Response Predictors for Breast Cancer Immunotherapy

Masters in Therapeutic Oncology Summit

Isaac S. Chan, M.D., Ph.D.

UT Southwestern Medical Center

Departments of Internal Medicine and Molecular Biology

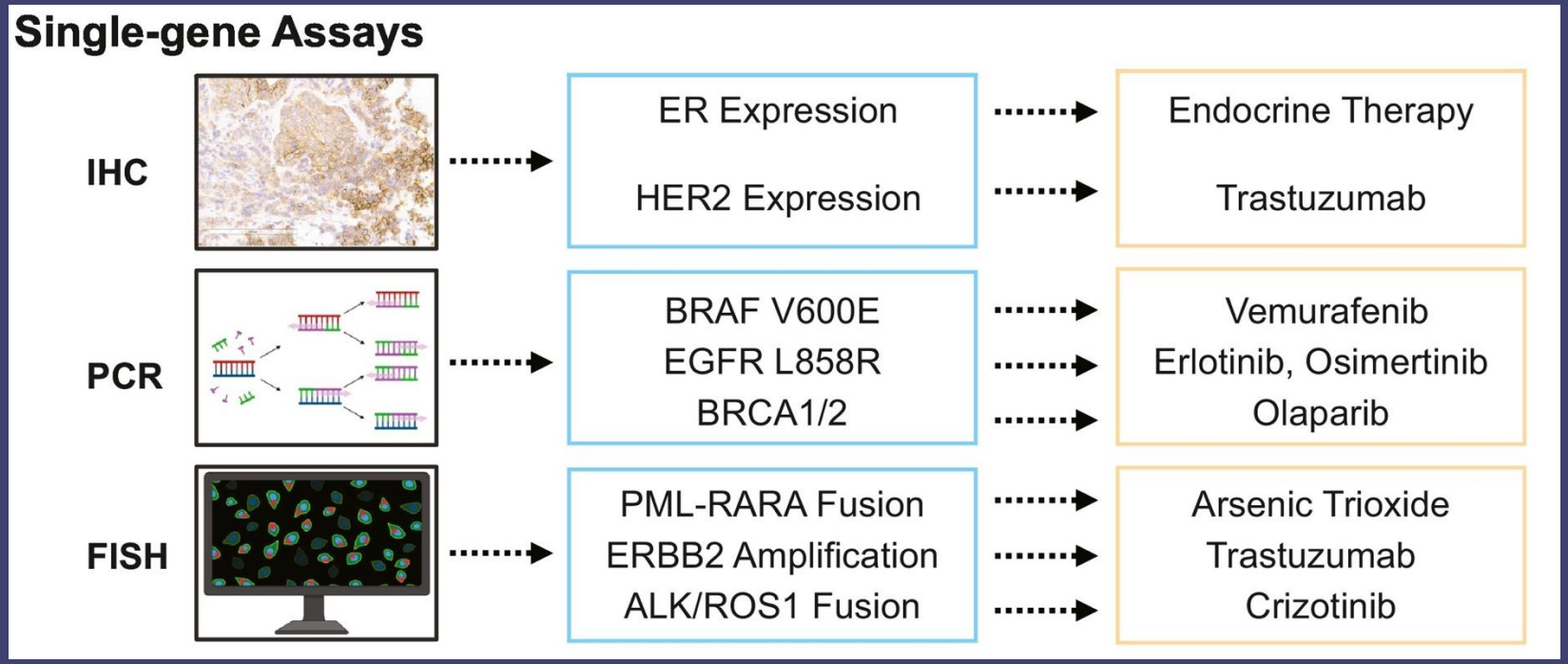
Simmons Comprehensive Cancer Center

March 29, 2025

UT Southwestern Medical Center

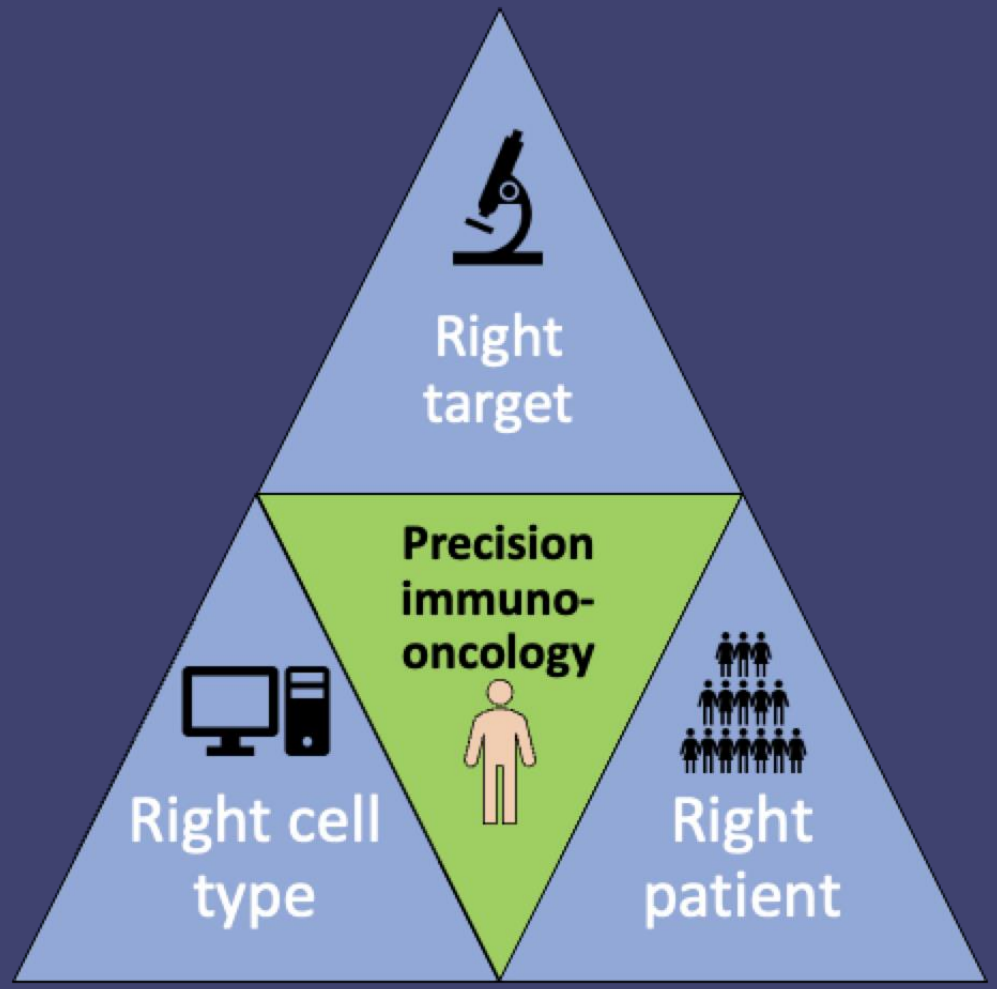
What is precision oncology?

Precision oncology is governed by the principle that select molecular alterations that increase cancer cell fitness, so-called drivers, may represent therapeutic vulnerabilities and opportunities for prognostic or predictive assays.

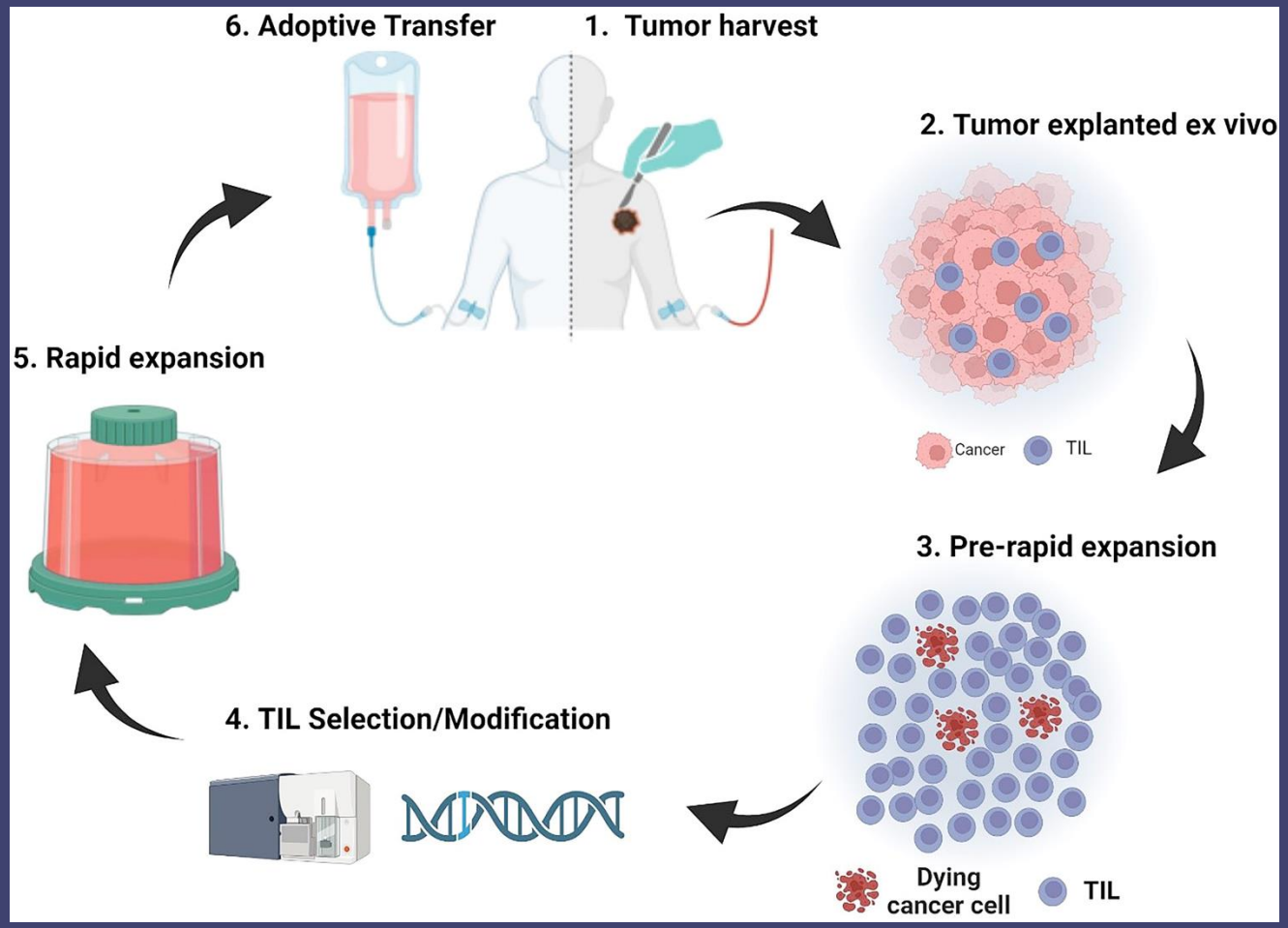


1. Repetto, Cancer Discv, 2024; 2. Tang, Trends in Can., 2024

What about precision immuno-oncology?



Tumor infiltrating lymphoctyes



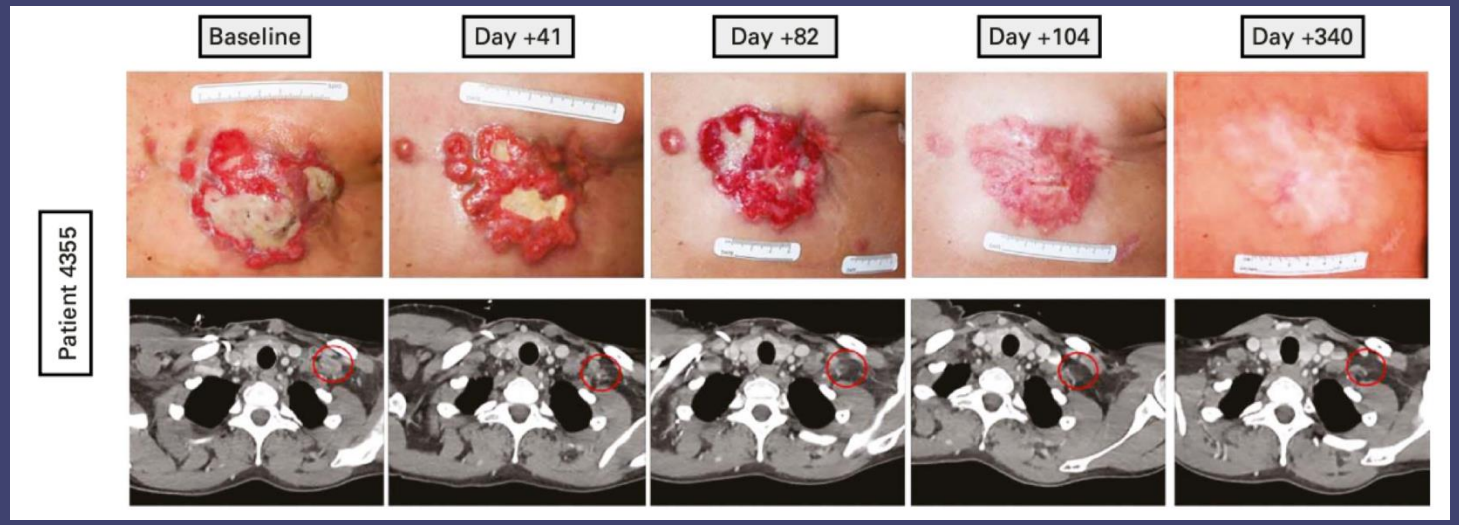
Sariak, Can Cell, 2024

Tumor infiltrating lymphoctyes

original reports

Breast Cancers Are Immunogenic: Immunologic Analyses and a Phase II Pilot Clinical Trial Using Mutation-Reactive Autologous Lymphocytes

Nikolaos Zacharakis, PhD[1]; Lutfi M. Huq, BA[1]; Samantha J. Seitter, DO[1]; Sanghyun P. Kim, PhD[1]; Jared J. Gartner, MSc[1]; Sivasish Sindiri, MSc[1]; Victoria K. Hill, PhD[1]; Yong F. Li, BS[1]; Biman C. Paria, PhD[1]; Satyajit Ray, PhD[1]; Billel Gasmi, MD[2]; Chyi-chia Lee, MD, PhD[2]; Todd D. Prickett, PhD[1]; Maria R. Parkhurst, PhD[1]; Paul F. Robbins, PhD[1]; Michelle M. Langhan, BS[1]; Thomas E. Shelton, BS[1]; Anup Y. Parikh, MD[1]; Shoshana T. Levi, MD[1]; Jonathan M. Hernandez, MD[3]; Chuong D. Hoang, MD[4]; Richard M. Sherry, MD[1,5]; James C. Yang, MD[1]; Steven A. Feldman, PhD[1,6]; Stephanie L. Goff, MD[1]; and Steven A. Rosenberg, MD, PhD[1]



Tumor infiltrating lymphoctyes

Identification of Somatic Mutations in Resected Metastatic Deposits

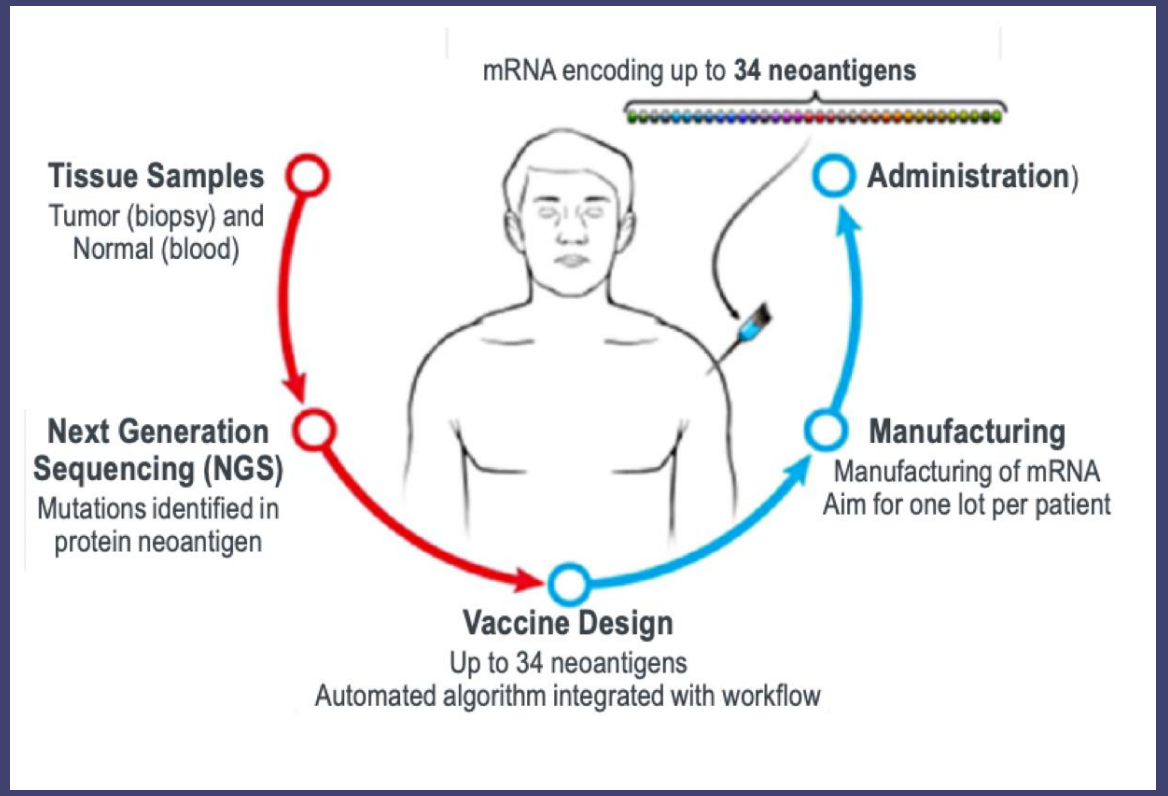
Somatic mutations were identified in all patients, with a median 1.45 mutations per Mb (range: 0.14-14.62; Fig 3A). The median number of nonsynonymous mutations was 112 per patient (range: 6-563; Fig 3B). The number of mutations was not associated with the clinical receptor (HR, HER2) status, presence or absence of known deleterious *BRCA* germline variants, or the site of the resected tumor (Fig 3B). There was no association with the number of prior lines of treatment with mutation burden (data not shown). The majority of mutated variants were nonsynonymous single-nucleotide variants (SNVs, median 61% per patient). Synonymous SNVs (median 26% per patient) and rare SNVs (median 3.4% per patient) leading to stop codons (nonsense mutations) were excluded from screening (Fig 3C). A median of 86 (range: 4-220) unique mutated variants per patient were synthesized for testing of tandem minigenes (TMGs) and long peptides (12-25AA) using techniques previously described (Fig 3D and Data Supplement).[3]

