IASLC 2022 World Conference on Lung Cancer
AUGUST 6-9, 2022 | VIENNA, AUSTRIA

Tumor Biology, Pathology, Novel Diagnostics

Tianhong “Tina” Li, MD, PhD

**UC Davis Comprehensive Cancer Center,
Sacramento, CA, United States**

Presented by Tianhong “Tina” Li; UCDCCC, USA; thli@ucdavis.edu

DISCLOSURES

Company	Relationship
Merck, OncoC4, LabyRx Immuno-Oncology, Genentech, Novartis, AbbVie Inc, Astelas, Atlas Medx, AstraZeneca, RasCal, Jounce, Tempus, Lung Cancer Mutation Consortium	Contracted Support/Research

Presented by Tianhong “Tina” Li; UCDCCC, USA; thli@ucdavis.edu

OUTLINE

1. Genomic analysis of neuroendocrine transformation of EGFR-mutant lung adenocarcinoma
--MA02.04 Triparna Sen

2. Standardization of histopathological evaluation of resected NSCLC
--PL04.03 Douglas J. Hartman

3. Spatial high-dimensional tissue imaging for novel biomarker assessment
--ES35.03 David Rimm

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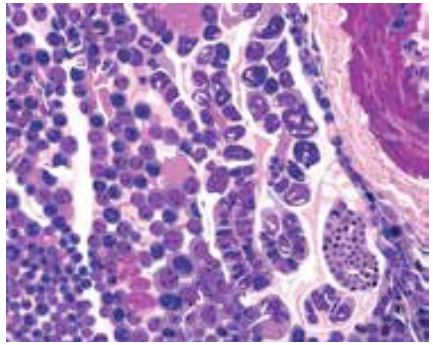
Molecular Drivers and Therapeutic Targets for Neuroendocrine Transformation in Lung Cancer

Triparna Sen, PhD
Icahn School of Medicine at Mount Sinai
New York, United States

NE transformation as a mechanism of targeted drug resistance

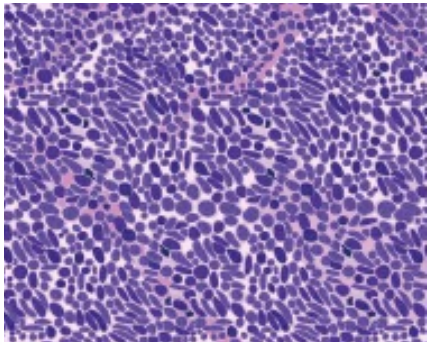
EGFR-mutant lung adenocarcinoma

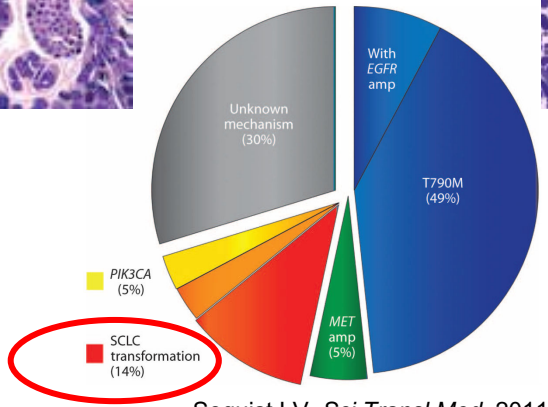


TKI

? ?

Small cell lung cancer





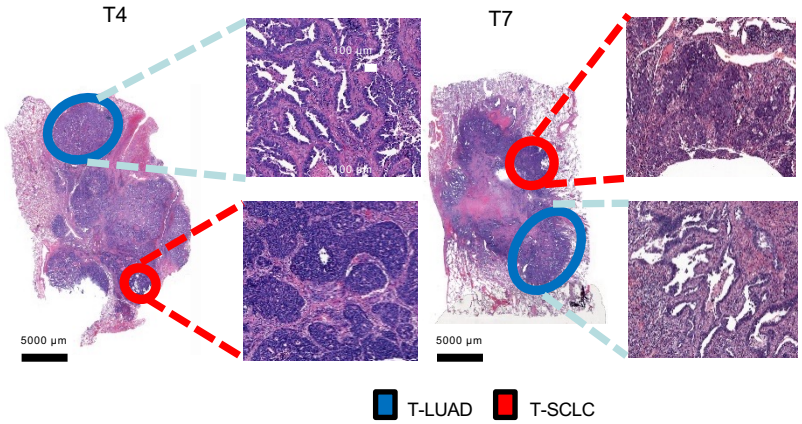
Sequist LV, *Sci Transl Med*. 2011

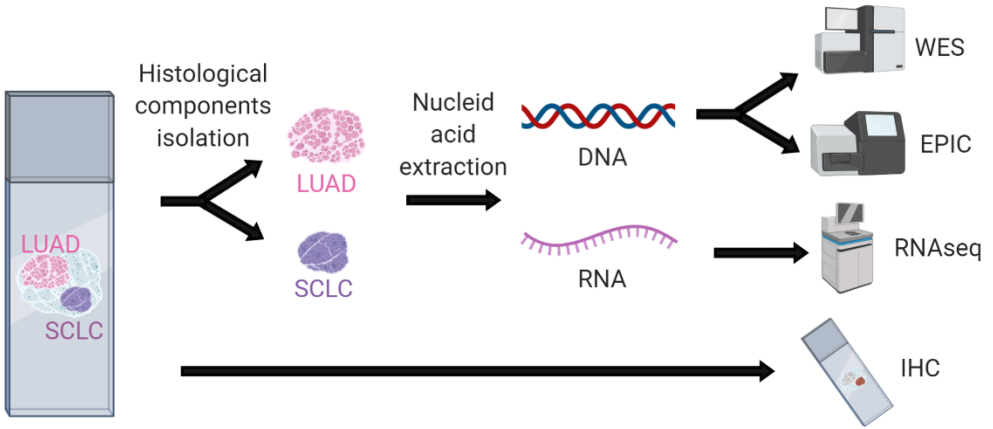
Cancer Discovery, 2021
Journal of Hematology and Oncology, 2021
Nature Reviews Clinical Oncology, 2020

Molecular characterization of combined histology LUAD/SCLC clinical samples

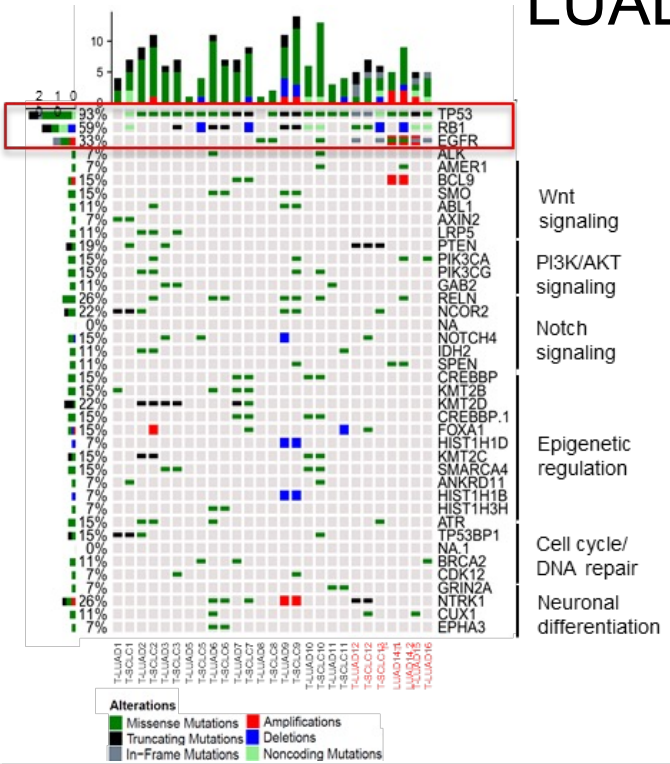
CASES

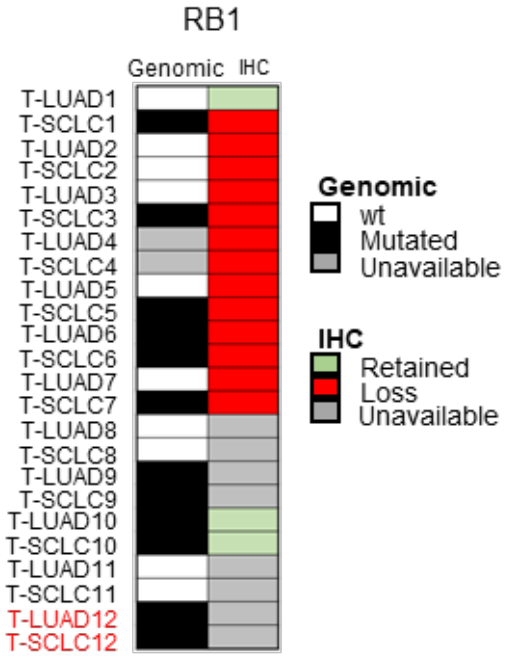
- combined LUAD/SCLC 22
- pre-transformation LUAD 5
- post-transformation SCLC 3
- never-transformed LUAD 15
- *de novo* SCLC 18

