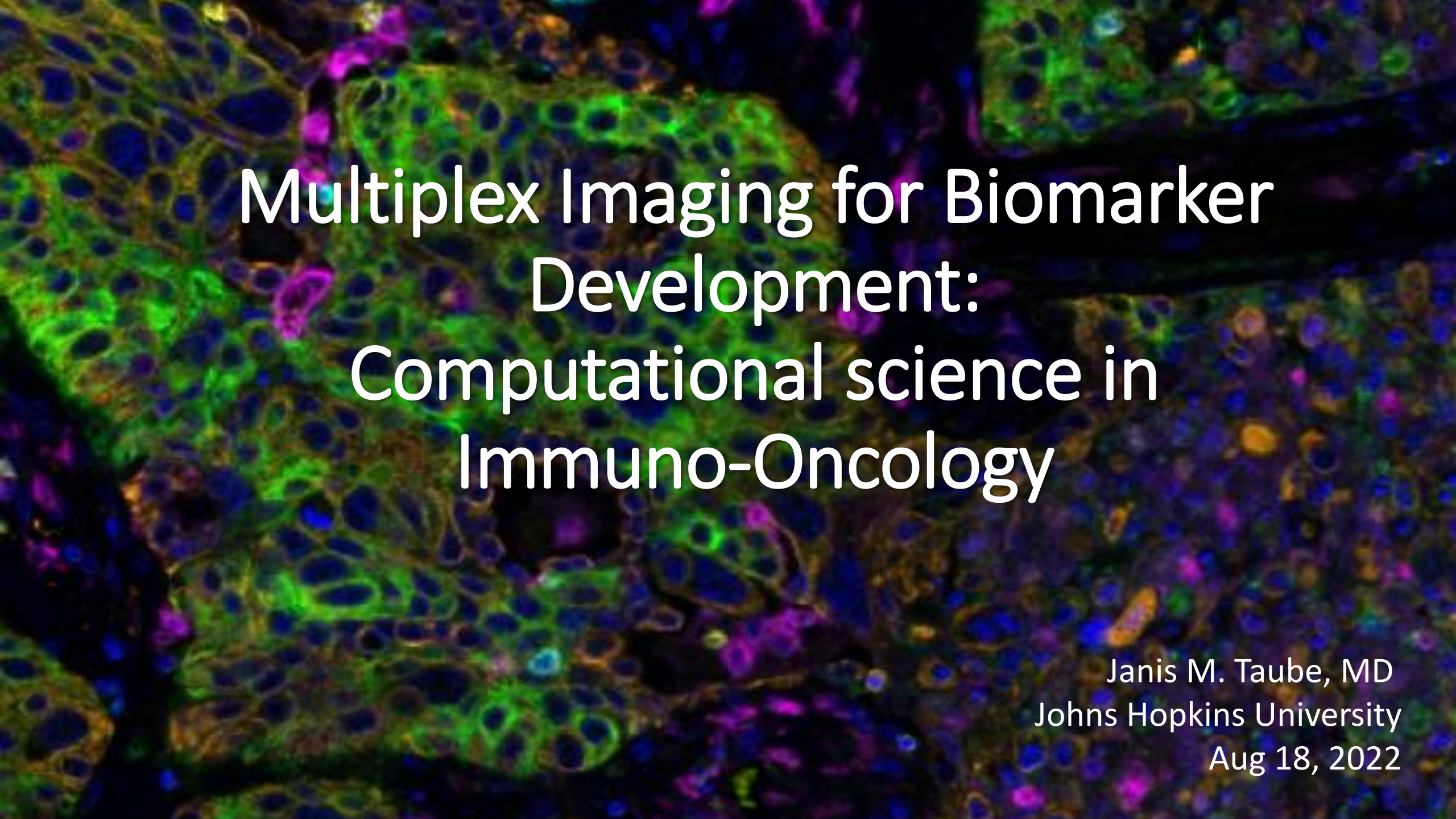

Multiplex Imaging for Biomarker Development: Computational science in Immuno-Oncology

Janis M. Taube, MD
Johns Hopkins University
Aug 18, 2022

Disclosures

- Research funding: BMS, Akoya Biosciences (JT and AS)
- Vectra imaging system loan, reagent provision, and stock options from Akoya Biosciences (JT and AS)
- Consultant/Advisory boards: BMS, Merck, Astra Zeneca, and Compugen (JT) and Akoya Biosciences (JT and AS)

Guiding principles:

- The next generation of tissue-based biomarkers will likely include the identification and quantification of multiple cell types and their spatial interactions.

- Next generation of tissue-based biomarkers are likely to be identified using large, well-curated datasets and are likely to include not just multiplex, but multimodality approaches.