Neoantigen Characteristics

Screening for neoantigen-reactive TIL was performed for each of the 42 patients. Twenty-eight of 42 (67%) patients contained TIL recognizing at least one neoantigen (median: 3 neoantigens [neoAgs] per patient [pt], range: 1-11; Data Supplement). Overall, 2.3% (95 of 4,131) of all tested unique somatic mutations were found to be immunogenic. Each neoantigen identified was unique among all patients (Data Supplement). *TP53* was the only mutated gene that was recognized by more than one patient (n = 3); however, the specific TP53 neoepitopes were encoded by three different *TP53* mutations (p.Y220C, p.R273C, and p.Q331H). Overall, 76% (68 of 89) of the identified neoantigens were recognized by CD4+ T lymphocytes (for 6

mRNA-4157 (V940)

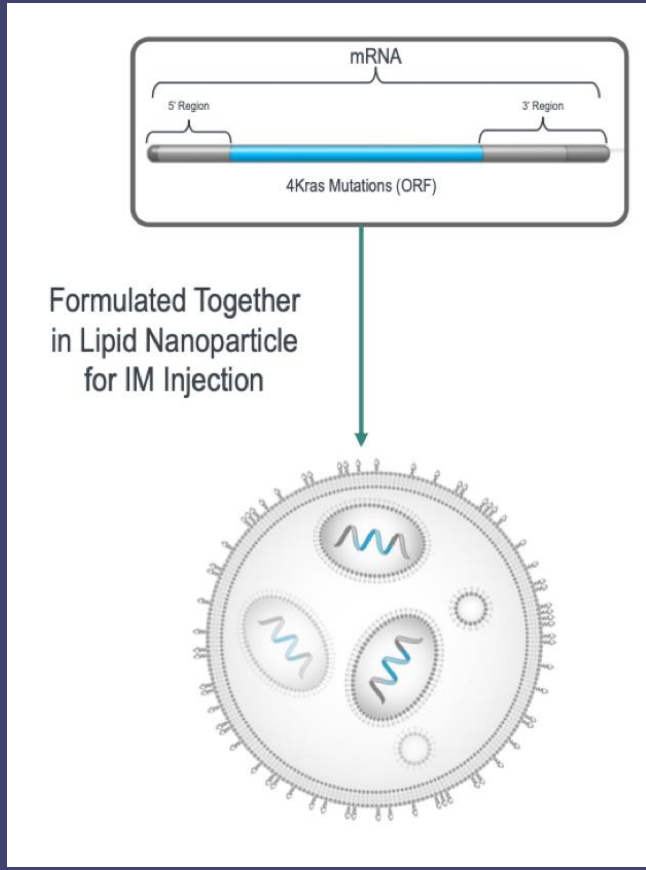
- Cells from the patient's tumor are analyzed, and genetic sequencing is used to identify neoantigen epitopes that may elicit the strongest immune response in the patient.
- The sequences encoding the patient-specific epitopes are transcribed and loaded onto a single mRNA molecule.



Merck

mRNA-4157 (V940)

- Upon administration, mRNA-4157 is taken up and translated by antigen presenting cells (APCs).
- Then, the expressed epitopes are presented via MHC molecules on the surface of the APCs.
- Induces cytotoxic T-lymphocyte- and memory T-cell-dependent immune responses that specifically target and destroy the patient's cancer cells that express these neoantigens.



1. NCI Drug Dictionary. mRNA-4157. Accessed June 2021. 2. Bauman JE et al. Presented at SITC 2020.

NCT03897881 KEYNOTE-942: Study Design

Glossary

Objectives: Phase 2, randomized, open-label study to assess whether postoperative adjuvant therapy with mRNA-4157 (V940) and pembrolizumab improves RFS compared to pembrolizumab alone in patients with complete resection of cutaneous melanoma and a high risk of recurrence[1-4]

Key Eligibility Criteria

- Stage IIIB,[a] IIIC, IIID, or IV resectable cutaneous melanoma metastatic to a lymph node and at high risk of recurrence
- Complete surgical resection within 13 weeks prior to first pembrolizumab dose
- Disease free at study entry (after surgery) with no loco-regional relapse or distant metastasis and no clinical evidence of brain metastases
- Tissue available for next-generation sequencing
- ECOG PS 0 or 1
- Normal organ and marrow function reported at screening

R 2:1

mRNA-4157 (V940) 1 mg IM Q3W (up to 9 doses) + Pembrolizumab 200 mg IV Q3W (up to 18 cycles)[b] (n = 107)

Pembrolizumab 200 mg IV Q3W x 18 cycles (n = 50)

Until disease recurrence, unacceptable toxicity, or up to approximately 1 year of treatment

RFS follow-up: up to 3 years following the first dose of pembrolizumab

Stratification Factor

- Disease stage (per AJCC 8th edition)

Primary Endpoint

- RFS[c] (ITT population)

Secondary Endpoints

- DMFS[d]
- Safety and tolerability

- Median follow-up (Data cut off: November 14, 2022): 23 months for mRNA-4157 (V940) + pembrolizumab and 24 months for pembrolizumab only
- The study had 80% power to detect an HR of 0.5 with ≥40 RFS events (with a 1-sided alpha of 0.1)

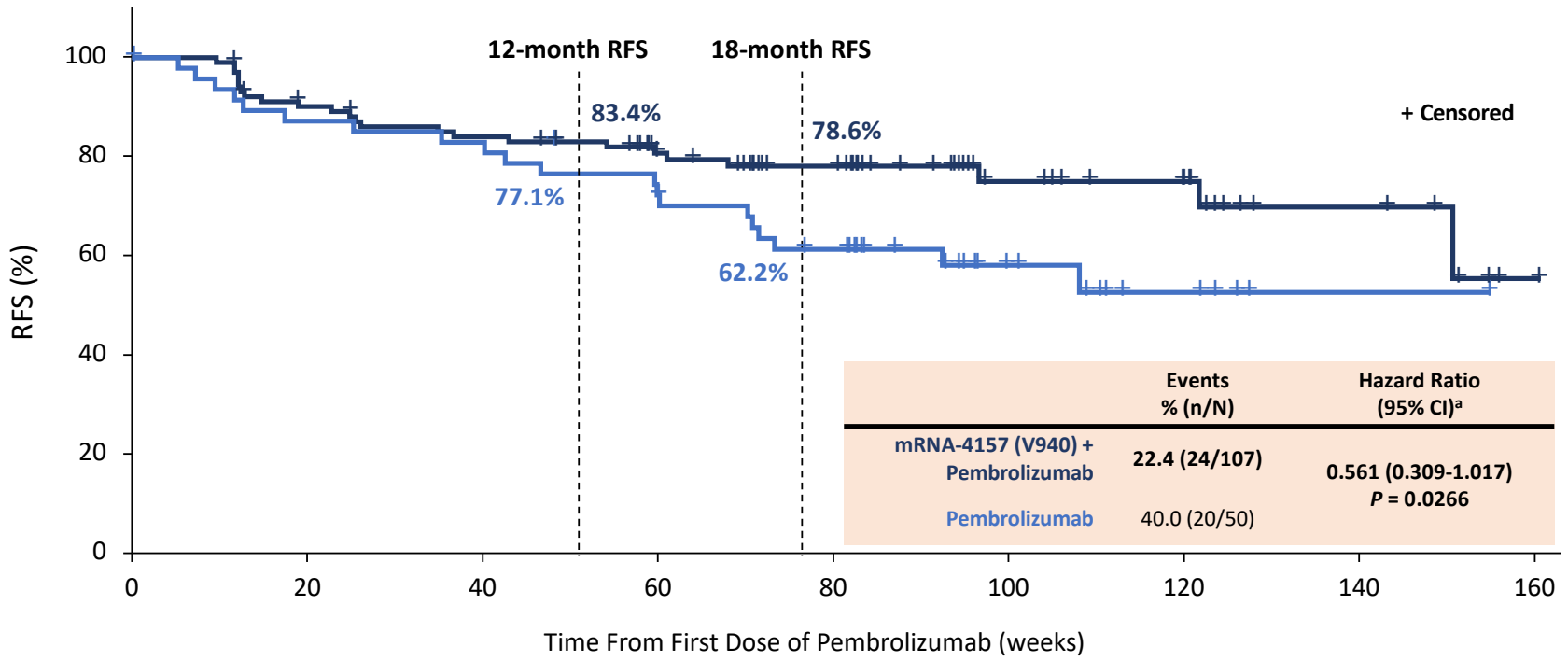
[a]Patients with Stage IIIB disease were eligible only if relapse occurred within 3 months of prior surgery of curative intent [b]Patients assigned to the combination arm received pembrolizumab 200 mg IV (typically two 3-week cycles) **while mRNA-4157 is being manufactured**. The combination treatment period began upon availability of mRNA-4157 (V940). The first dose of mRNA-4157 (V940) was administered with the next dose of pembrolizumab to achieve synchronous combination dosing in 21-day cycles. Typically, the first dose of mRNA-4157 (V940) was administered with the third dose of pembrolizumab but the first dose of mRNA-4157 (V940) may also have been delayed until the fourth or fifth dose of pembrolizumab.[1] [c]Investigator-assessed RFS was defined as the time from first dose of pembrolizumab until the date of first recurrence (local, regional, or distant metastasis), a new primary melanoma, or death from any cause in the ITT population. [d]Investigator assessed DMFS was defined as the time from first dose of pembrolizumab until the date of first distant recurrence or death from any cause. DMFS analysis was prespecified for testing following positive RFS in the ITT population.

1. ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT03897881. Accessed May 30, 2023. 2. Khattak A, et al. Presented at AACR 2023. 3. Merck. Data on file. 4. Khattak A, et al. Presented at ASCO 2023.

NCT03897881 KEYNOTE-942: RFS (ITT Population)[1,2]

Glossary

Primary Endpoint



	Events % (n/N)	Hazard Ratio (95% CI)[a]
mRNA-4157 (V940) + Pembrolizumab	22.4 (24/107)	0.561 (0.309-1.017) *P* = 0.0266
Pembrolizumab	40.0 (20/50)	

Number at Risk

mRNA-4157 (V940) + Pembrolizumab	107	92	85	73	49	24	20	8	1
Pembrolizumab	50	42	40	37	28	13	6	1	0

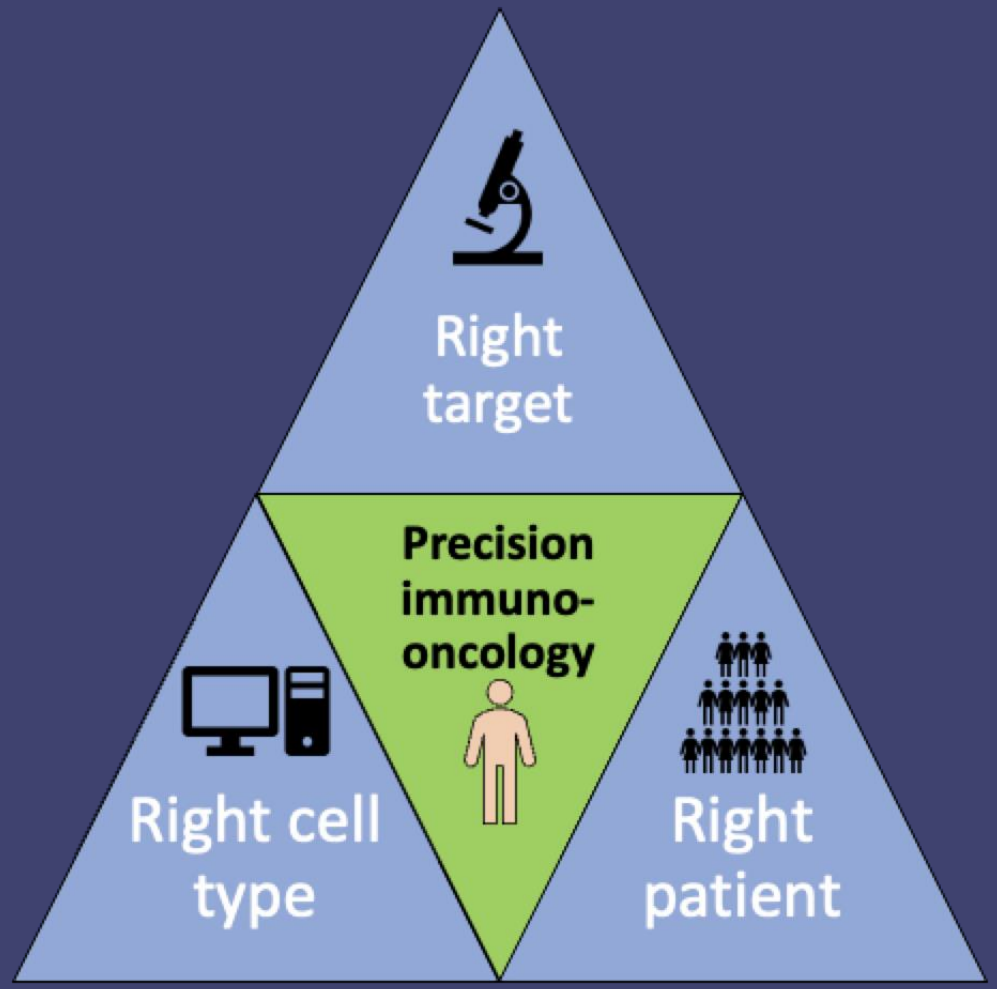
Median follow up: 23 months (combination arm); 24 months (monotherapy arm). Data cutoff: November 14, 2022.

[a]The hazard ratio and 95% CI for mRNA-4157 (V940) plus pembrolizumab versus pembrolizumab is estimated using a Cox proportional hazards model with treatment group as a covariate, stratified by disease stage (stages IIIB or IIIC or IIID vs stage IV) used for randomization. The *P* value is based on a 1-sided log-rank test stratified by disease stage (stages IIIB or IIIC or IIID vs stage IV) used for randomization.

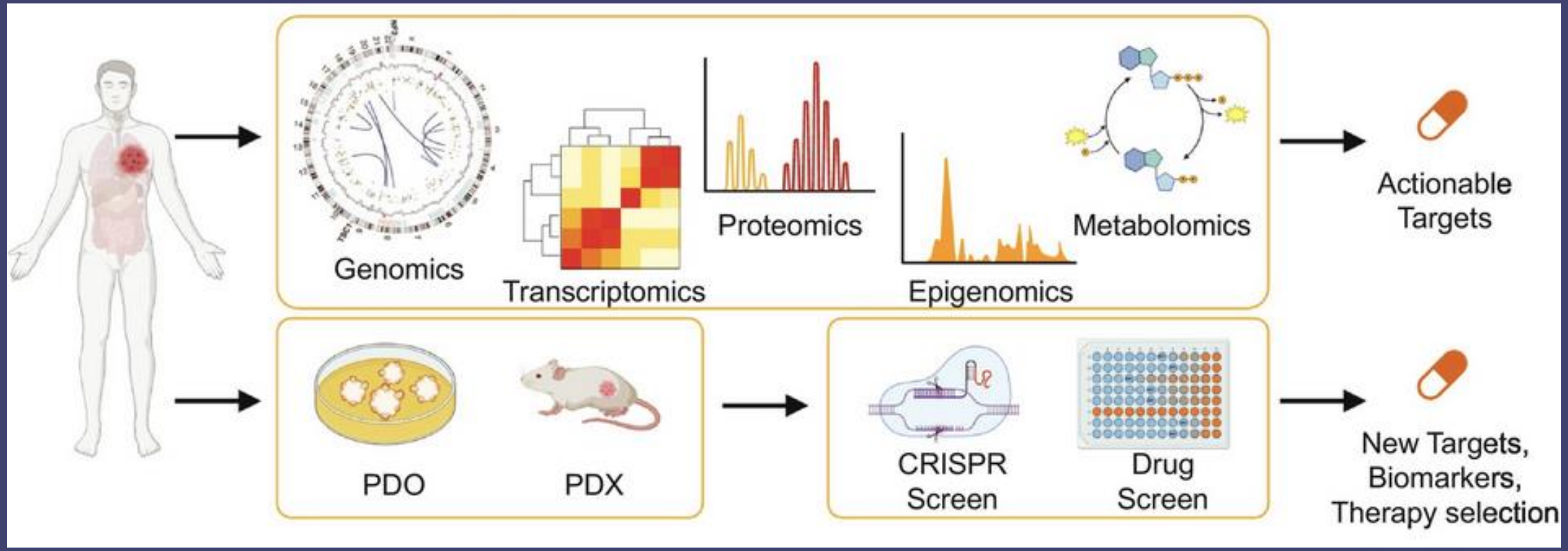
1. Khattak A, et al. Presented at AACR 2023. 2. Khattak A, et al. Presented at ASCO 2023.