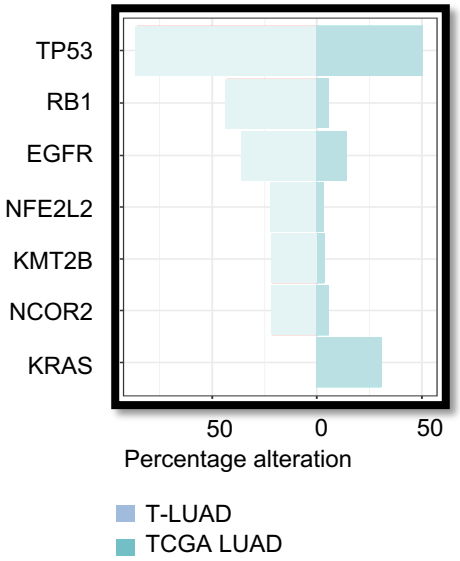




Genomic characterization (WES) of combined histology LUAD/SCLC clinical samples

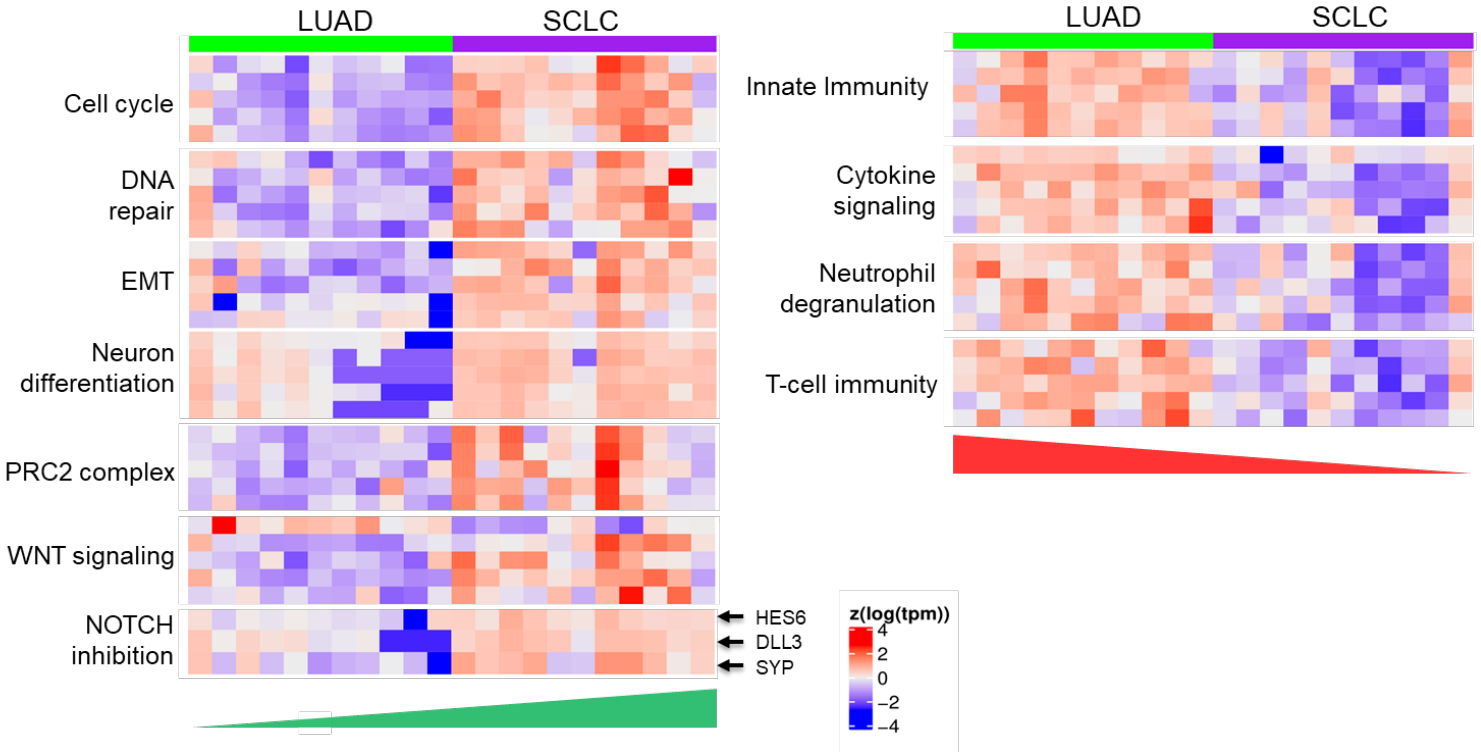






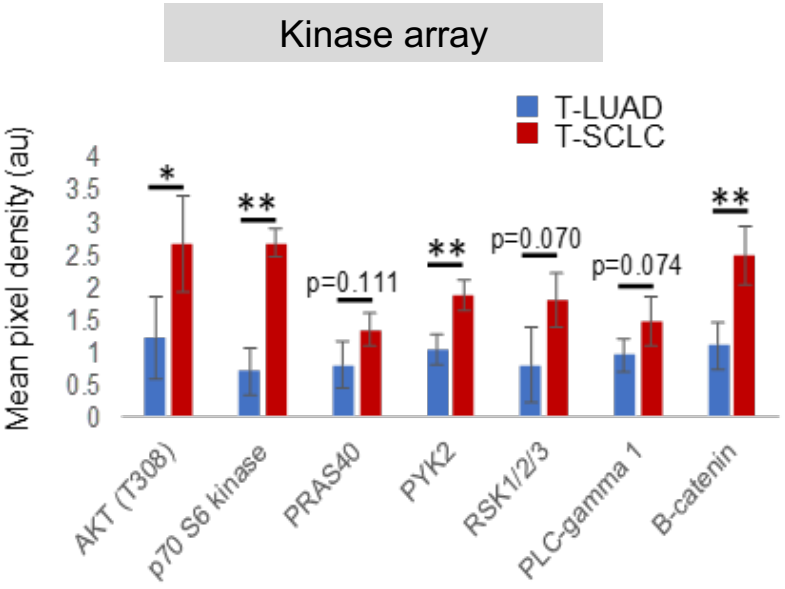
1. *TP53, RB1* alteration are common and shared between the LUAD and SCLC component.
2. Loss of RB1 protein expression in samples showing RB1 WT status in WES
3. *EGFR* alteration is not a pre-requisite for NE transformation

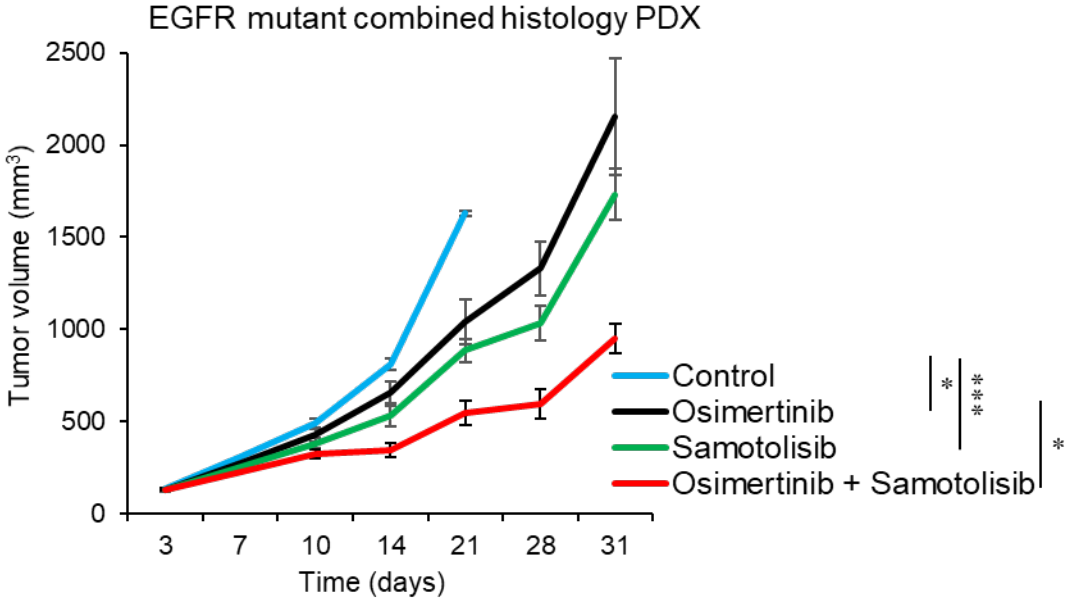
Gene expression changes in combined histology LUAD/SCLC clinical samples



MA02.04 Triparna Sen, Icahn School of Medicine at Mount Sinai, United States.