Acknowledgements

Taube Lab members

Sneha Berry, MS

Benjamin Green

Liz Engle, MS

Tricia Cottrell, MD, PhD

Haiying Xu

Aleksandra Ogurtsova

Nicholas Giraldo, MD, PhD

AI/Computer Vision

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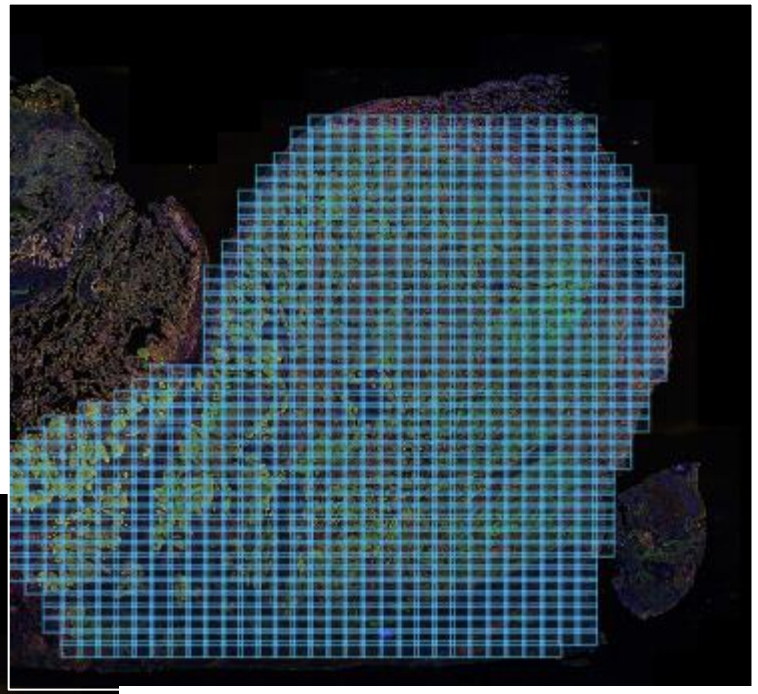
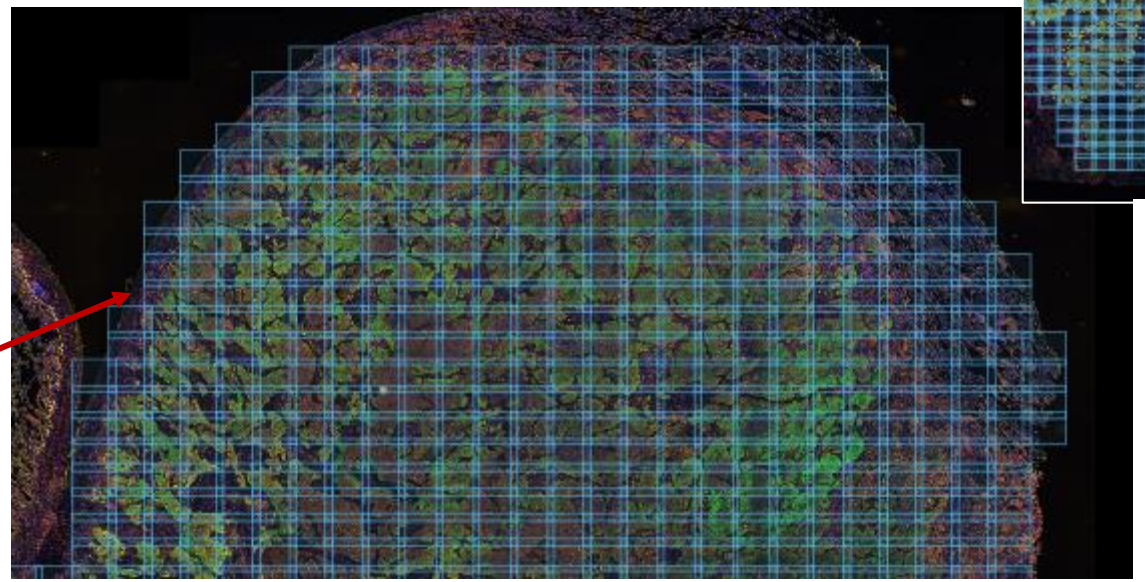
- One tumor >1000 fields and >300 GB disk space