This information concerns investigational products and/or investigational uses of approved products, the safety and effectiveness of which have not been

Can we capture all aspects of precision-oncology?

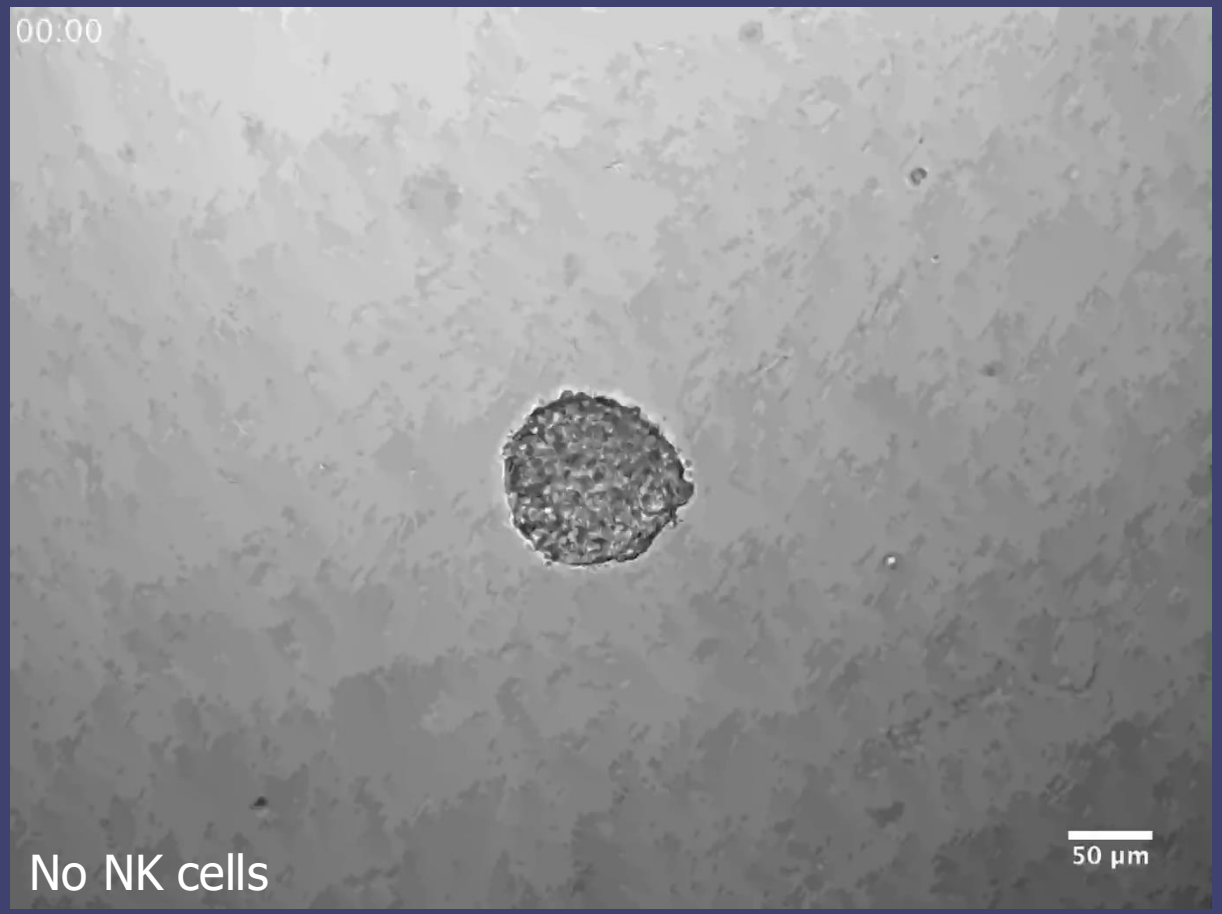


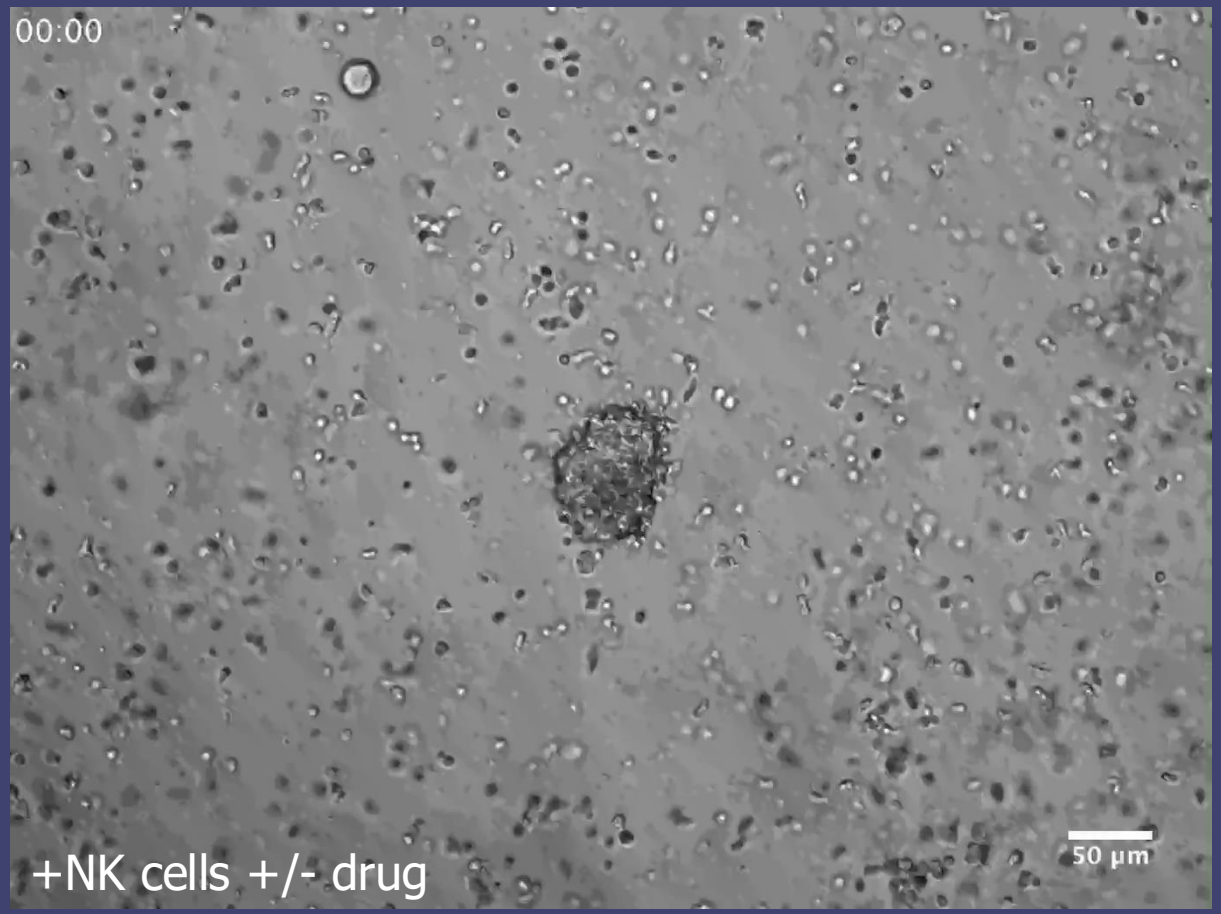
Is more data better for precision oncology?



Tang, Trends in Can., 2024

A platform to model NK cell-cancer cell interactions





Chan et. al., Journal of Cell Biology, 2020; Chan and Ewald, Methods in Molecular Biology, 2022; Cornelius et. al.,..Chan, JoVE, 2022; Lake et. al.,..Chan, Front. Dev. Biol., 2024

Are 43 proteins enough?

Article

Spatial predictors of immunotherapy response in triple-negative breast cancer

https://doi.org/10.1038/s41586-023-06498-3

Received: 28 June 2022

Accepted: 28 July 2023

Published online: 06 September 2023

Open access

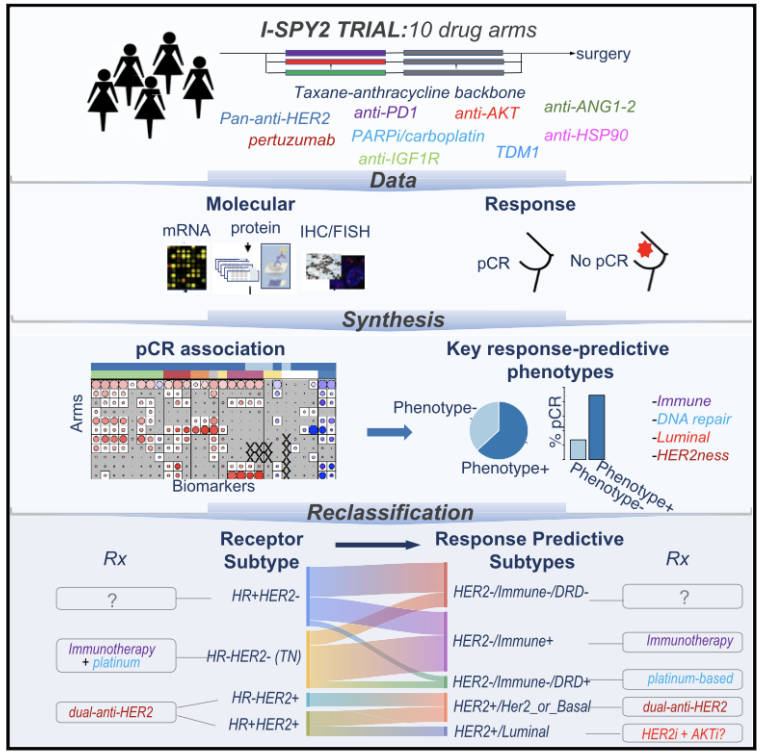
Xiao Qian Wang[1], Esther Danenberg[1], Chiun-Sheng Huang[2], Daniel Egle[3], Maurizio Callari[4], Begoña Bermejo[5,6,7], Matteo Dugo[8], Claudio Zamagni[9], Marc Thill[10], Anton Anton[11], Stefania Zambelli[8], Stefania Russo[12], Eva Maria Ciruelos[13], Richard Greil[14,15,16], Balázs Győrffy[17,18], Vladimir Semiglazov[19], Marco Colleoni[20], Catherine M. Kelly[21], Gabriella Mariani[22], Lucia Del Mastro[23,24], Olivia Biasi[20], Robert S. Seitz[25], Pinuccia Valagussa[4], Giuseppe Viale[20,26], Luca Gianni[4,28], Giampaolo Bianchini[4,8,28] ✉ & H. Raza Ali[1,27,28] ✉

tumours early on-treatment. We used imaging mass cytometry[3] to profile the in situ expression of 43 proteins in tumours from patients in a randomized trial of neoadjuvant ICB, sampled at three timepoints (baseline, $n = 243$; early on-treatment, $n = 207$; post-treatment, $n = 210$). Multivariate modelling showed that the fractions of proliferating $CD8^+TCF1^+$ T cells and $MHCII^+$ cancer cells were dominant predictors of response, followed by cancer–immune interactions with B cells and granzyme B^+ T cells. On-treatment, responsive tumours contained abundant granzyme B^+ T cells,

Incorporate multiple gene signatures into prediction

Redefining breast cancer subtypes to guide treatment prioritization and maximize response: Predictive biomarkers across 10 cancer therapies

Graphical abstract



Authors

Denise M. Wolf, Christina Yau, Julia Wulfkuhle, ..., Gillian L. Hirst, Laura J. Esserman, Laura J. van 't Veer

Correspondence

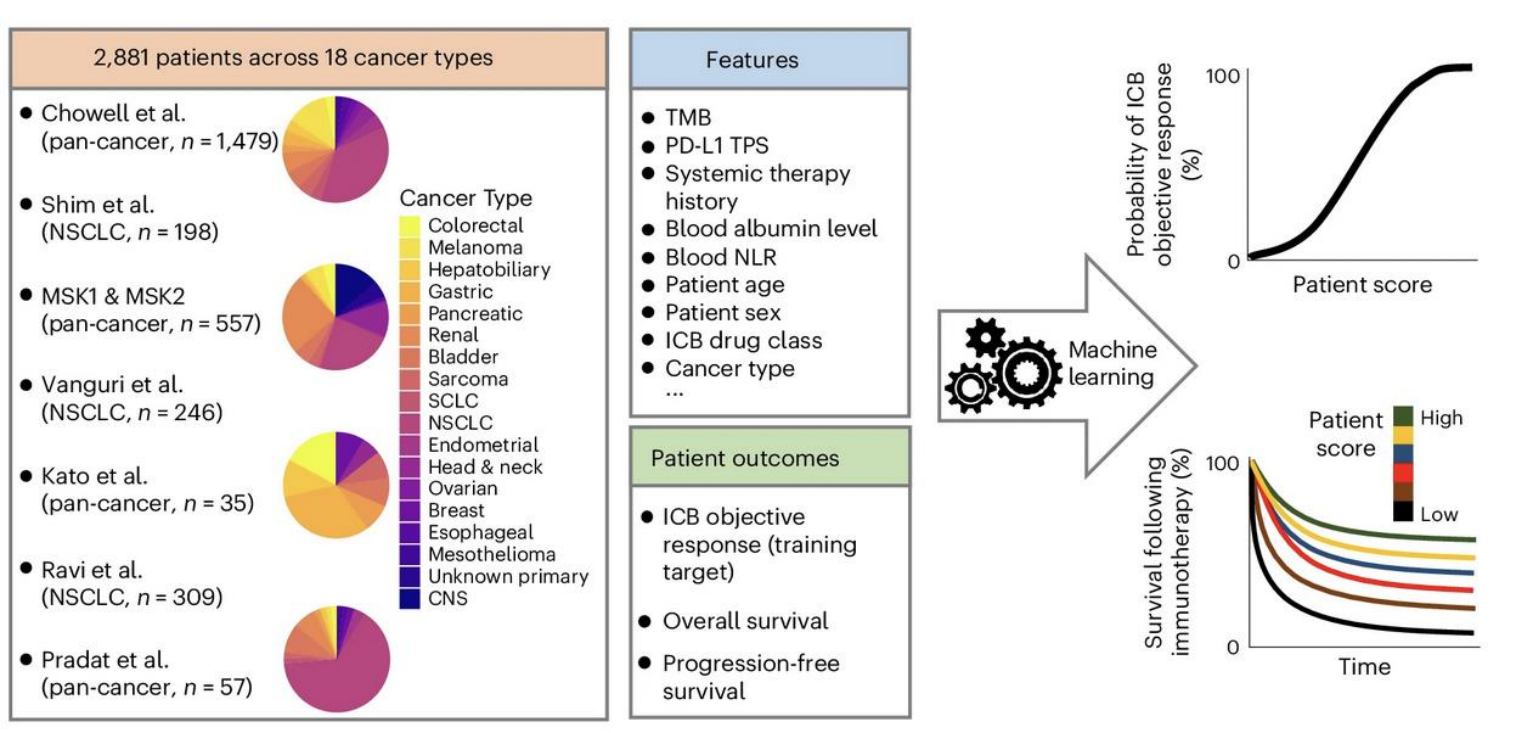
denise.wolf@ucsf.edu (D.M.W.), cyau@buckinstitute.org (C.Y.), laura.vantveer@ucsf.edu (L.J.v.V.)

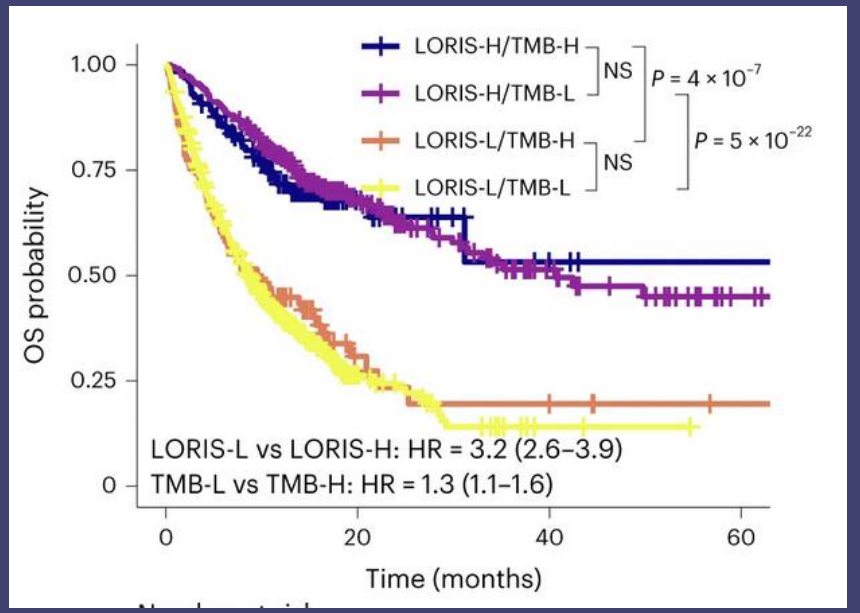
In brief

Wolf et al. use gene expression, protein levels, and response data from 10 drug arms of the I-SPY2 neoadjuvant trial to create new breast cancer subtypes that incorporate tumor biology beyond clinical hormone receptor (HR) and HER2 status. Use of these response-predictive subtypes to guide treatment prioritization may improve patient outcomes.