AKT inhibition delays tumor growth and augments the anti-tumor effect of osimertinib in an EGFR mutant PDX model of NE transformation





MA02.04 Triparna Sen, Icahn School of Medicine at Mount Sinai, United States.

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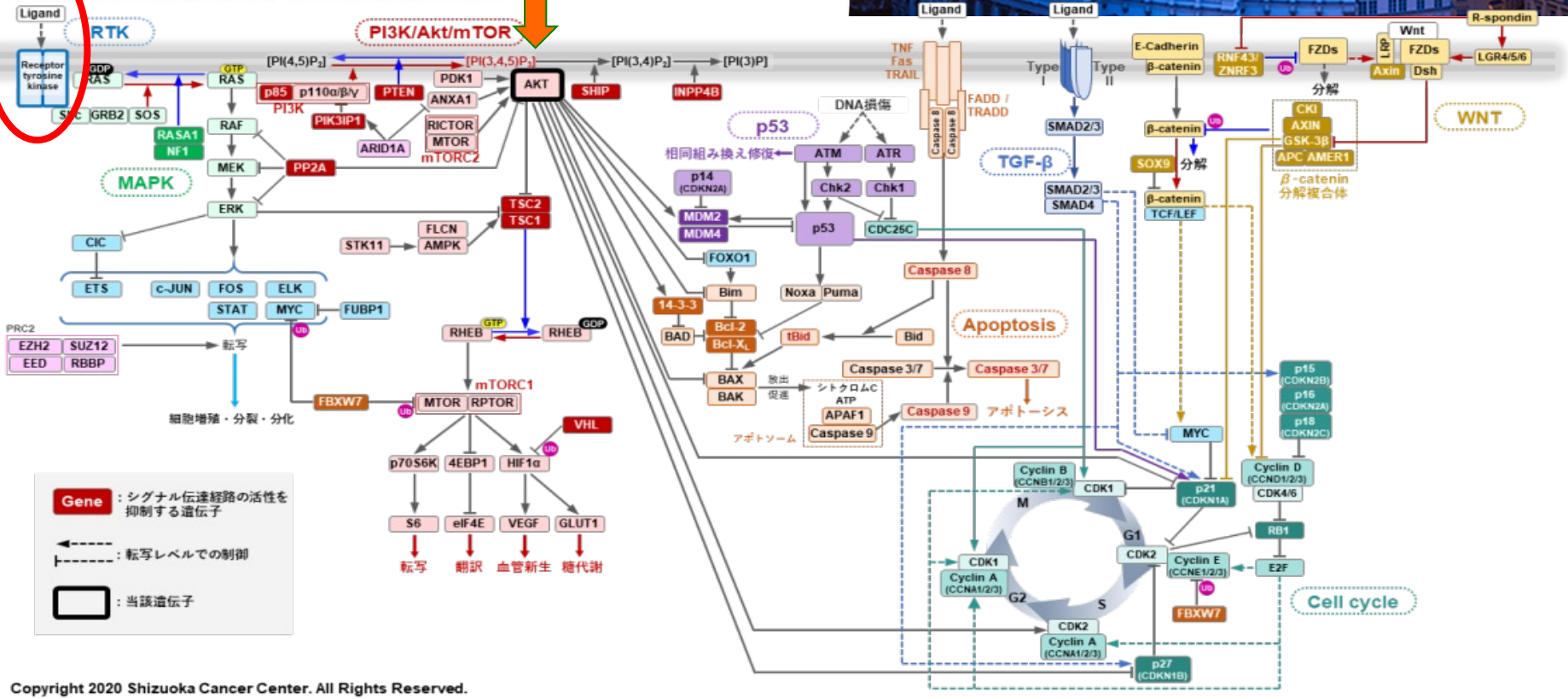

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https://www.jcga-scc.jp/ja/gene/AKT1

Take home messages:

- NE transformation may not be driven by specific mutations.
- WNT, AKT signaling, and the PRC2 complex may be drivers of NE transformation.
- NE transformation is associated with the suppression of the anti-tumor immune response.
- AKT signaling and EZH2 (PRC2 complex) are potential therapeutic targets to prevent/delay/treat NE transformation.

Pathological evaluation of NSCLC

Implications for neoadjuvant chemoimmunotherapy in early-stage NSCLC

- "The goal is to use only image-level annotations to achieve pixel-level prediction of three common and meaningful tissue types, **tumor epithelial tissue, tumor stromal tissue** and **normal tissue**."
- **Molecular and immune biomarker assessment**
- Difficult and Time-consuming task
- Interobserver variability and relatively low reproducibility
- AI assistance to traditional pathology work to overcome these limitations

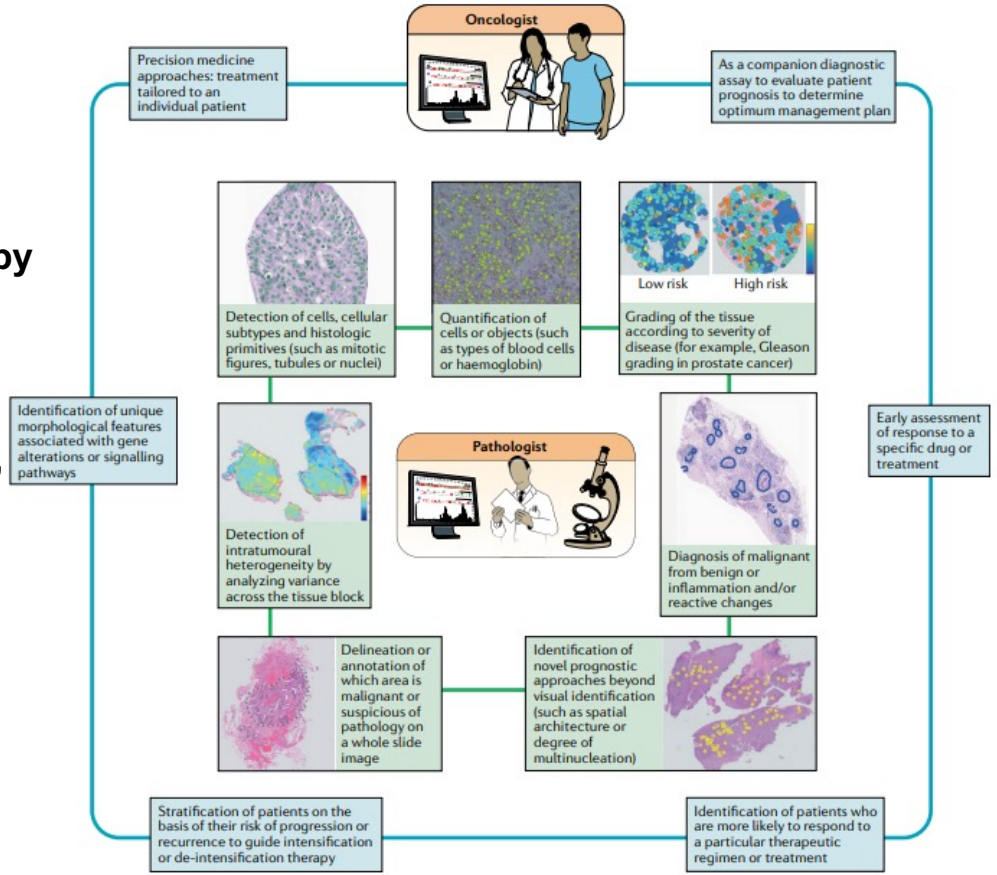


Fig. 4 | **Artificial intelligence (AI) and machine learning approaches complement the expertise and support the pathologist and oncologist.** Some of the existing AI approaches currently used by pathologists to analyse images from tumours are depicted. For the practicing oncologist, AI approaches can be used to aid decision making for different aspects of the management of patients with cancer.

