mass cytometry of the chemokine milieus in melanoma characterizes features of the re-sponse to immunotherapy

TOBIAS HOCH, DANIEL SCHULZ, NILS ELING, JULIA MARTÍNEZ GÓMEZ, MITCHELL P. LEVESQUE, AND BERND BODENMILLER Authors Info & Affiliations







UNIVERSITY OF MARYLAND

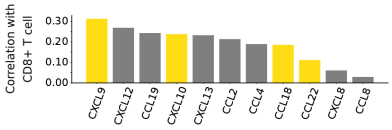
Melanoma Test Case

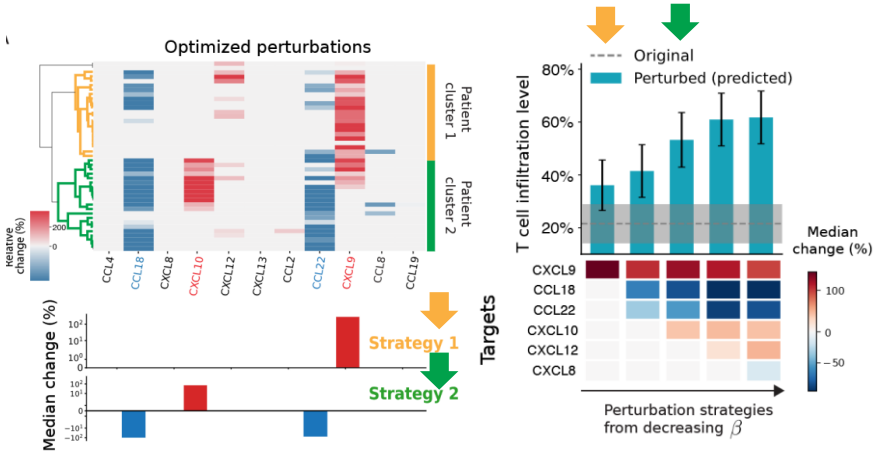
Melanoma + chemokine data set

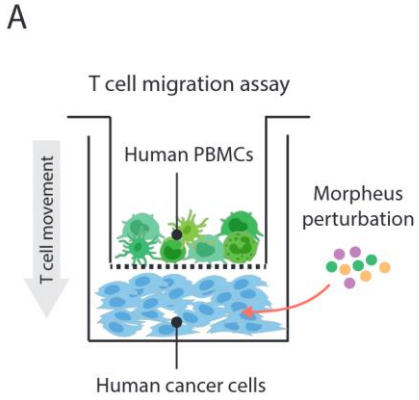
In vitro validation

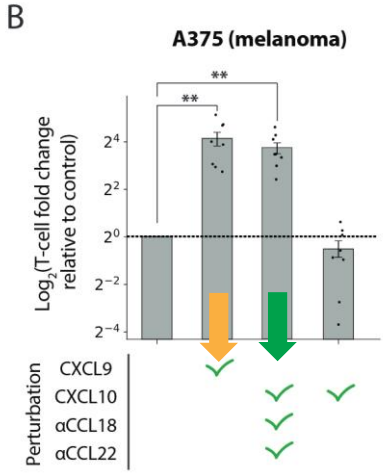
Multiplexed imaging mass cytometry of the chemokine milieus in melanoma characterizes features of the re-sponse to immunotherapy

TOBIAS HOCH, DANIEL SCHULZ, NILS ELING, JULIA MARTÍNEZ GÓMEZ, MITCHELL P. LEVESQUE, AND BERND BODENMILLER Authors Info & Affiliations









UNIVERSITY OF MARYLAND

Conclusions

In silico testing of perturbations

Constrained by spatial library and observed tissue space

Single and combination therapy candidates based on spatial hypotheses

Wang, … **Xu**, Thomson, **Nature Biomedical Engineering, 2025**

UNIVERSITY OF MARYLAND

Conclusions

In silico testing of perturbations

Const

Single

Wang

More other things:

- Lymphoma Spatial Biomarkers
  - … Xu et al, Blood Advances, 2022
  - … Xu et al, Am. J. of Hematology, 2025
- CRC Cancer: Metastasis to Liver
  - … Xu* et al, Cell Metabolism, 2023
  - … You†, Xu†, Seki†, JCI, 2025
- HIV+ Immunocompromised DLBCL
  - Coelho, Roush, Xu et al, British Journal of Haematology, 2024
  - Roush, Coelho, Xu et al, JCI Insight, 2024

Acknowledgements

Career Development Award Funding

NCATS UCLA KL2

Tower Cancer Research Foundation

Christian Steidl Lab

Tomohiro Aoki

Aixiang Jiang

Lauren Chong

Yifan Yin

Akil Merchant Lab

Alicia Gamboa

Sandra Orsulic Lab

Marcela Haro

Matthew Thomson Lab

Jerry Zitong Wang

Abdullah Farooq

ITCR

Contact

alexmxu@umd.edu