Wolf, Cancer Cell., 2022

Interpreting multi-modal data





Chang, Nat Can., 2024

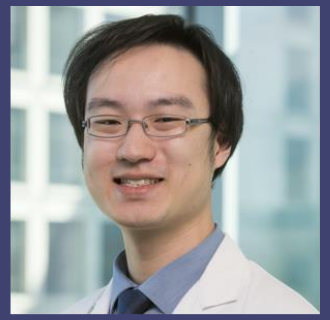
Philosophical questions

- How much complexity is required to model human tumor biology? What is *truth*?
- How accurately do these models capture temporal changes that occur within microenvironments?
- What are the key cellular and architectural components within a tumor that influences treatment response?

Systems-biology approaches for precision immuno-oncology

Lily Xu
UTSW Med Student
Chan Lab

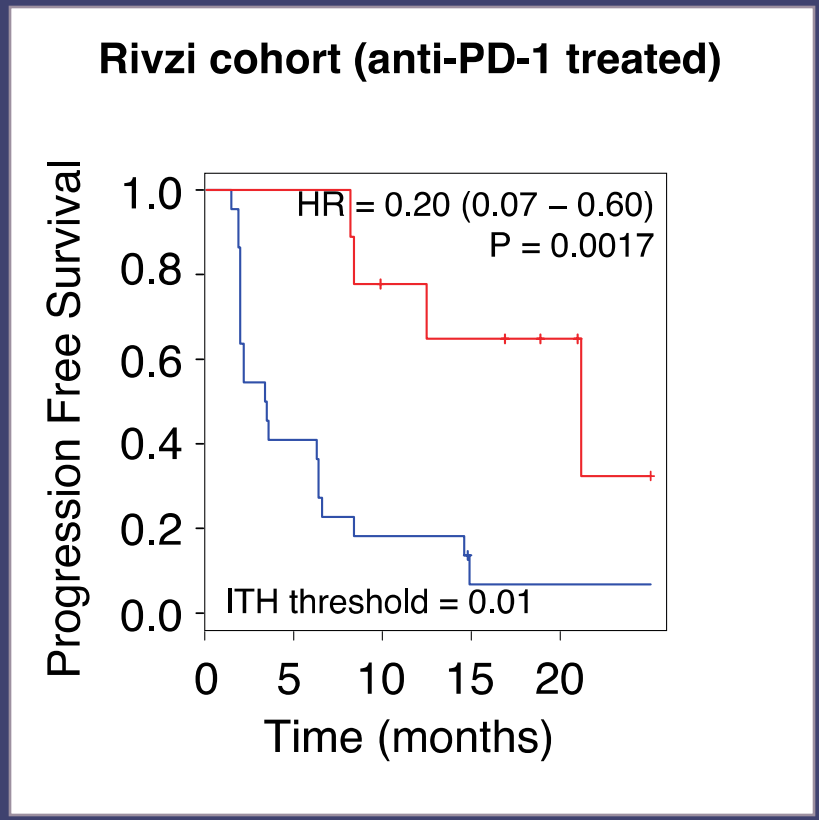
Kaitlyn Saunders
UTD Master's in Bioinformatics
Chan Lab



Shao-Po Huang
UTSW MSTP
Chan Lab

Intratumoral heterogeneity influences ICI response

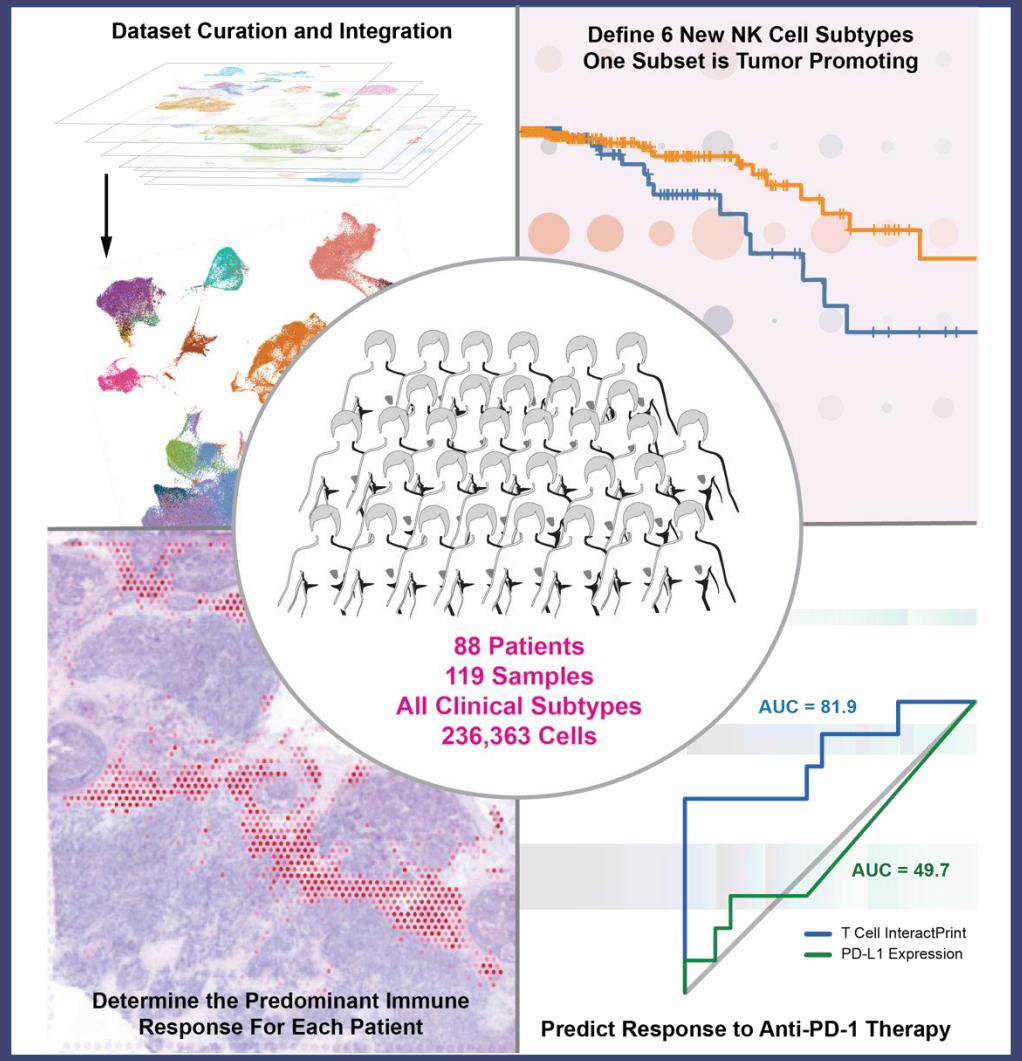
- Different immune cell types, patient selection, and intratumoral heterogeneity (ITH) influence ICI response[1].
- ITH is negatively correlated with response to ICI[2,3].
- How cancer epithelial cell heterogeneity influences immune interactions remains underexplored.

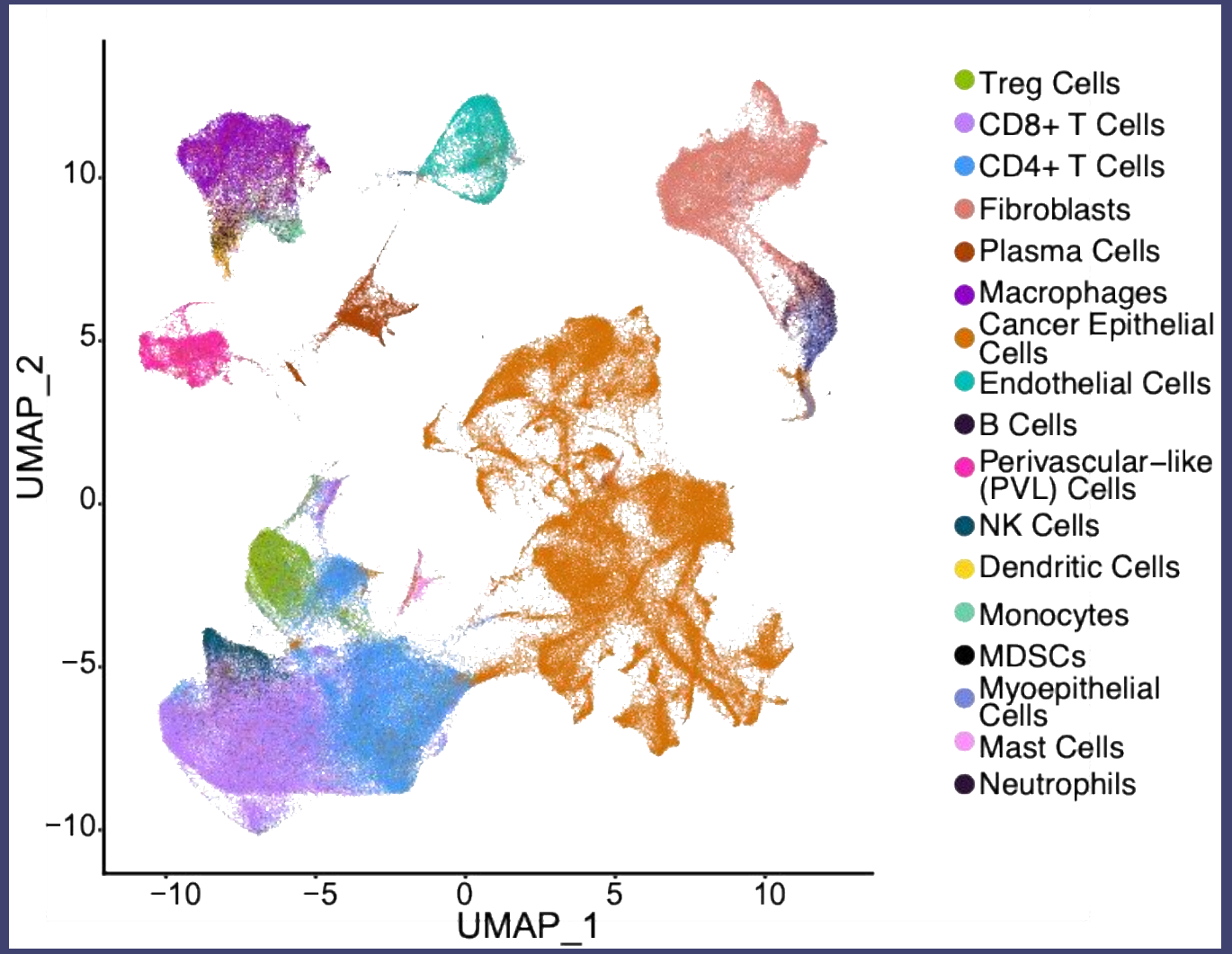


McGranahan et al., Science 2016.

[1] Cortes et al., NEJM 2022; [2] Wolf et al., Clin Cancer Res 2022; [3] McGranahan et al., Science 2016.

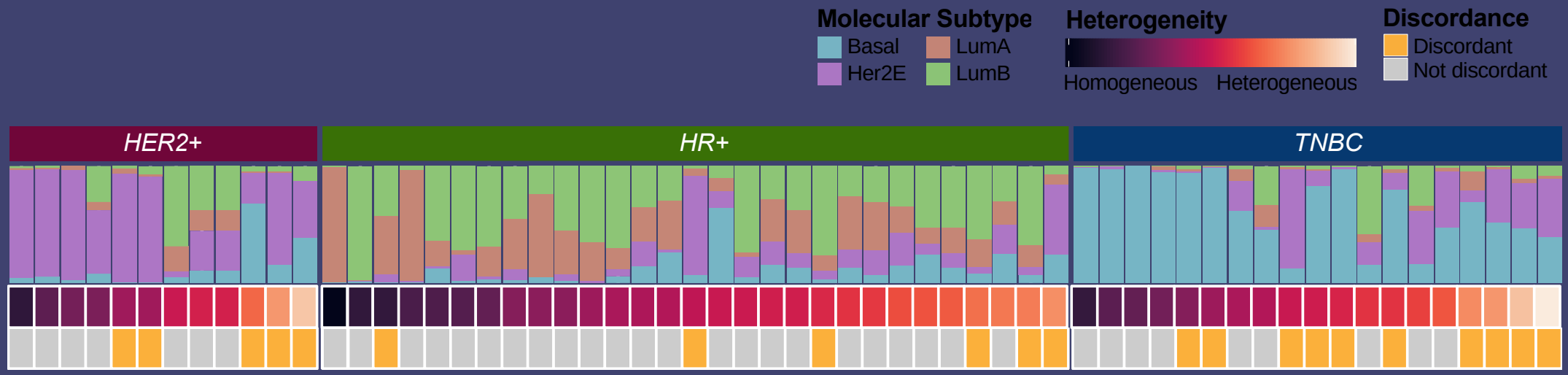
Large single-cell RNA-seq reference dataset of breast tumors





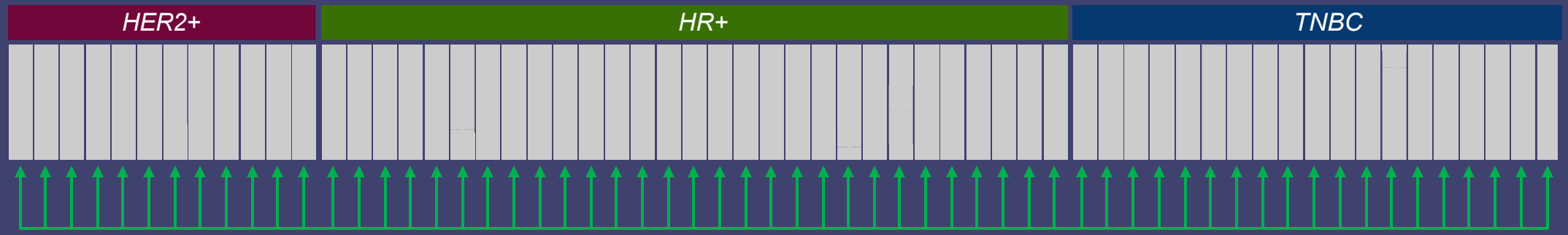
Xu, Saunders, and Huang et. al.,..Chan, Cell Reports Medicine, 2024

Cancer epithelial cell heterogeniety is driven by factors beyond traditional molecular subtypes



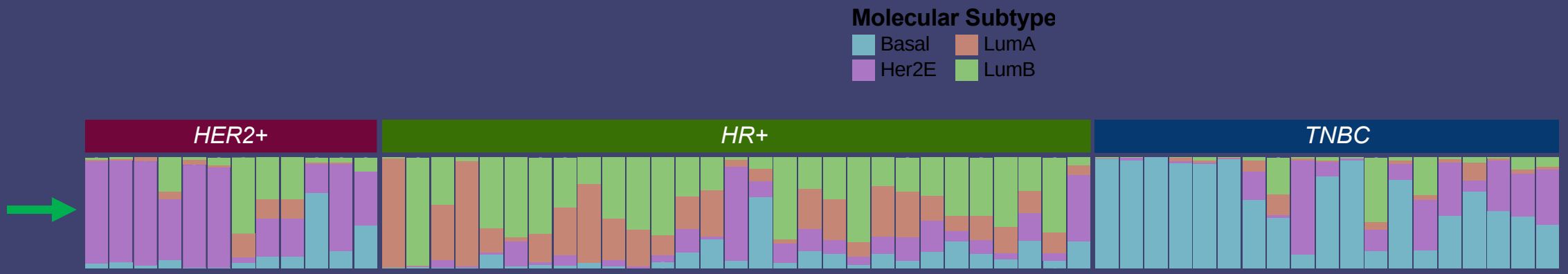
Cancer epithelial cell heterogeneity is driven by factors beyond molecular subtype

Cancer epithelial cell heterogeneiety is driven by factors beyond traditional molecular subtypes



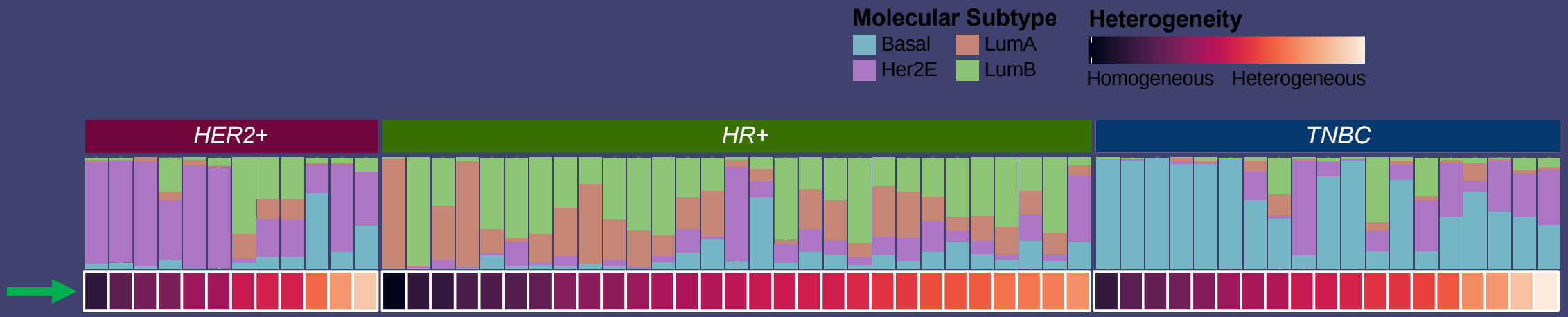
Each column is a sample

Cancer epithelial cell heterogeneiety is driven by factors beyond traditional molecular subtypes