Hartman DJ, Van Der Laak JAWM, Gurcan MN, Pantanowitz L. Value of public challenges for the development of pathology deep learning algorithms. J Pathol Inform 2020;11:7.
Kapil et al, PMID 30478349; Taylor et al, PMID 30640753; Wu et al, PMID 34518630

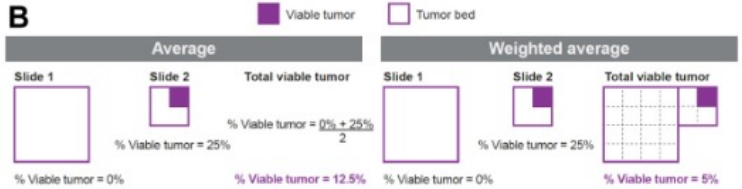
NATURE REVIEWS | CLINICAL ONCOLOGY VOLUME 16 | NOVEMBER 2019 | 703

Major Pathologic Response Assessment

- Proposed assessment of histologic features after neoadjuvant treatment of NSCLC
- Examines %viable tumor, %necrosis, and %stroma

A Major Pathologic Response Calculator Form

Study #									
Site #									
Subject ID									
		Tumor (Bed) Dimensions					Area		
Slide	Slide # (e.g; A1, A2)	Width, cm	Length, cm	Percentage Viable Tumor/ Tumor (Bed)	Percentage Necrosis	Percentage Stroma	Width × Length	Weighted	Mean Weighted Percentage Viable Tumor
1	A1	2.9	2	0%	50.00%	50.00%	5.8	25%	0.00%
2	A2	2	1.3	25%	25.00%	50.00%	2.6	11%	2.81%
3	A3	2.5	2	5%	30.00%	65.00%	5	22%	1.08%
4	A4	2	2.2	3%	20.00%	77.00%	4.4	19%	0.57%
5	A5	1	0.5	90%	0.00%	10.00%	0.5	2%	1.95%
6	A6	2.3	2.1	6%	10.00%	84.00%	4.83	21%	1.25%
							23.13	100%	7.66%
Weighted Percentage Viable Tumor	7.66%								
Non-Weighted Percentage Viable Tumor	21.50%								
Average Necrosis	22.50%								
Average Stroma	56.00%								



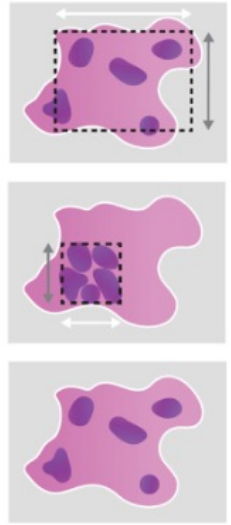
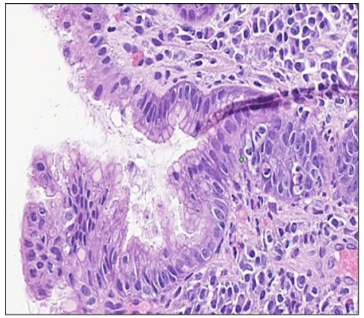


Figure 1. Using the MPRCT in microscopic assessment of pathologic response. (*A*) Example of MPRCT data collection form. Tumor bed = viable tumor + necrosis + stroma. Default of individual percentage stroma is 100%; the actual value is displayed after values are entered for percentage viable tumor and percentage necrosis. (*B*) Schematic revealing differences in obtaining the unweighted (21.50%) and weighted (7.66%) average MPRs. (*C*) How to determine the length and width of viable tumor (purple foci) in the tumor bed (pink). White outline borders an irregularly shaped tumor bed. The black dashed rectangle provides the best-fitted regular shape to assess width (white arrow) and height (gray arrow). #, number; ID, identification; MPR, major pathologic response; MPRCT, major pathologic response calculator tool.

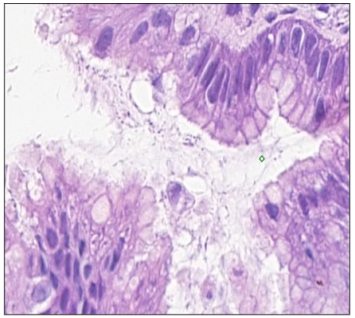
Saqi A, Leslie KO, Moreira AL et al. Assessing Pathologic Response in Resected Lung Cancers: Current Standards, Proposal for a Novel Pathologic Response Calculator Tool, and Challenges in Practice, JTO Clin and Res Reports, Vol 3, Iss 5, 2022

Data Limitations

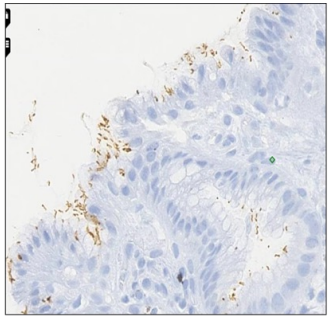
- Multiple Laboratories
  - Tissue processing variability
  - Staining variability
  - Slide preparation
  - Digital platform
- Robust outcomes
  - Clinically meaningful
  - Prospective vs retrospective
- Data augmentation techniques
  - Rotate images
  - Alter scales



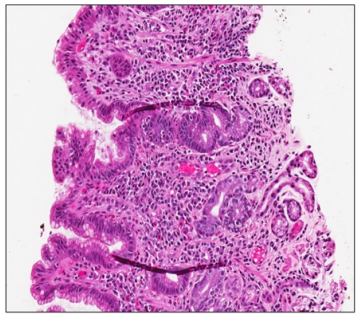
Omnyx - 40x



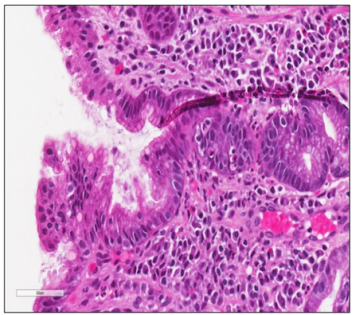
Omnyx - 60x



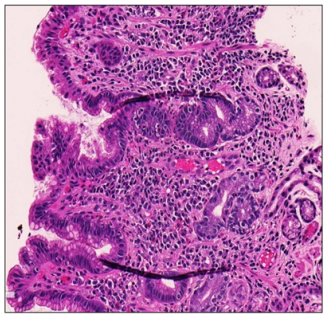
Omnyx - 40x (IHC)



Aperio - 20x



Aperio - 40x

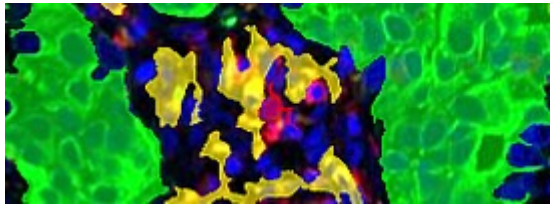


NanoZoomer - 20x

Discovery of Predictive Biomarkers for Immunotherapy Using Deep Spatial Tissue Analysis in Lung Cancer

- Measuring vs Counting
- High-plex **(Deep) Spatial** Discovery tools
- **Predictive** Markers vs Prognostic Markers
- **Discovery** in Lung Cancer (using QIF, IMC and DSP)
    - **Discovery** Related to Tumor Infiltrating Lymphocytes (TIL)
    - **Discovery** of Other Biomarkers

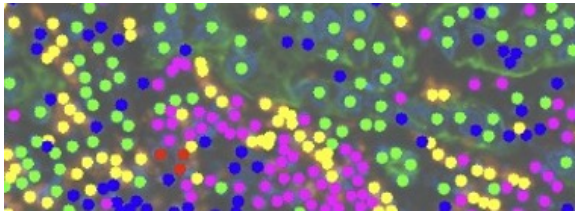
Measure Tissue



$$\frac{\Sigma \text{ target intensity in compartment}}{\Sigma \text{ compartment pixel area}}$$

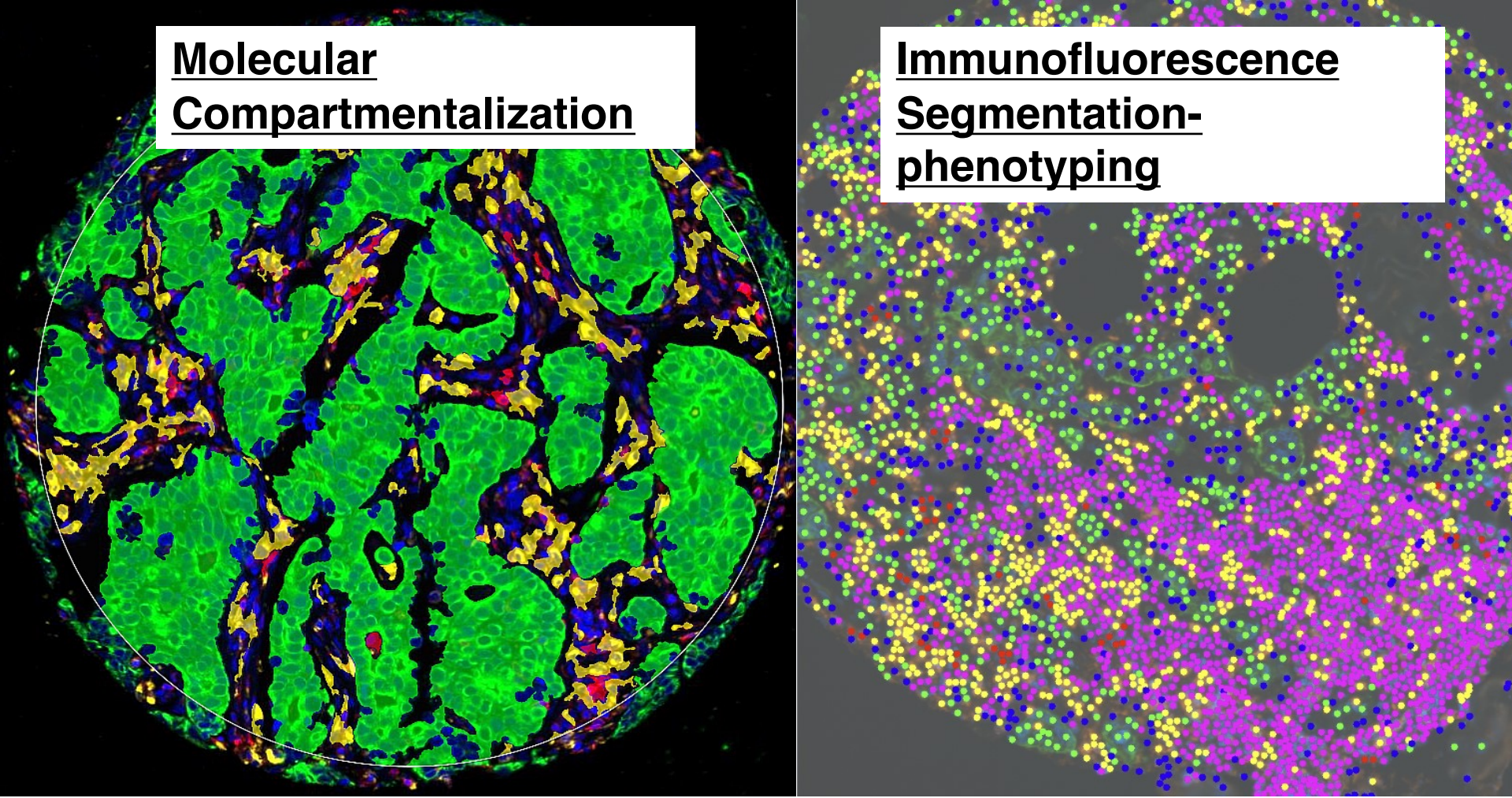
A concentration – Biochemistry:
Attomol/mm^2

Count Cells



$$\frac{\Sigma \text{ target positive cells}}{\Sigma \text{ cells of interest}}$$

A cell count – Pathology:
Percent cells positive (above threshold)


Molecular Compartmentalization
Immunofluorescence Segmentation-phenotyping

Summary of Protein High-Plex Methods

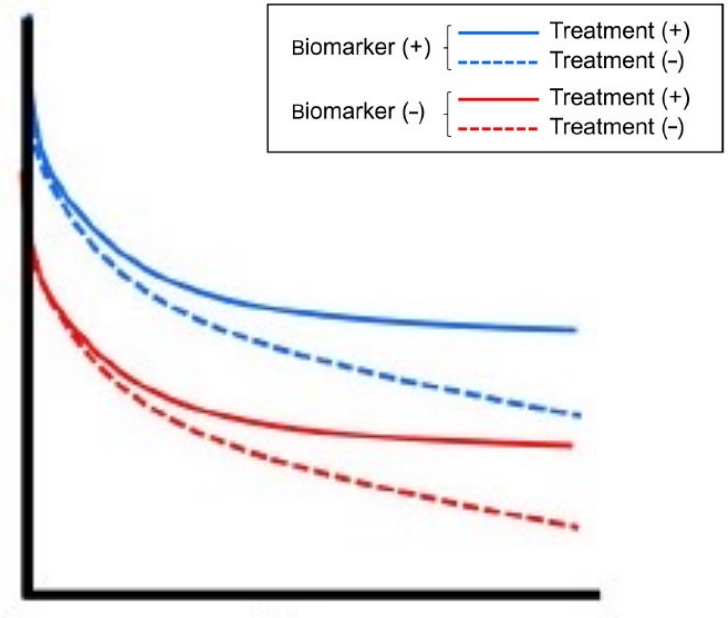
Technology	Plex	Cell vs Tissue	Resolution
Immunohistochemistry	1-3	both	0.2 microns
Immunofluorescence	5-9	both	0.2 or better with confocal, two photon etc
MICSSS	20-30	cell	0.2 microns
CyCIF	20-50	cell	0.2 microns
MultiOmyx	30-50	cell	0.2 microns
CODEX	30-50 (now 100)	cell	0.2 microns
Other Cycling	10-50	cell	0.2 microns
IMC	30-55	both	1 micron
MIBI	30-55	cell	**0.01 micron**
DSP	**770**	tissue	10 micron (non-imaging)

Why are there no new companion diagnostic tests after the first test that was approved with the drug?

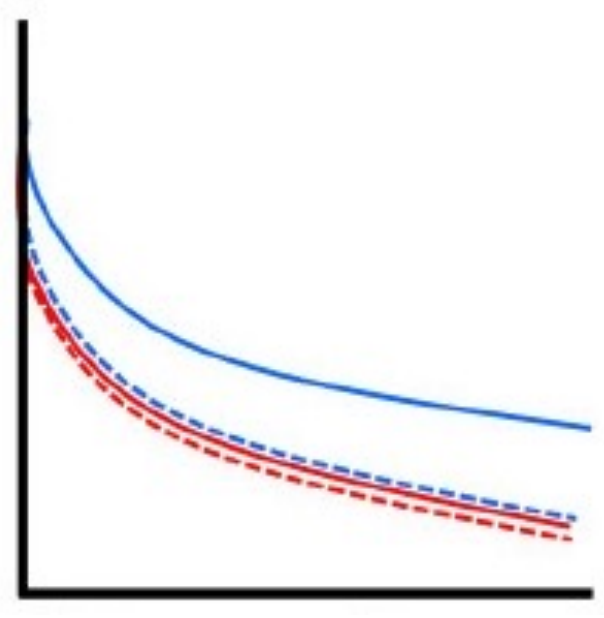
Prognostic	Predictive	Indicative*
• Separates groups with different **disease outcome**, regardless of treatment	• Separates groups that experience different **treatment effect (or response)**	• Associated with **treatment outcome**, but independent of disease prognosis in a control cohort
• Treatment effect does not depend on this biomarker	• Statistically significant **interaction test** • Requires **clinical trial with placebo arm**	• **Placebo arm is unethical** and unavailable