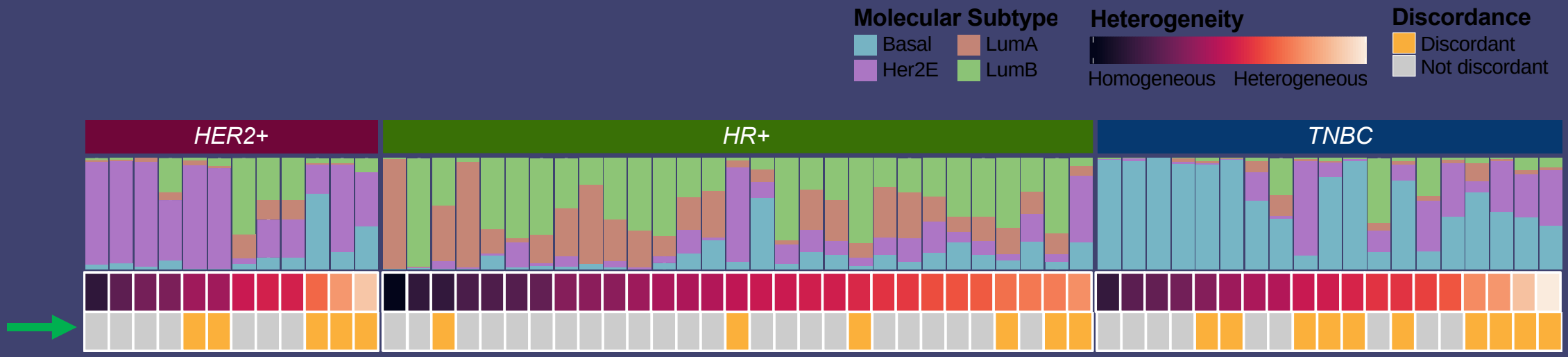
Varying degrees of molecular subtype heterogeneity exists across samples

Cancer epithelial cell heterogeneity is driven by factors beyond traditional molecular subtypes



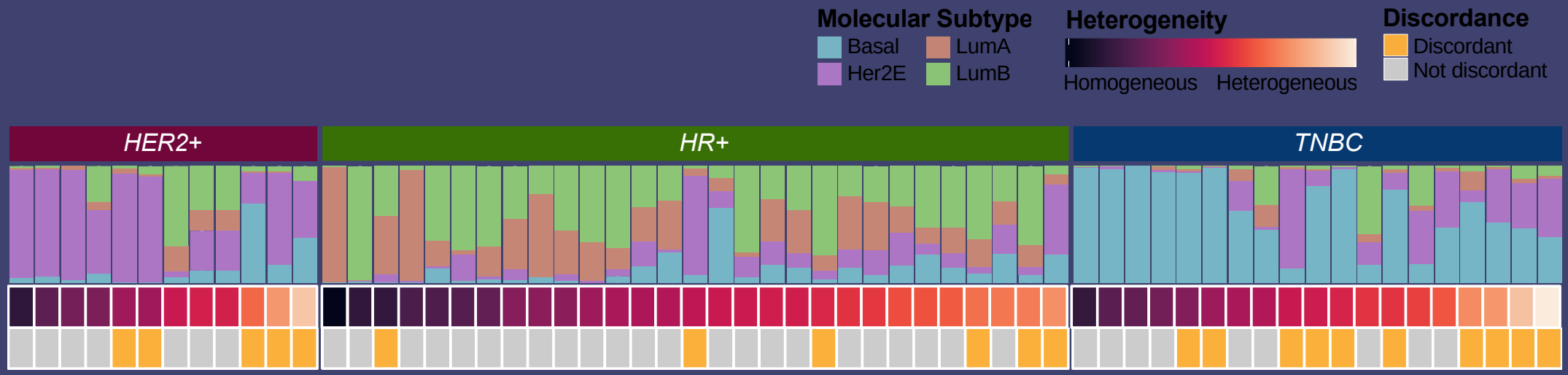
Single-cell transcriptional heterogeneity varies across samples

Cancer epithelial cell heterogeneity is driven by factors beyond traditional molecular subtypes



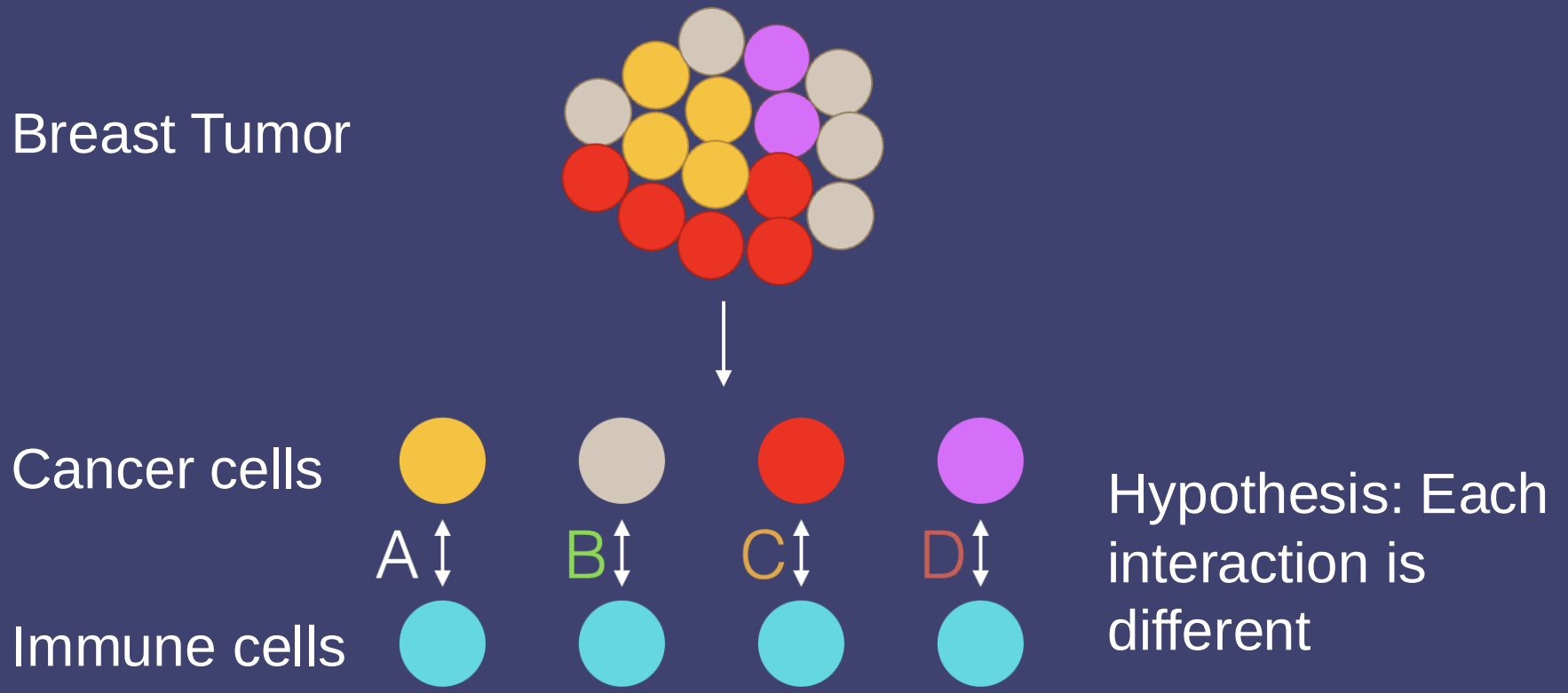
Discordance between molecular subtypes and single-cell transcriptional heterogeneity occurs in approximately 33% of samples

Cancer epithelial cell heterogeniety is driven by factors beyond traditional molecular subtypes



Cancer epithelial cell heterogeneity is driven by factors beyond molecular subtype

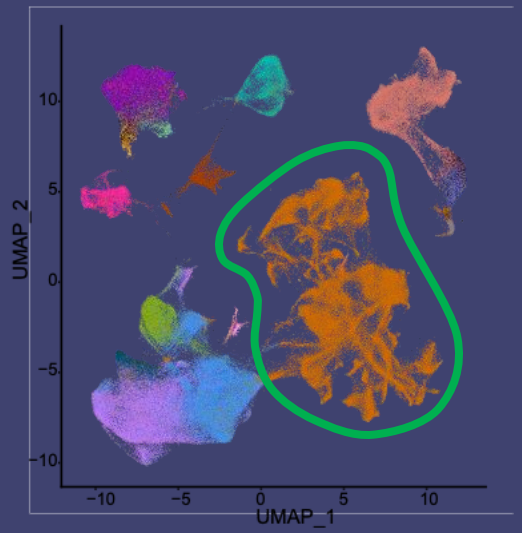
How does cancer epithelial cell heterogeneity influence immune interactions?

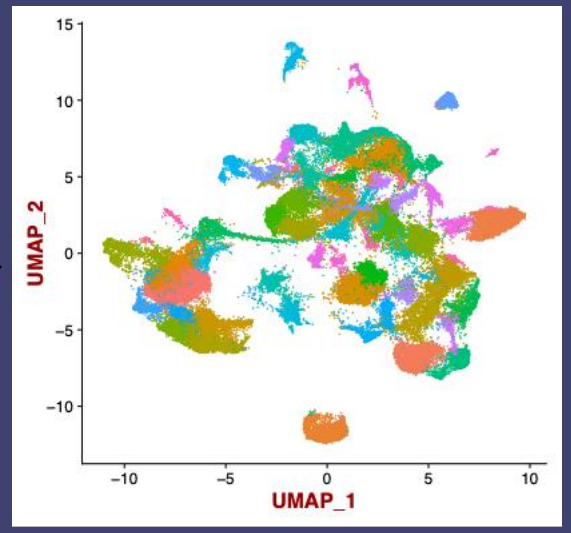


Hypothesis: Each interaction is different

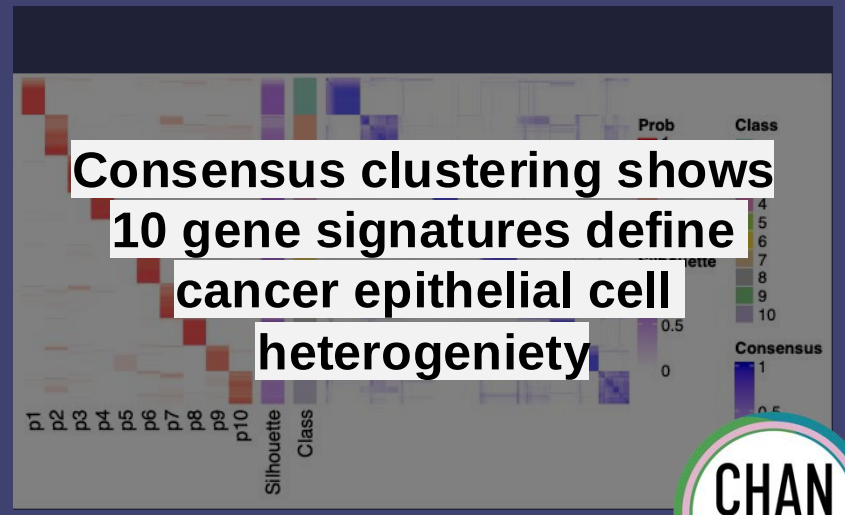
Cancer epithelial cell heterogeneity can be defined by 10 gene signatures

Leveraging this dataset, we generated an exhaustive collection of 10 gene signatures that reflect molecular features of different cancer epithelial cell clusters.



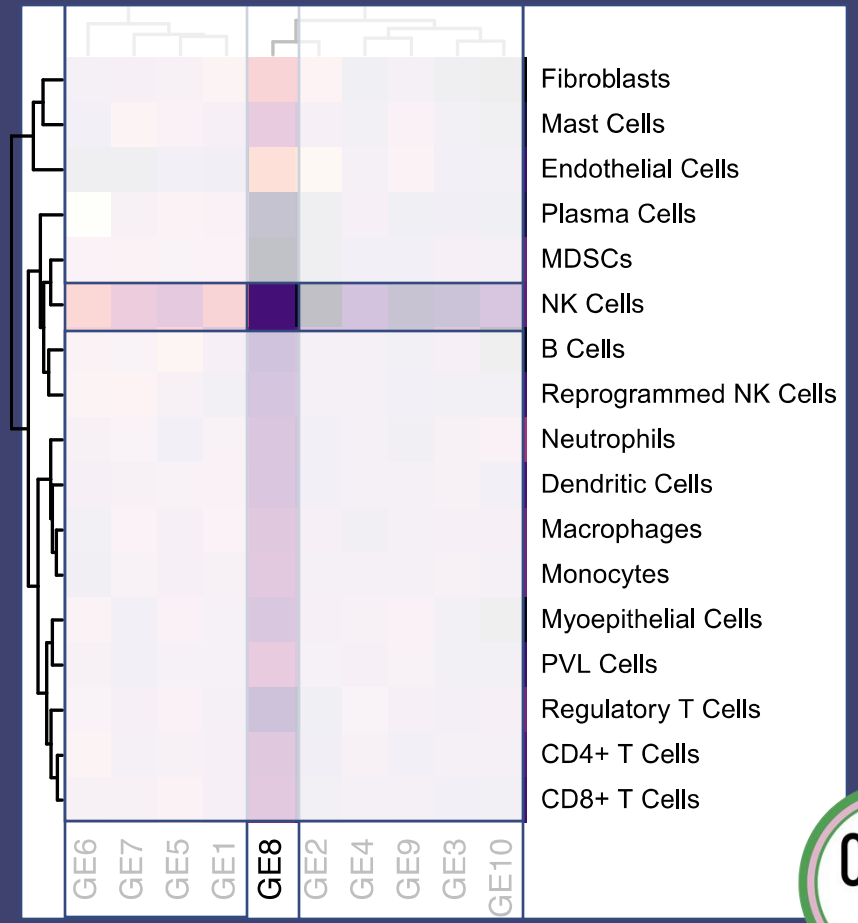


Unsupervised + supervised clustering analysis



Created a ‘decoder’ to predict cancer cell-immune interactions based on cancer epithelial cell heterogeneity

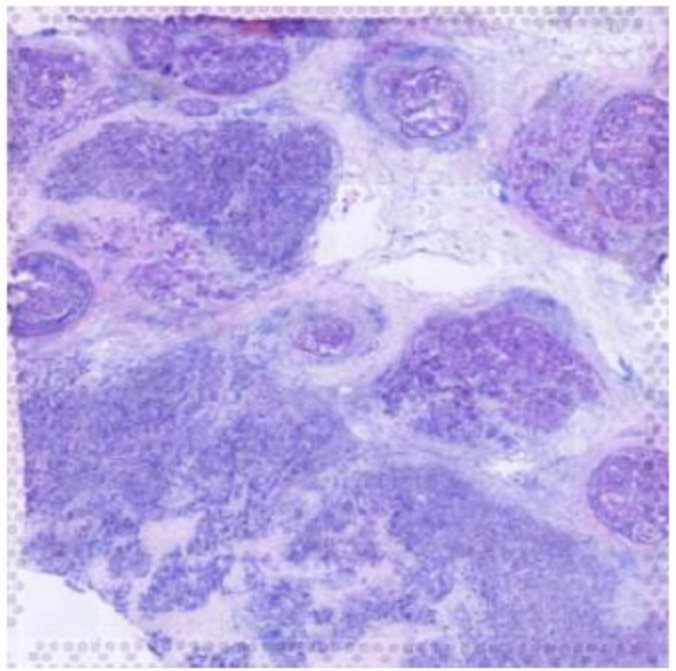
- Heterogenous breast cancer cells can be defined into 10 GEs.
- We predict immune interactions for each GE.
- GEs 1, 5, and 6 are predicted to be most interactive with T cells and NK cells
- Validated experimentally and using spatial transcriptomics.



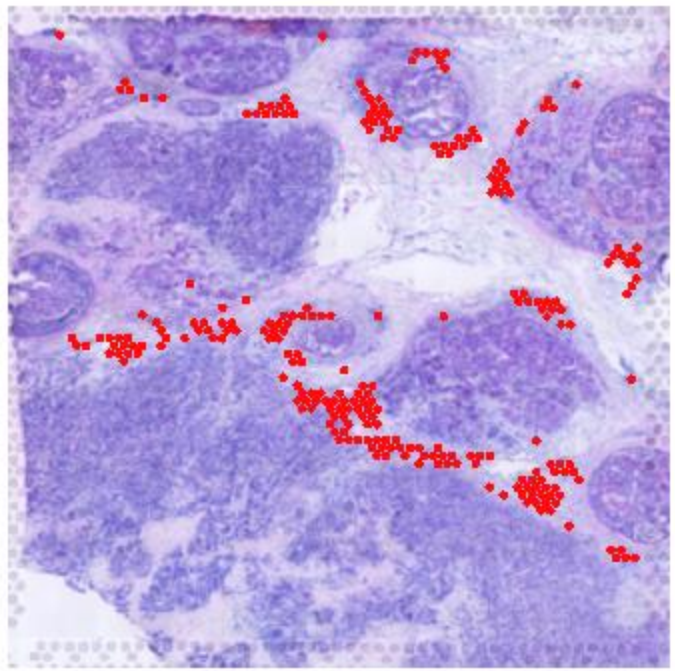
Spatial mapping of cancer epithelial cells validates predicted interactions

- Areas with **elevated GE5 expression were enriched for CD8+ T cells.**

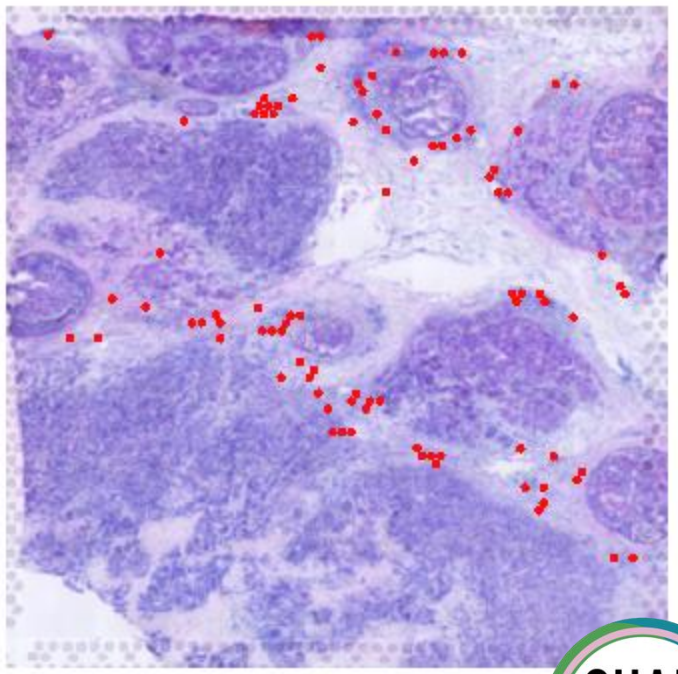
H&E



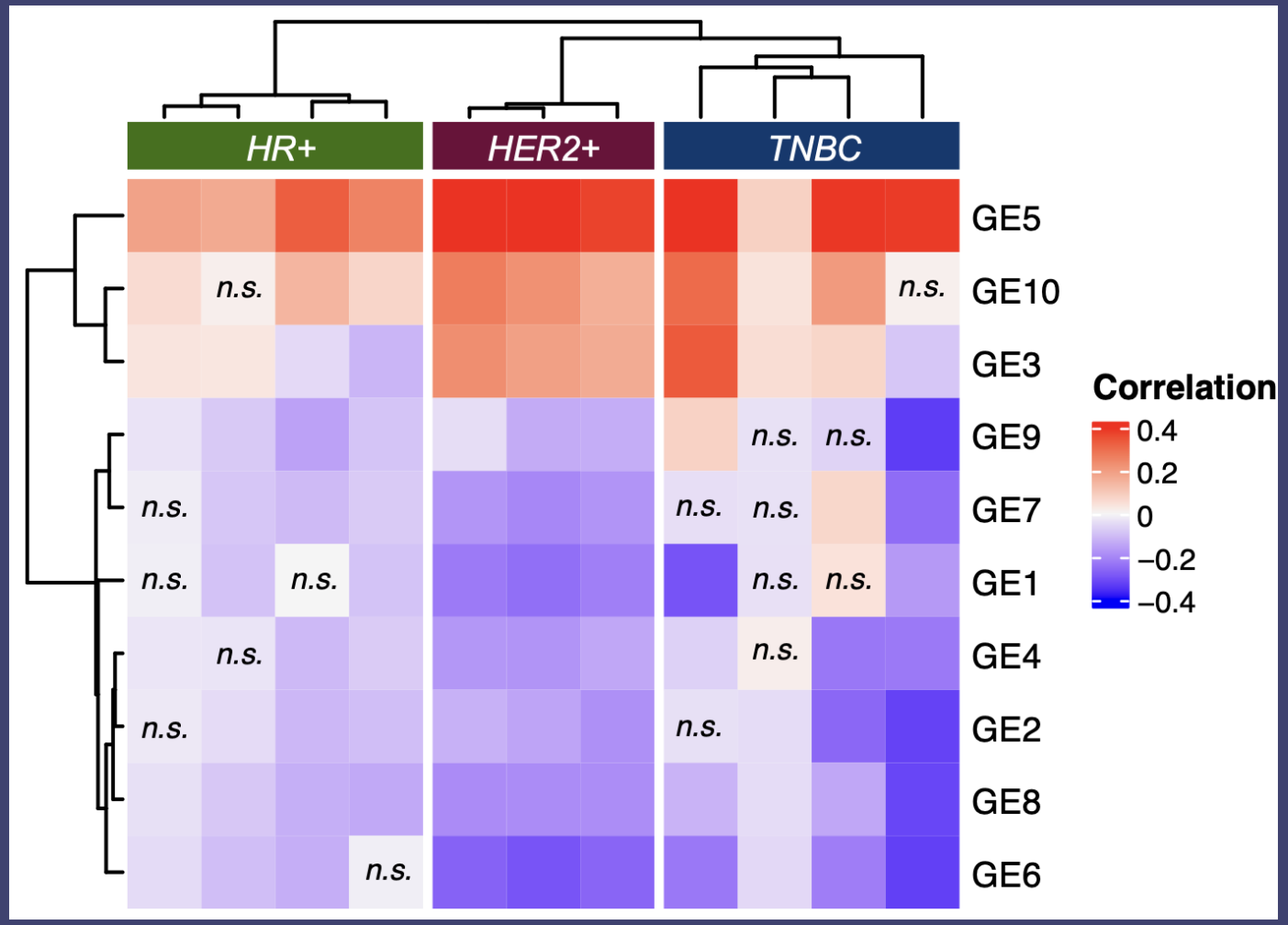
Areas with GE-5 labeled cells



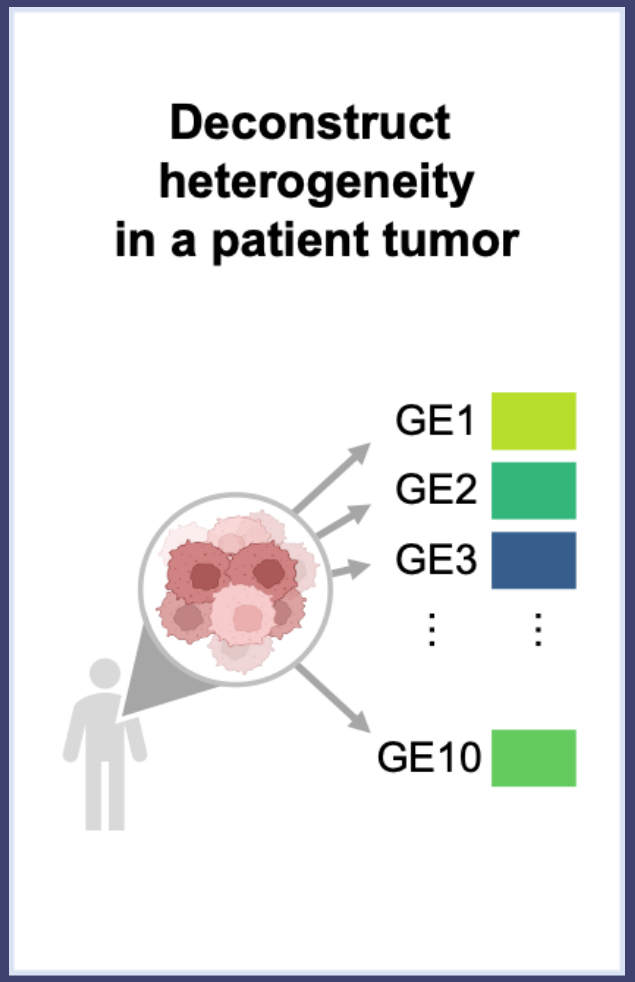
Areas with CD8+ T cells



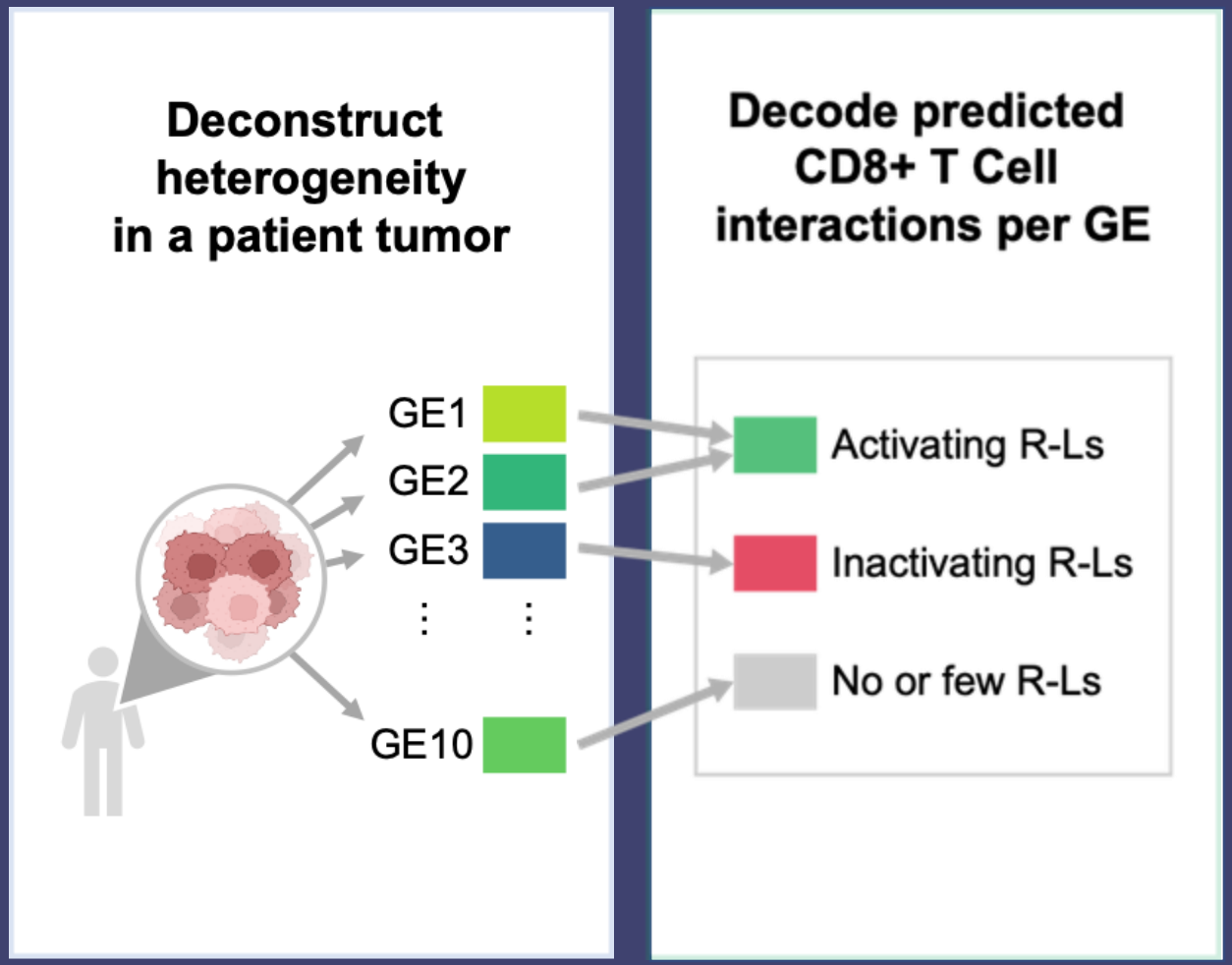
Spatial mapping of cancer epithelial cells validates predicted interactions



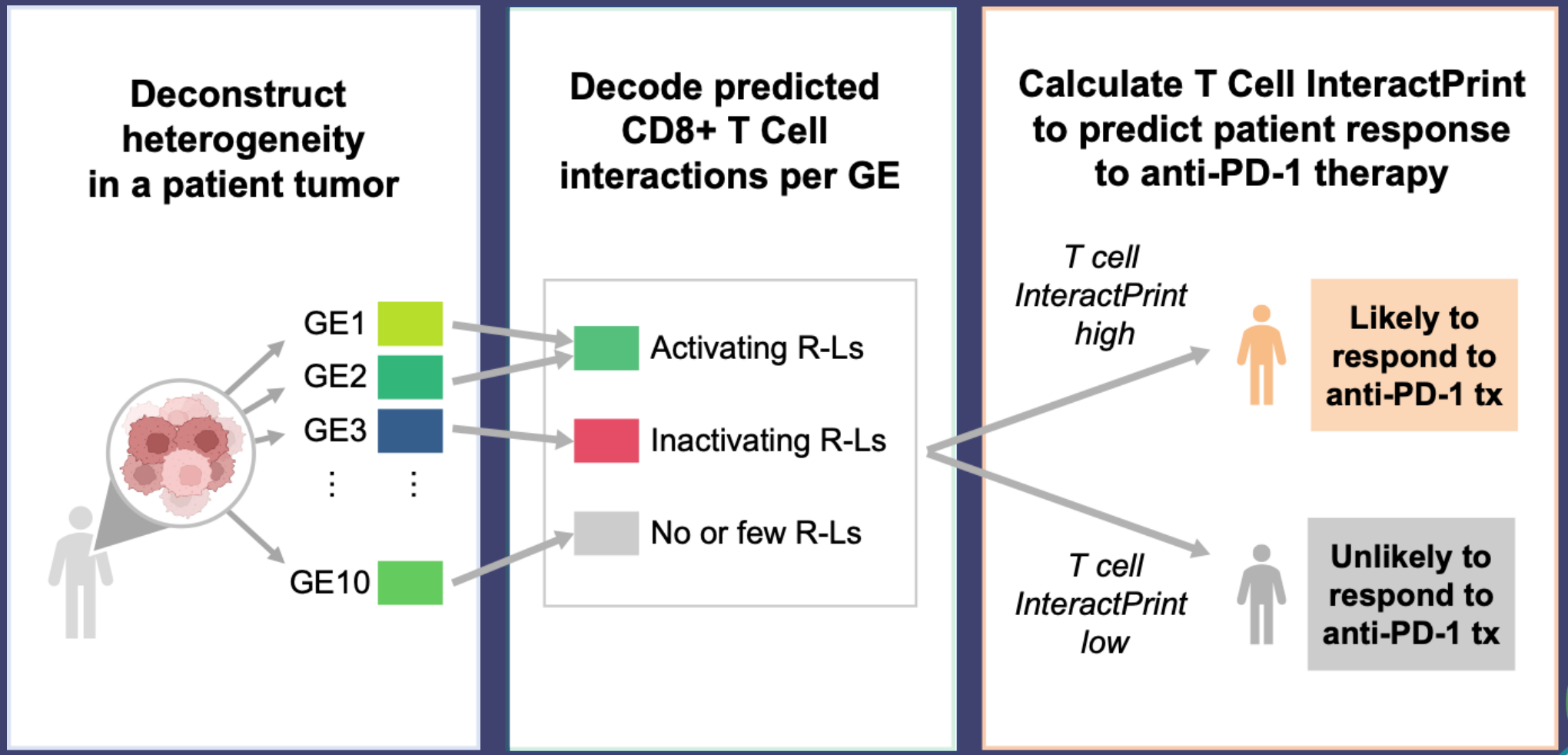
Applying InteractPrint to CD8+ T cells to predict response to anti-PD-1 therapy