* Term proposed by Daniel Zelterman in Wong et al, CCR 2019

Survival Curves for Prognostic and Predictive Biomarkers

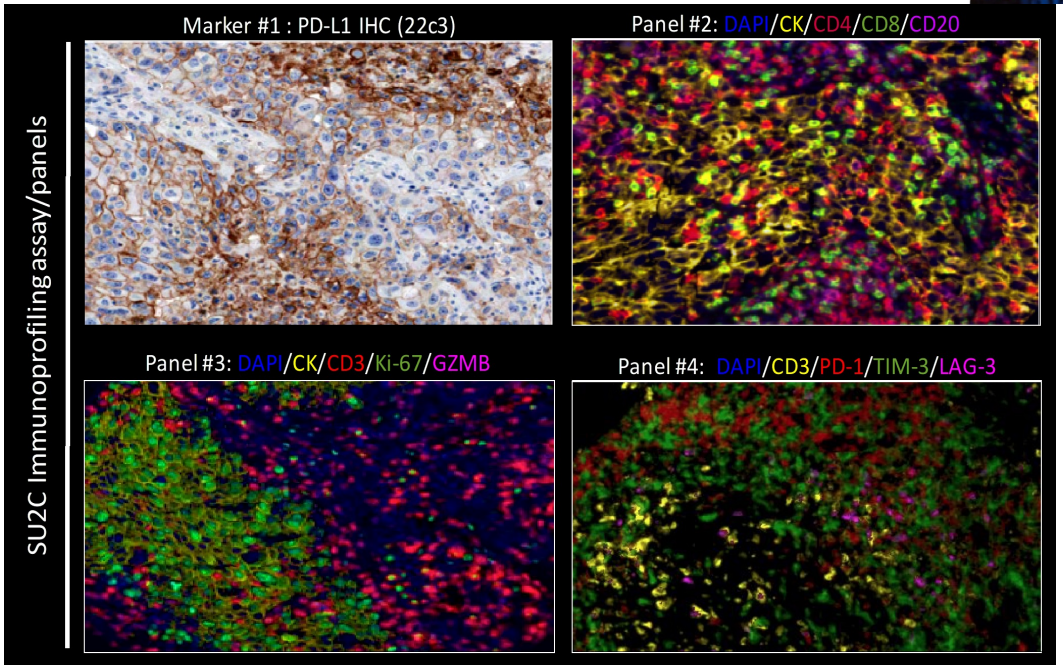


Prognostic



Predictive

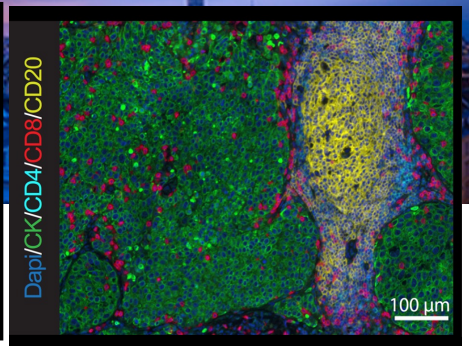
Spatial analysis of TILs and role in IO sensitivity in patients with advanced NSCLC