Applying InteractPrint to CD8+ T cells to predict response to anti-PD-1 therapy

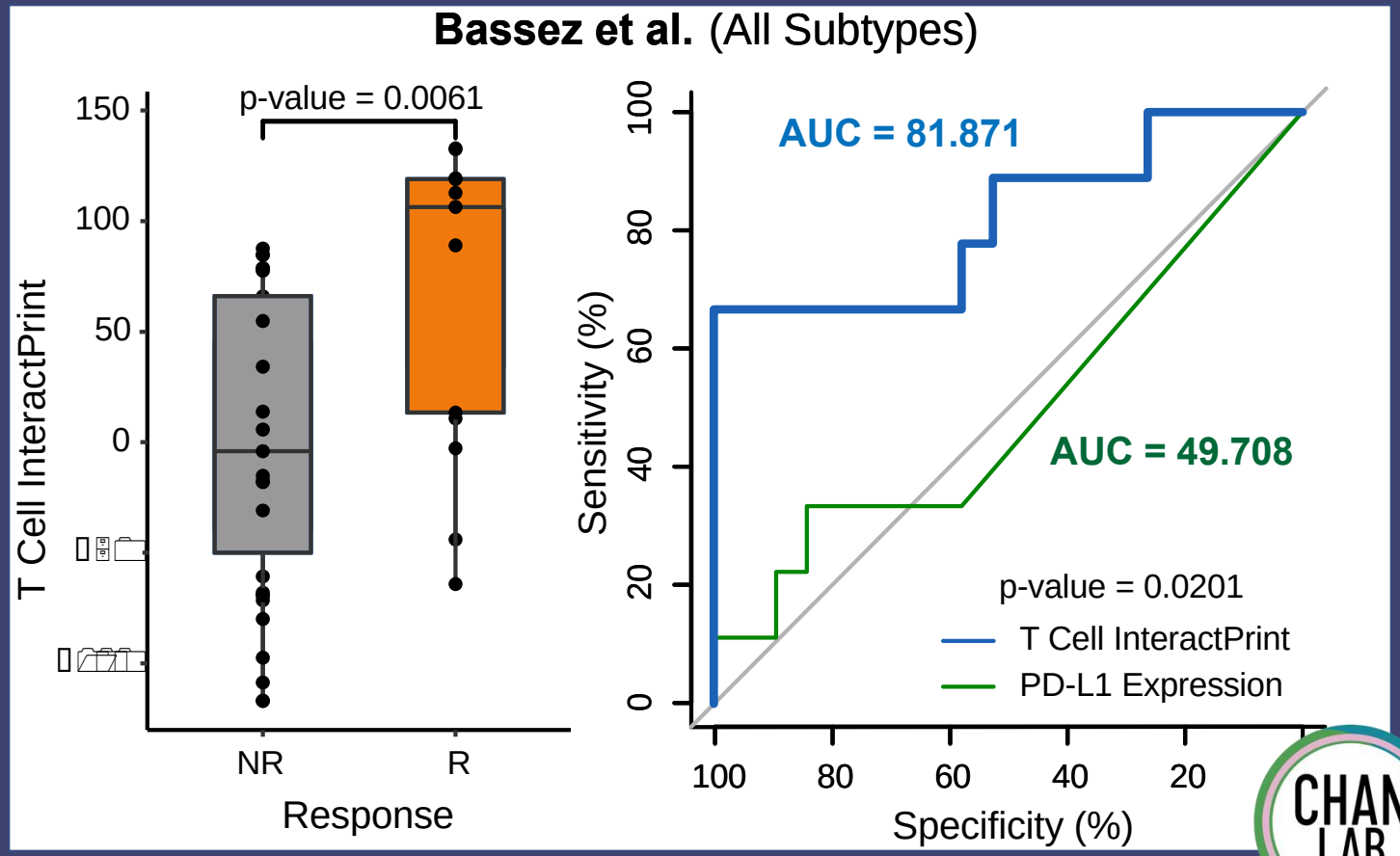


Applying InteractPrint to CD8+ T cells to predict response to anti-PD-1 therapy



T Cell InteractPrint predicts response in pembrolizumab-treated primary breast tumors

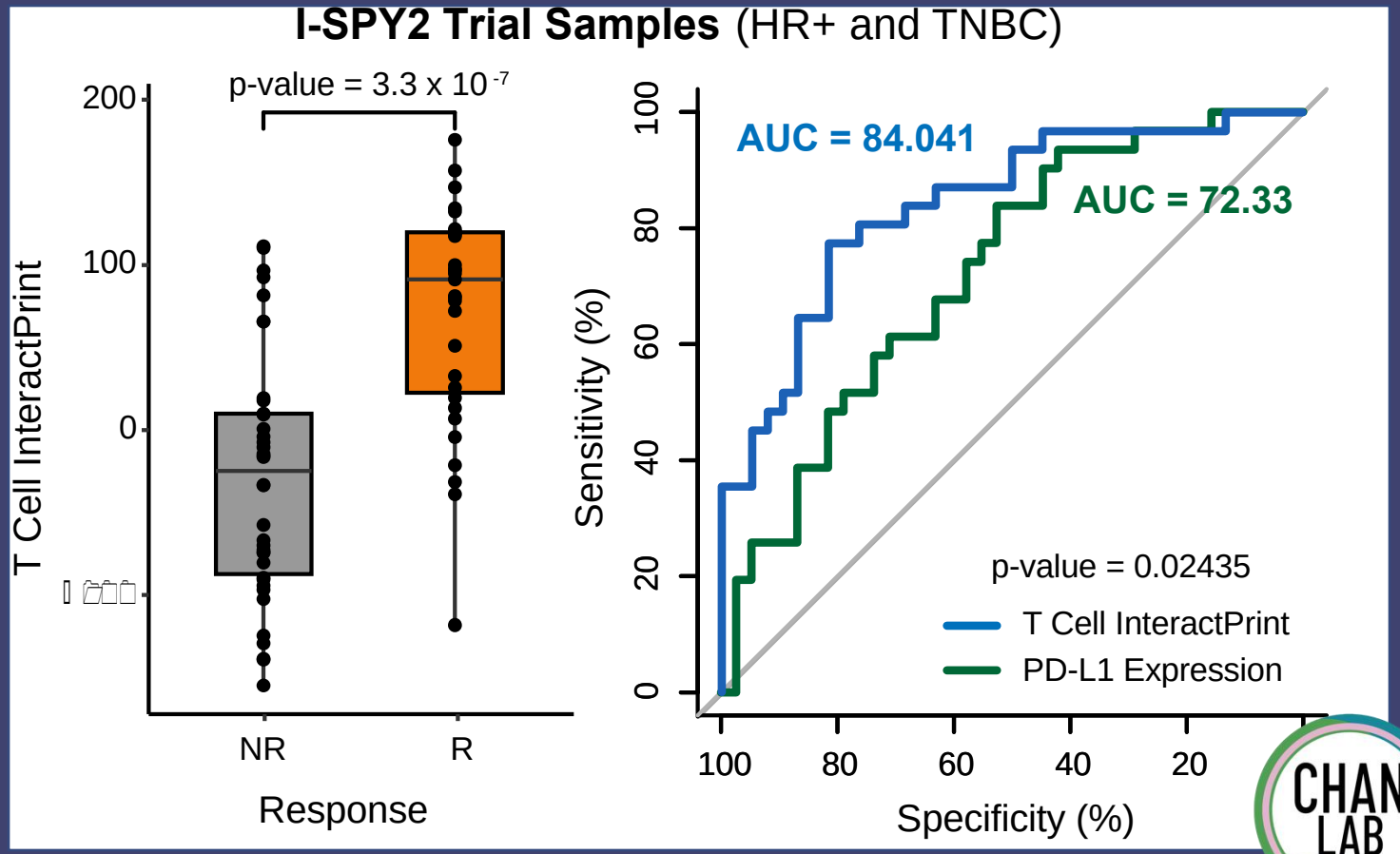
- In this trial, T Cell InteractPrint predicted response to anti-PD-1 therapy with an AUC of 81.9 ($p < 0.01$).



[4] Bassez et al., Nat Med 2021.

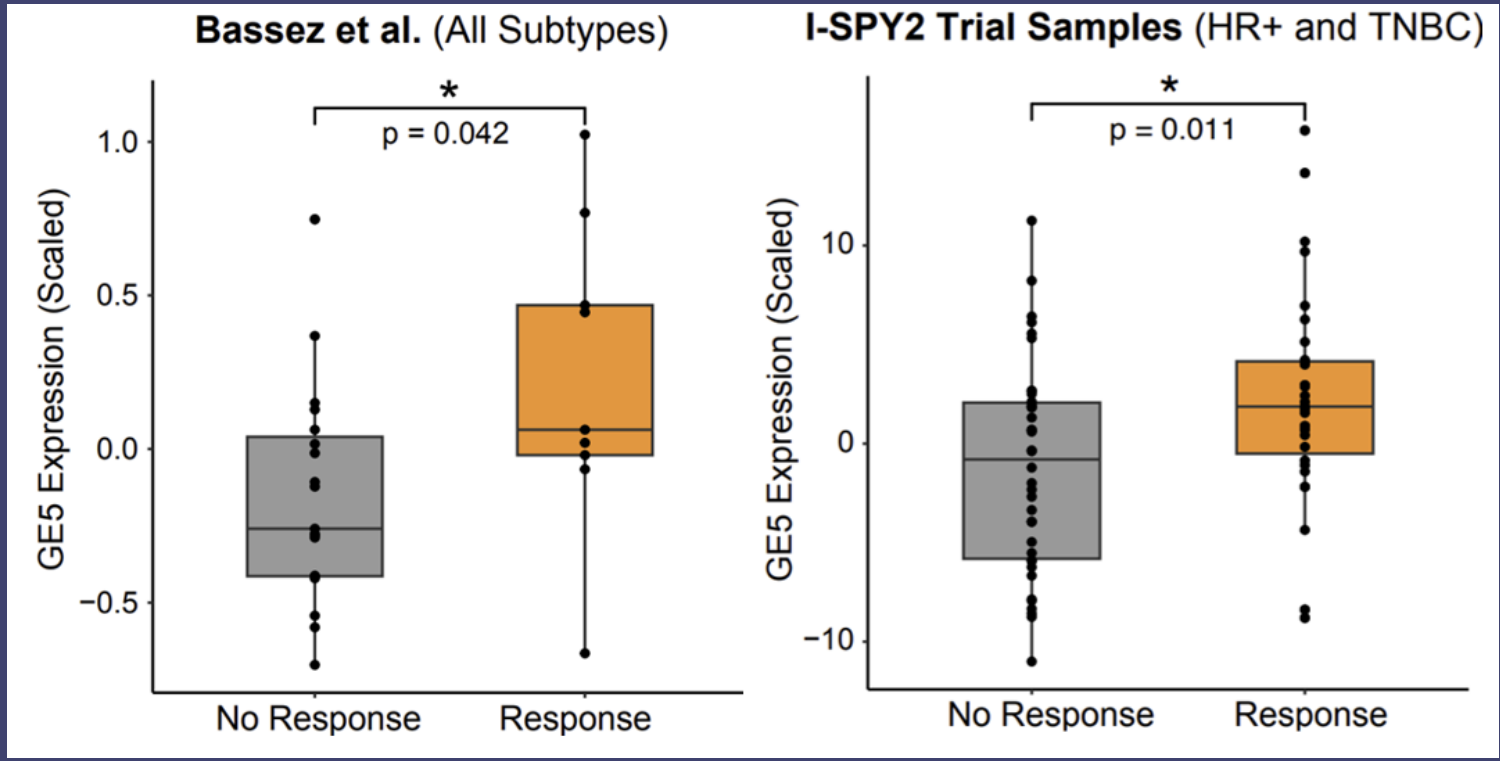
T Cell InteractPrint predicts response to anti-PD-1 therapy in I-SPY2

- In I-SPY2, T Cell InteractPrint predicted response to anti-PD-1 + neoadjuvant chemo with an AUC of 84.0 ($p < 1 \times 10^{-6}$).

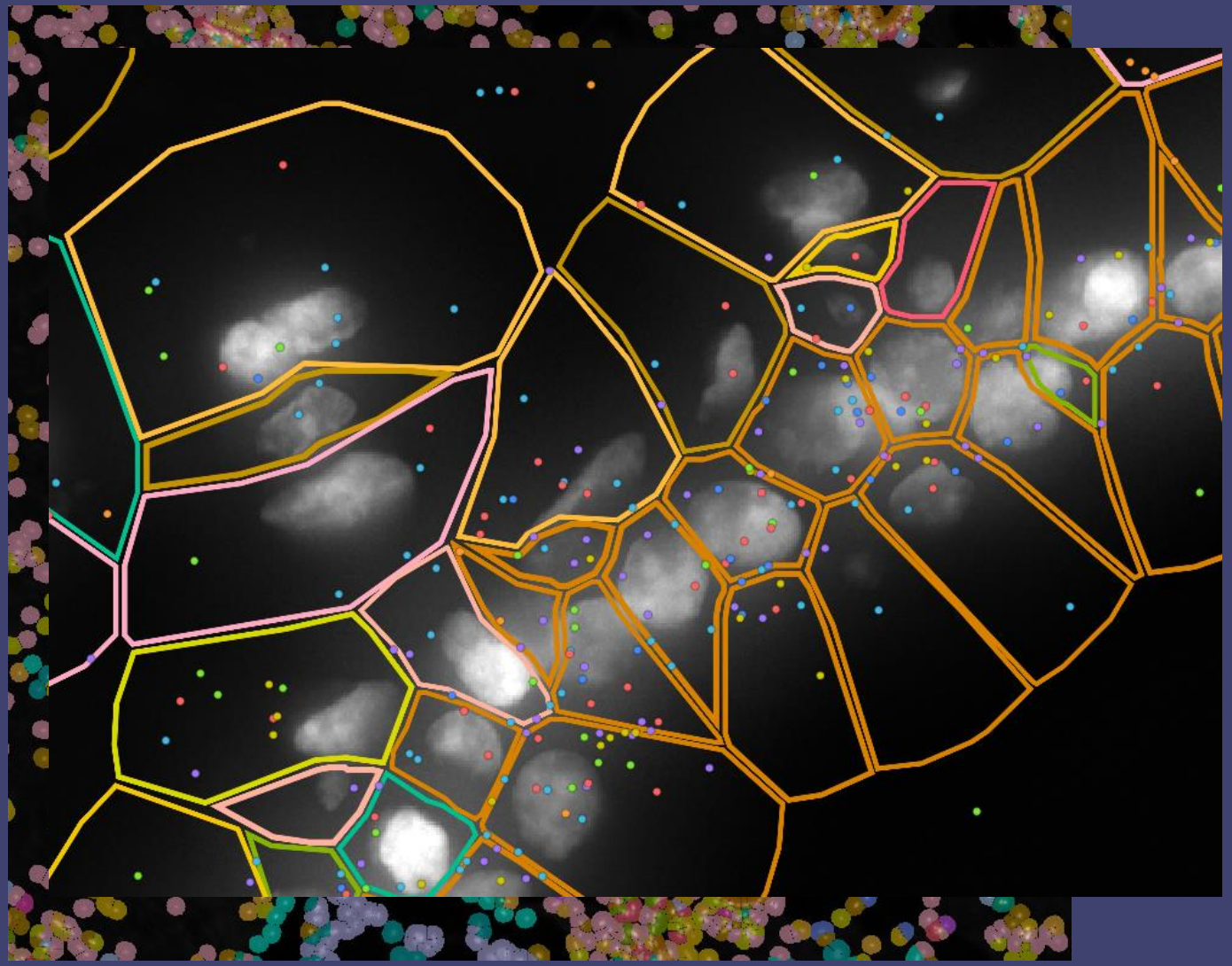


[5] Nanda et al., JAMA Oncol 2020.

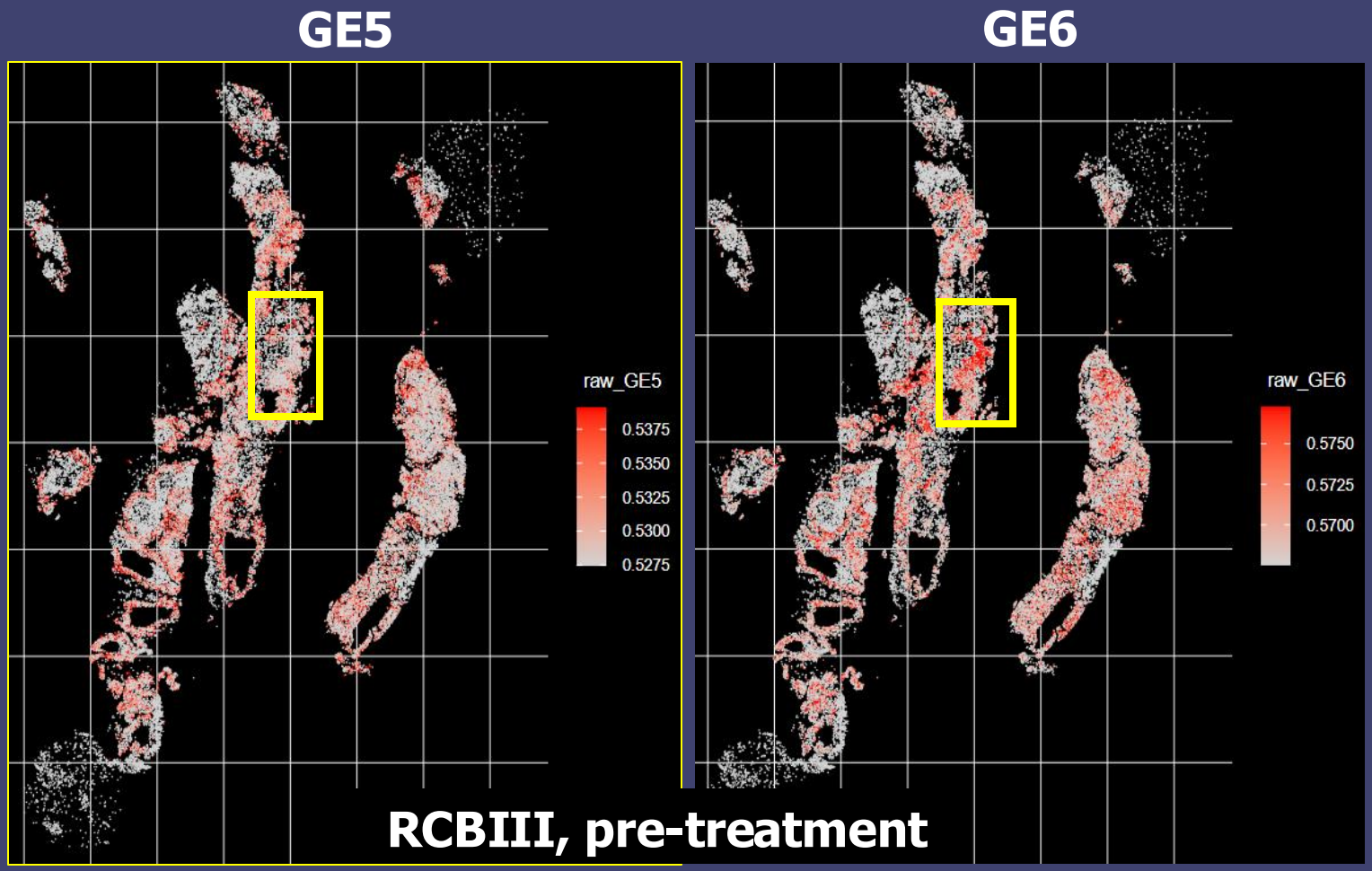
GE5 is enriched in IO responders

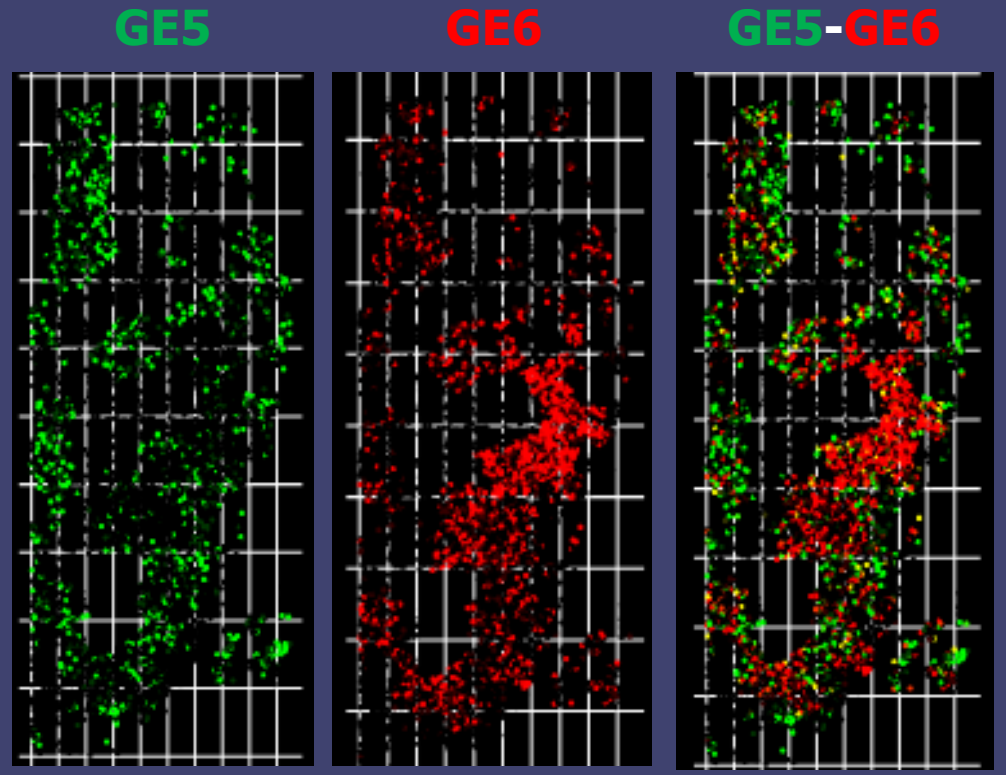


Single-cell spatial transcriptomics to improve InteractPrint

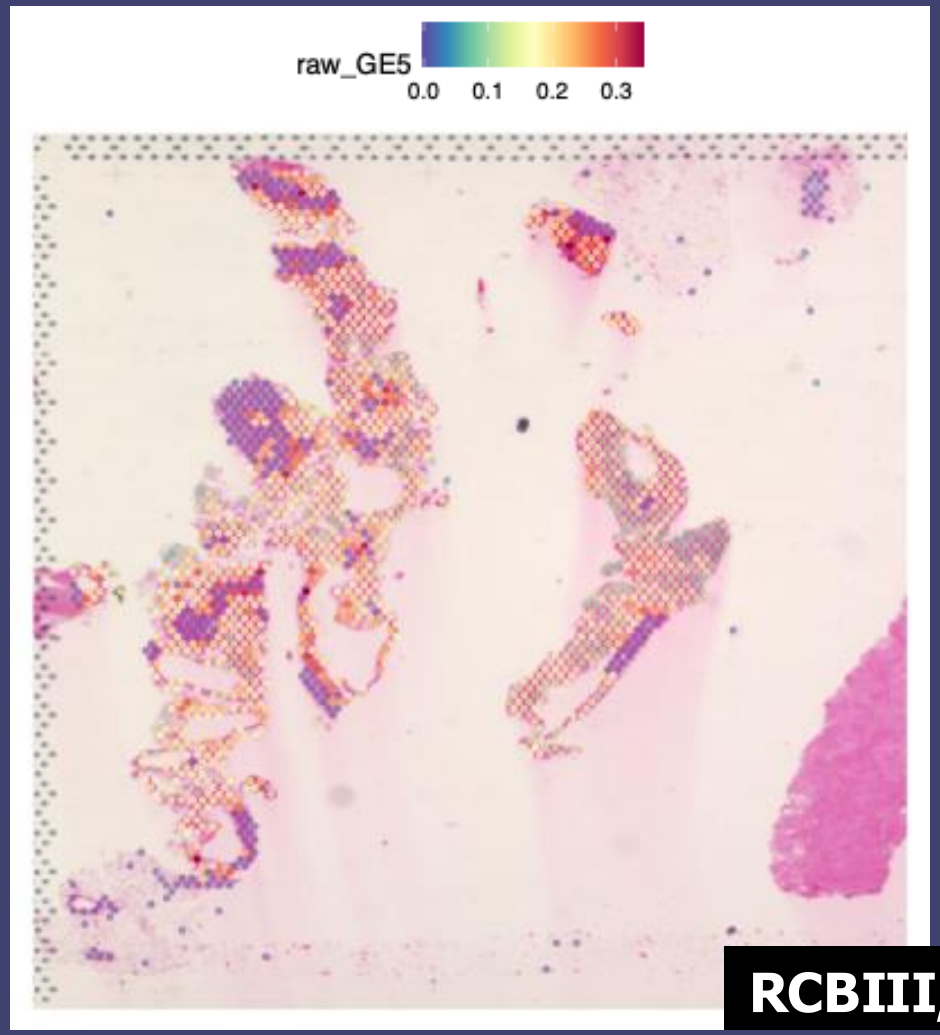


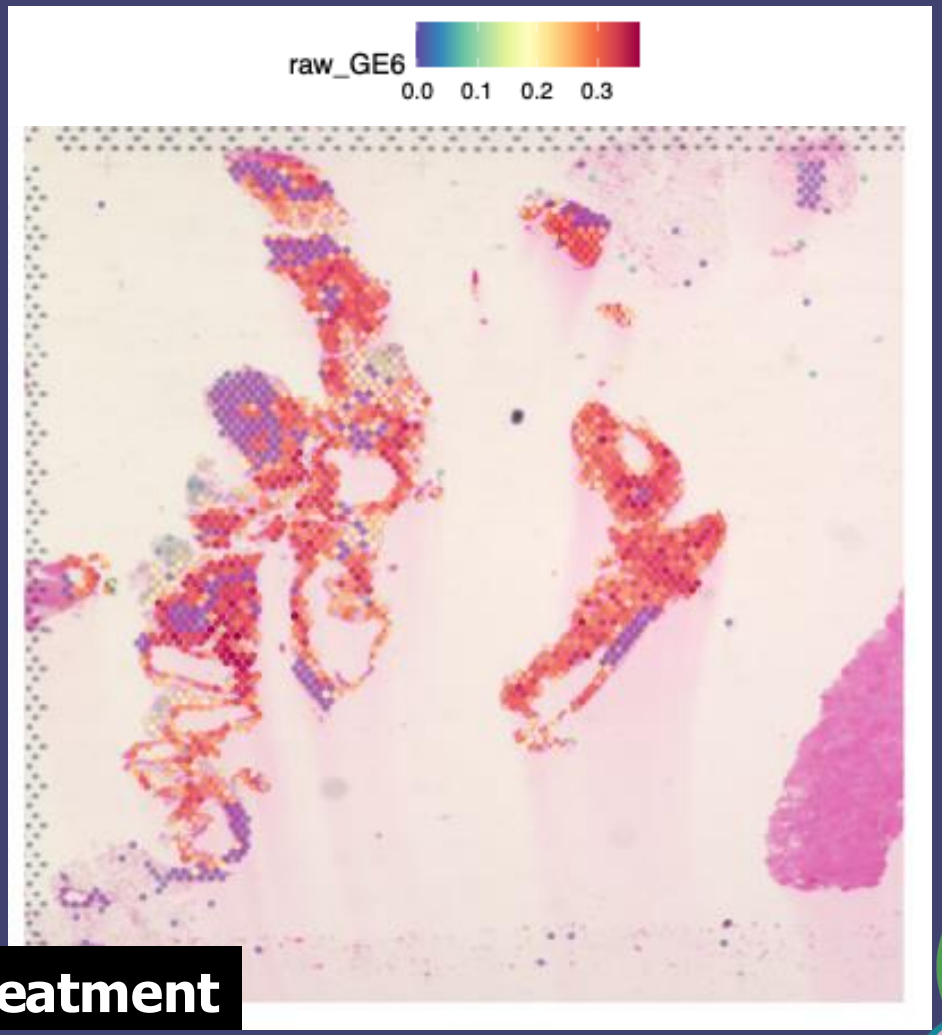
GE5 and GE6 on patient samples (Xenium)





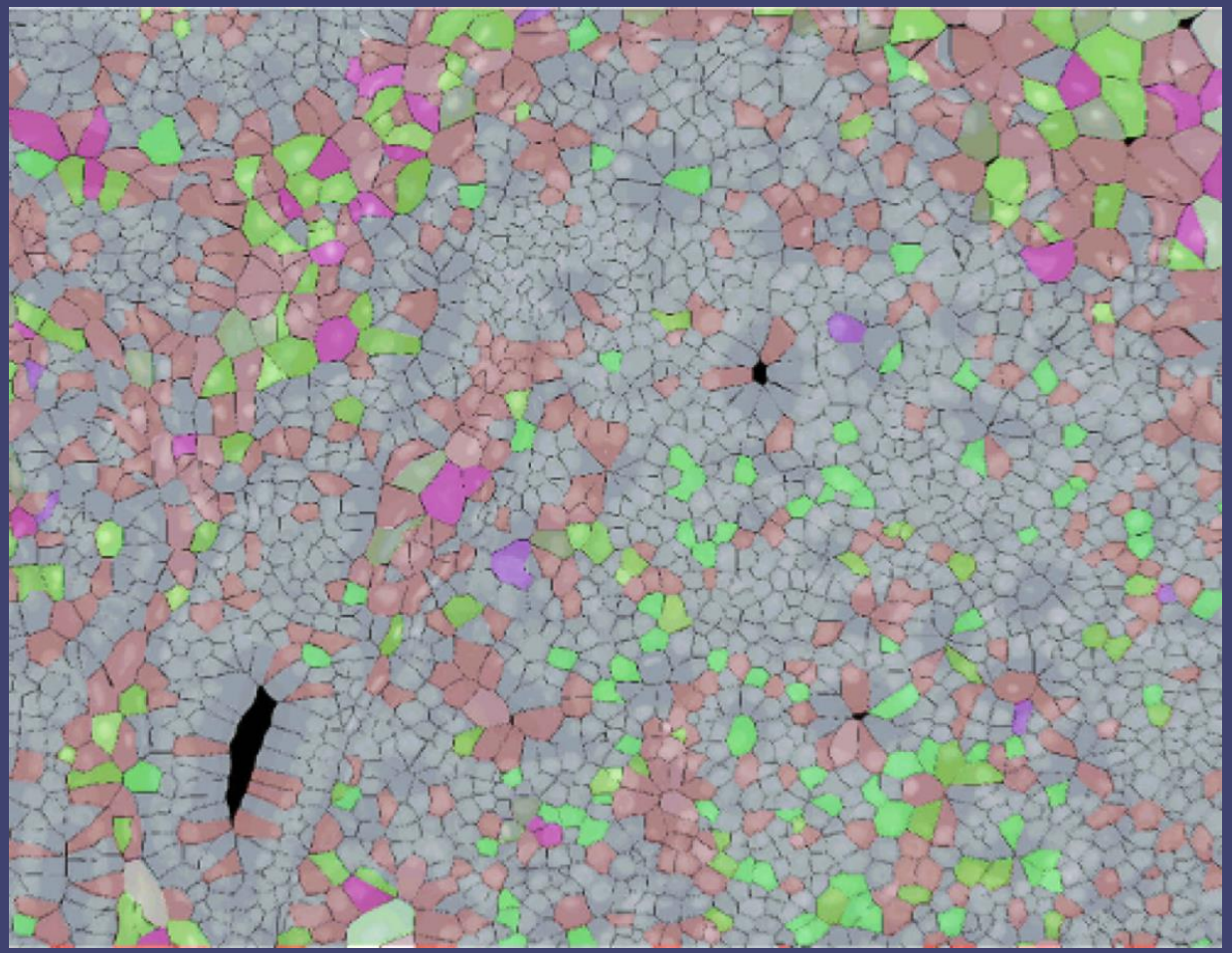
GE5 and GE6 on patient samples (Visium)





RCBIII, pre-treatment

Goal: Identify precise interactions



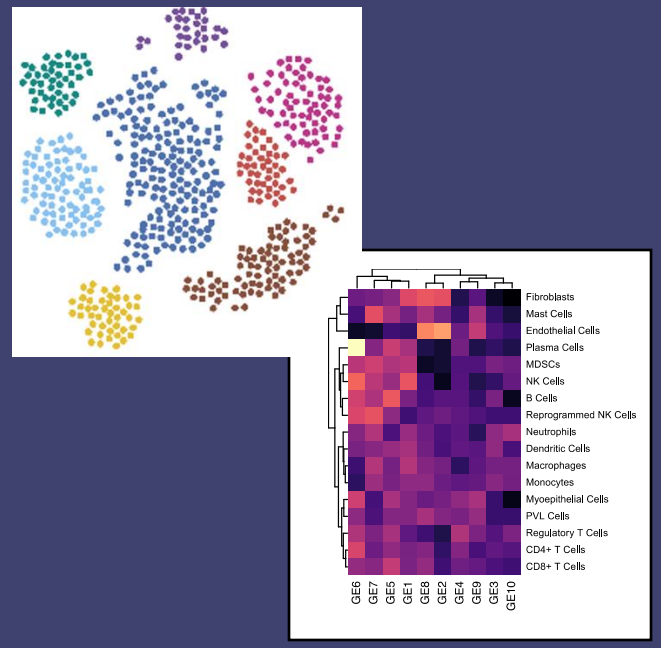
Brown = GE5 cancer cells

Purple = T cells

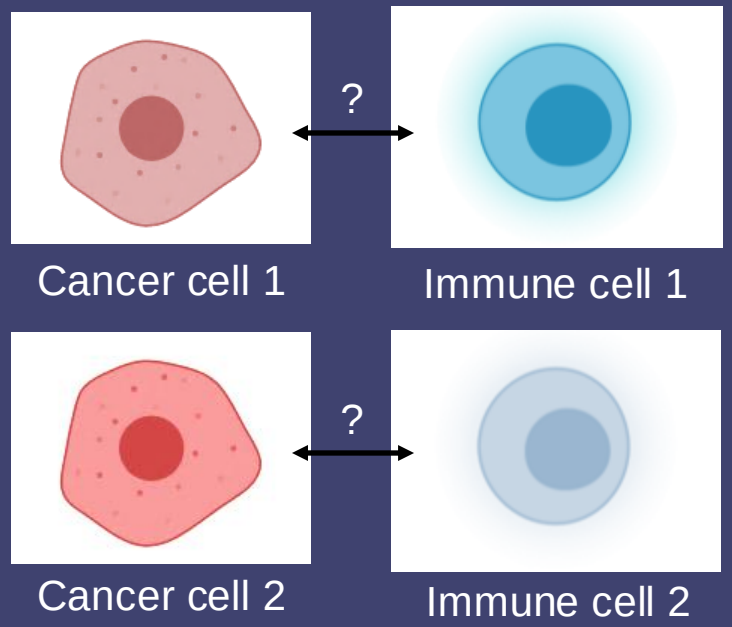
Green = NK cells

Future Directions – achieving precision IO

Use our InteractPrint to deconstruct ITH



Validate predicted interactions for other cell types



Predict and assess response to new immunotherapies

Will respond to new immunotherapies

Will not respond to new immunotherapies

Thank you!

UT Southwestern Medical Center

Chan Lab

Isaac Chan
Ayah Amed
Shaan Bandari
Hannah Chang
Luis Chinea
Flavia Fernandez
Caroline Hauer
Shao-Po Huang
Chris Kang
Sakshi Mohta
Khusboo Patel
Rina Sridharan
Isabella Terrazas
Lily Xu

Patient Advocates

Christine Hodgdon
Julia Maués

Funding

NIH
Susan G. Komen
Mary Kay Ash Foundation
Conquer Cancer Foundation
METAvivor
Theresa's Research Foundation
Pasarow Foundation

Collaborators/Mentors

Carlos Arteaga (UTSW)
Rani Bansal (Duke)
Rolf Brekken (UTSW)
Elizabeth Chen (UTSW)
Zhijian Chen (UTSW)
Christina Curtis (Stanford)
Kevin Dean (UTSW)
Jinming Gao (UTSW)
Hildur Knutsdottir (deCODE)
Heather McArthur (UTSW)
Donald McDonnell (Duke)
Lewis Lanier (UCSF)
Sangeetha Reddy (UTSW)
Marsha Rosner (Chicago)
Evanthia Torres (USC)
Reto Fiolka (UTSW)
Xiang Zhang (Baylor)
Lin Xu (UTSW)

CHAN LAB
CANCER HAS A NEMESIS