836 slides from 209 cases in 5 institutions

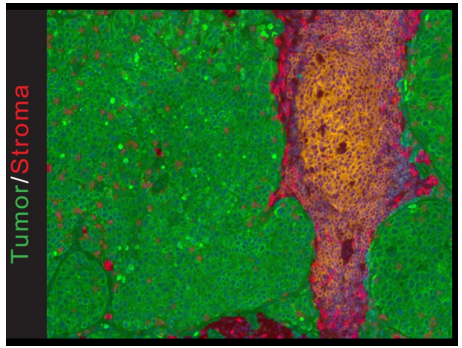
Slide Courtesy of Kurt Schalper

Multispectral imaging



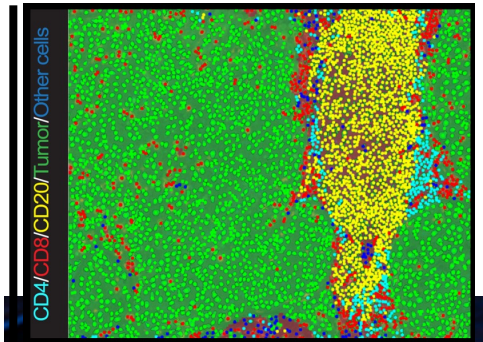
#1

Tissue segmentation



#2

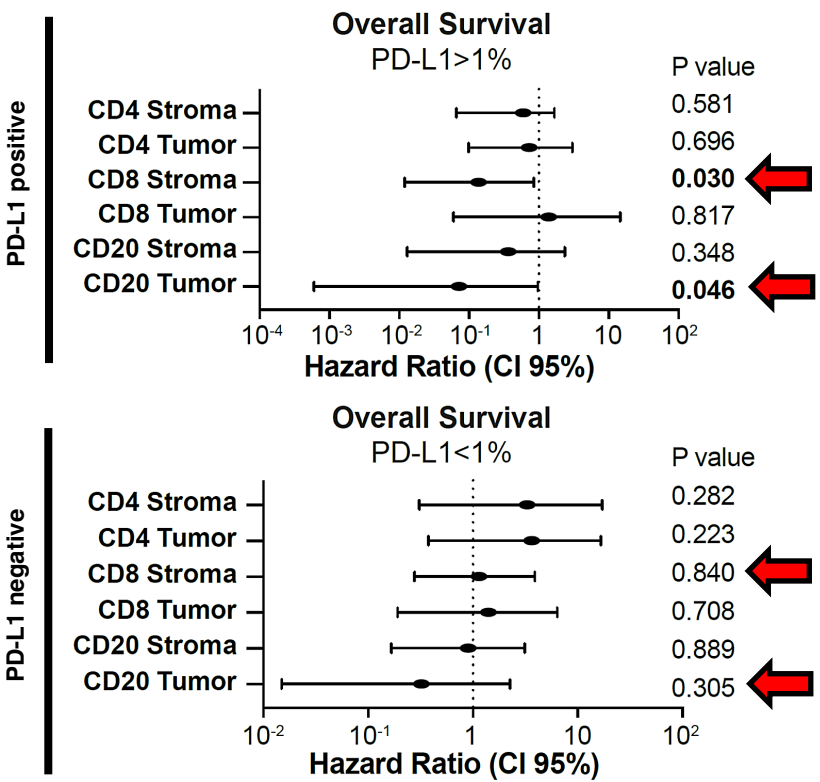
Cell segmentation

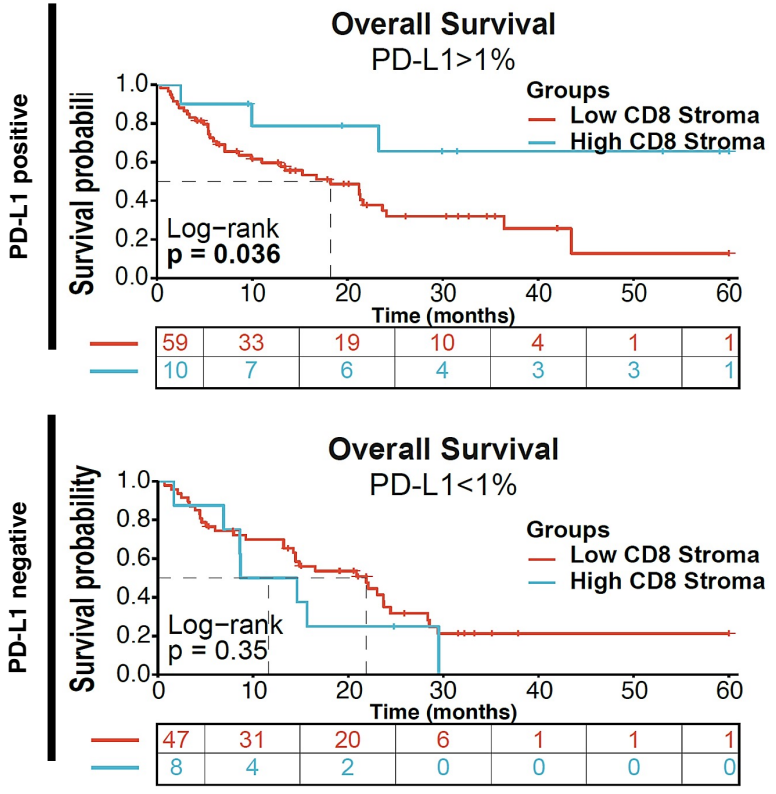


#3

Lopez de Rodas et al., 2022, JITC

TILs and survival after PD-1 axis blockade in patients with NSCLC





Lopez de Rodas.... Schalper, 2022, JITC

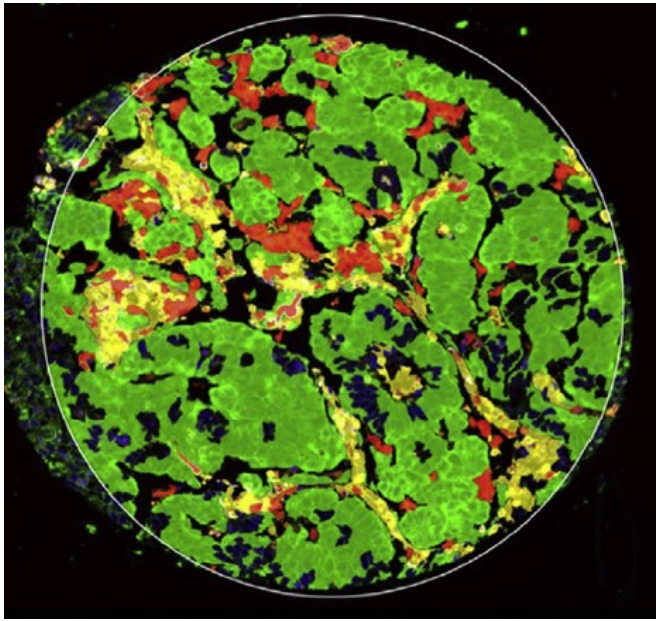
ORIGINAL ARTICLE

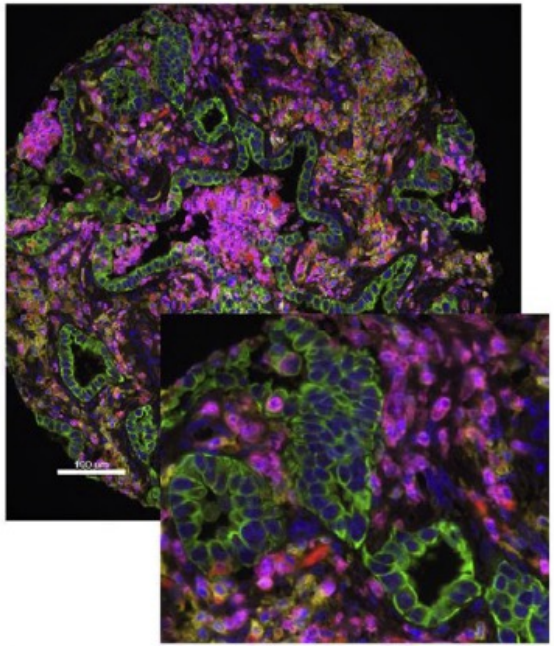
Journal of Thoracic Oncology

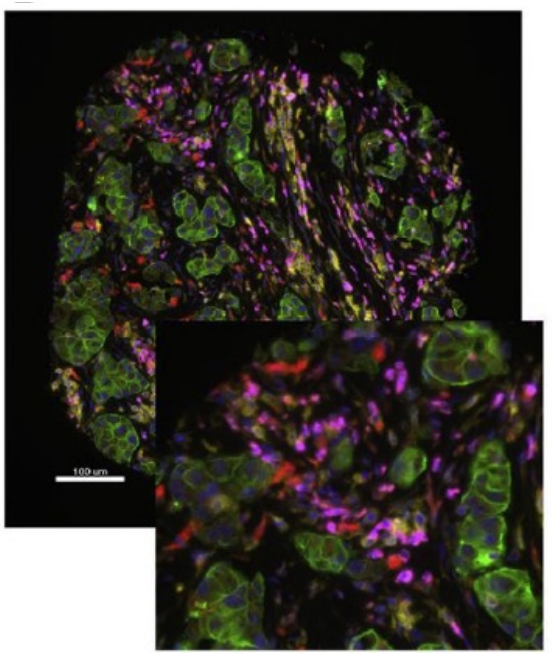
IASLC

Discovery of Biomarkers of Resistance to Immune Checkpoint Blockade in NSCLC Using High-Plex Digital Spatial Profiling

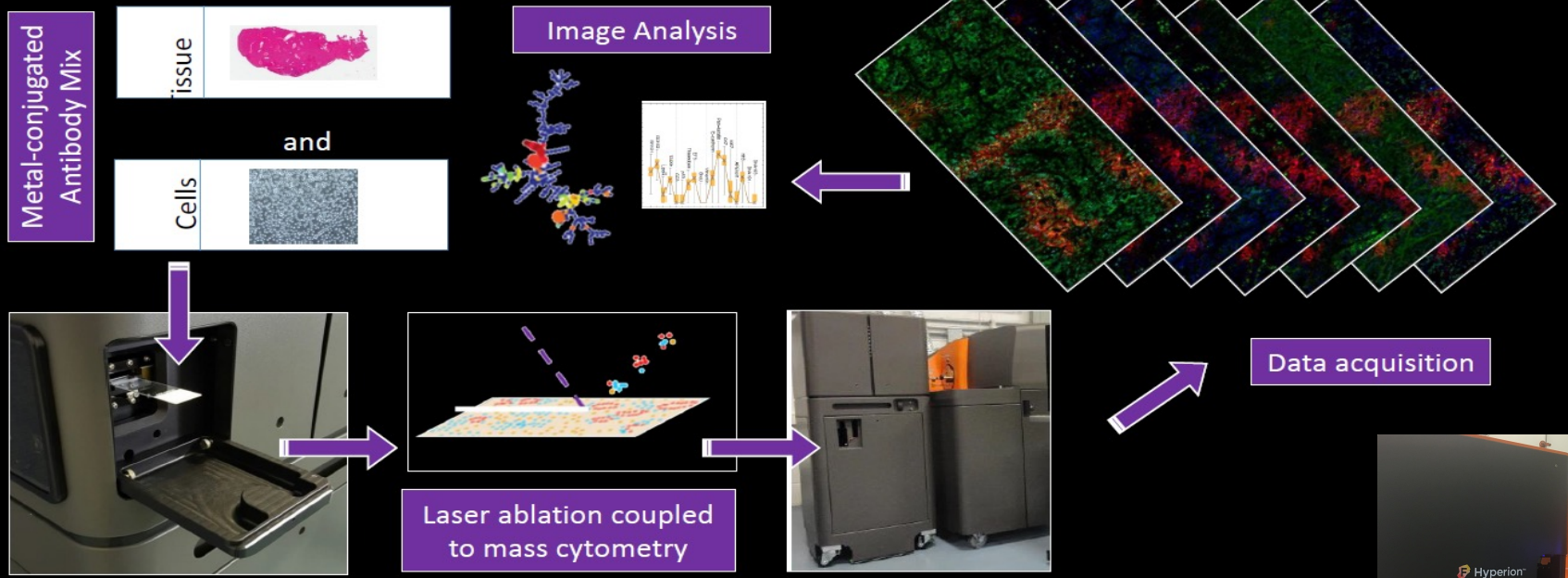
Myrto Moutafi, MD,[a] Sandra Martinez-Morilla, PhD,[a] Prajan Divakar, PhD,[b] Ioannis Vathiotis, MD,[a] Niki Gavrielatou, MD,[a] Thazin Nwe Aung, PhD,[a] Vesal Yaghoobi, MD,[a] Aileen I. Fernandez, PhD,[a] Jon Zugazagoitia, MD,[c] Roy S. Herbst, MD, PhD,[d] Kurt A. Schalper, MD, PhD,[a,d] David L. Rimm. MD. PhD[a,d,*]







Imaging Mass Cytometry (IMC)



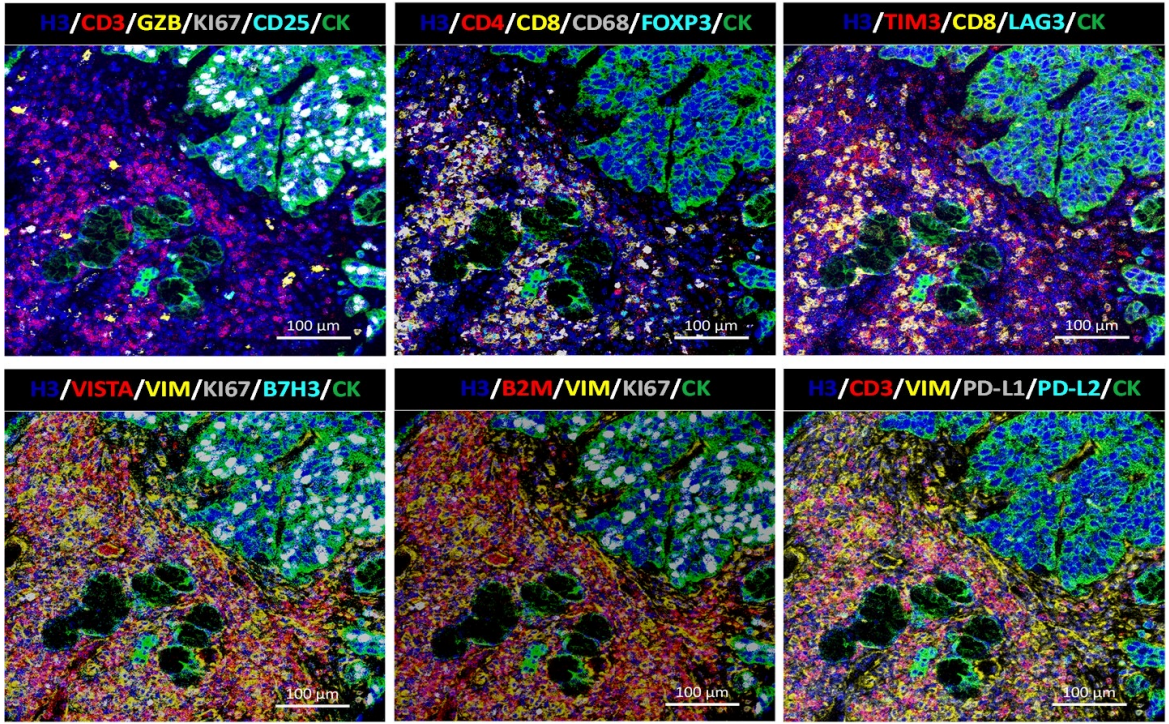
Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry

Charlotte Giesen[1,8], Hao A O Wang[2,3,8], Denis Schapiro[1,4], Nevena Zivanovic[1,5], Andrea Jacobs[1], Bodo Hattendorf[2], Peter J Schüffler[6], Daniel Grolimund[3], Joachim M Buhmann[6], Simone Brandt[7], Zsuzsanna Varga[7], Peter J Wild[7], Detlef Günther[2] & Bernd Bodenmiller[1]

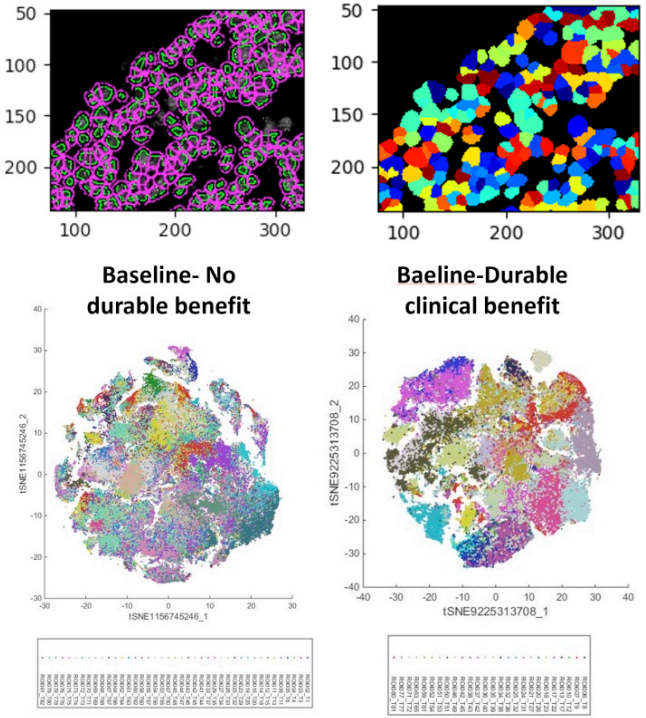
NATURE METHODS | VOL.11 NO.4 | APRIL 2014 | 417

Deep high-plex protein spatial NSCLC analysis

Integrated spatial visualization of selected markers

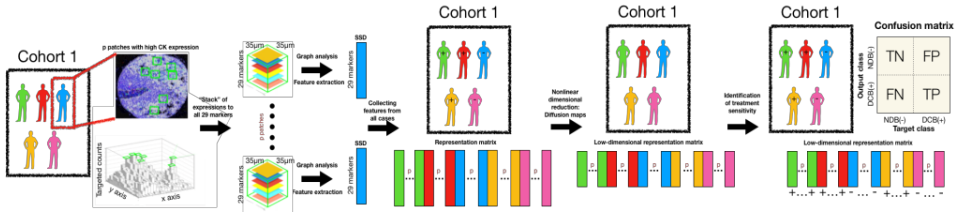


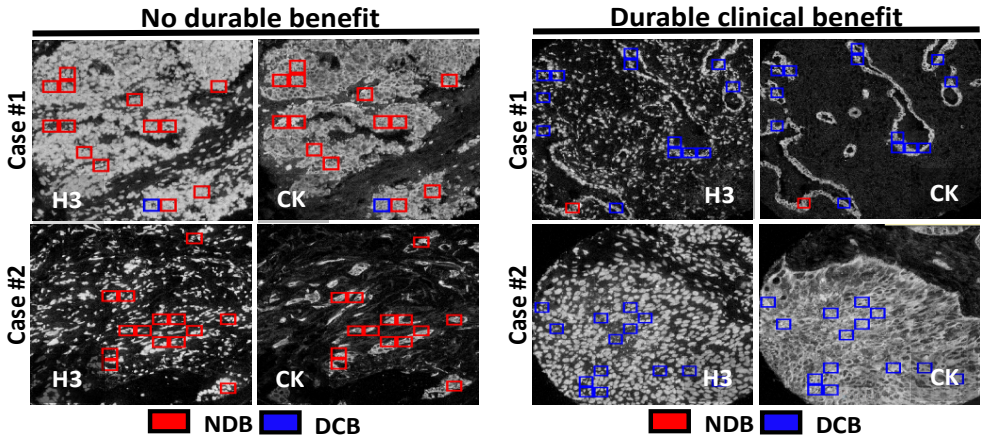
Segmentation and single-cell analysis

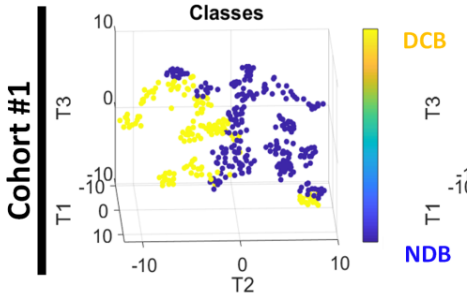


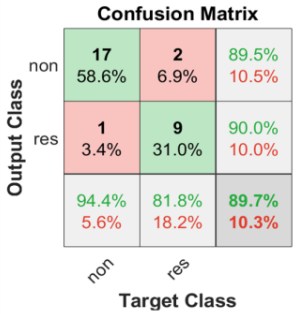
Shruti Desai and Kurt Schalper (submitted)

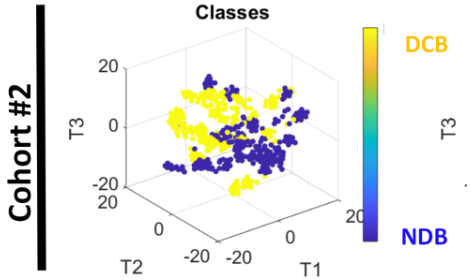
Integration of TME components using deep learning

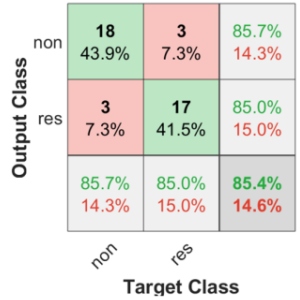












Summary:

1. Most High-plex methods have focused on **Cells** for discovery of new Predictive Markers
2. “Deep” Tissue Analysis may find **Indicative** Markers but is unlikely to find **Predictive** Markers
3. Combinations of Immune Cells may be associated with response to Immunotherapy, but so far only **CD8 in combination with PD-L1** seems promising (and possibly CD20)
4. Other Immune Cells (**Neutrophils**) may be associated with resistance to Immunotherapy

TAKE HOME MESSAGES

1. Neuroendocrine transformation of EGFR-mutant lung adenocarcinoma is associated with the suppression of anti-tumor immune response. AKT signaling and EZH2 (PRC2 complex) are potential therapeutic targets to prevent/delay/treat neuroendocrine transformation.
2. Major Pathologic Response (MPR) assessment requires the assessment of histologic features after neoadjuvant treatment of NSCLC, which examines %viable tumor, %necrosis, and %stroma.
3. Deep Spatial Tissue Analysis allows assessment of multiple predictive biomarkers in TME predictive of response to immunotherapy.