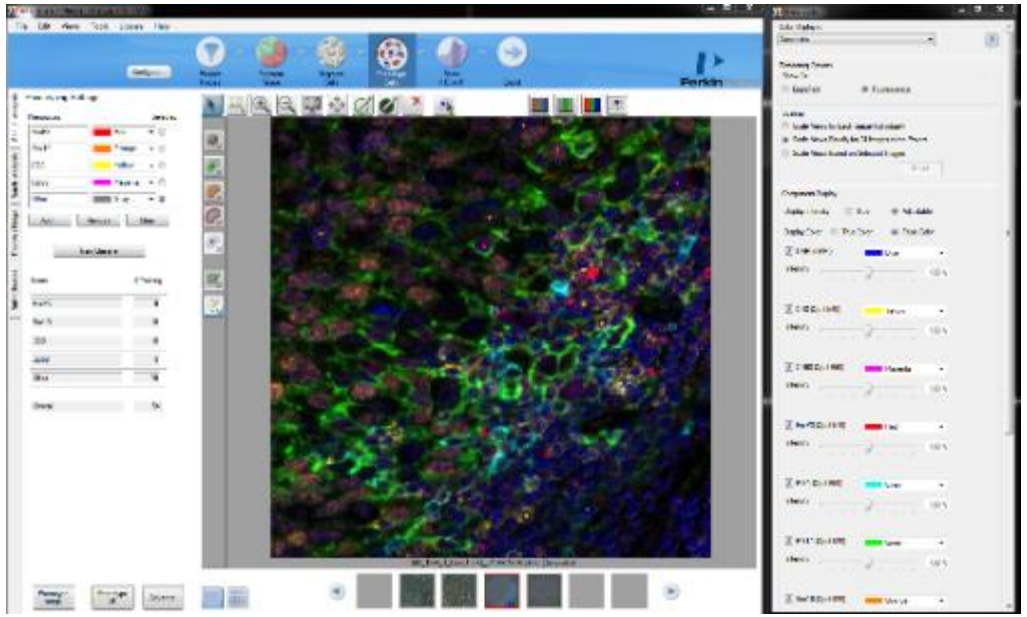


Each square= 1 HPF*

Stars and galaxies are like cells in pathology

Astronomy viewer

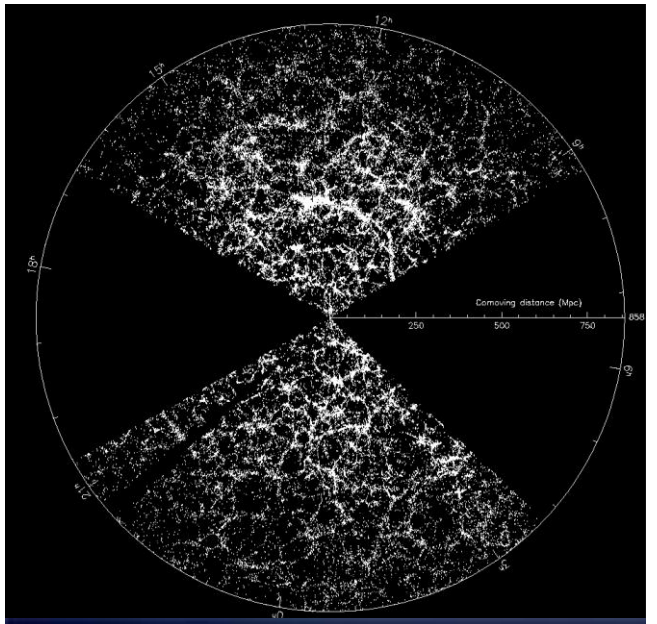
Pathology viewer

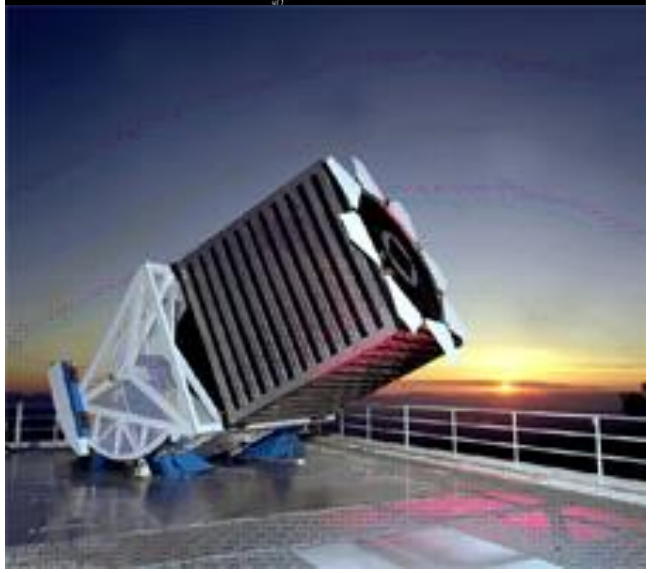


- Strong parallels between mIF today and astronomy 25 years ago
- Need to scale and organize data, multicolor photometry, image segmentation, spatial statistics

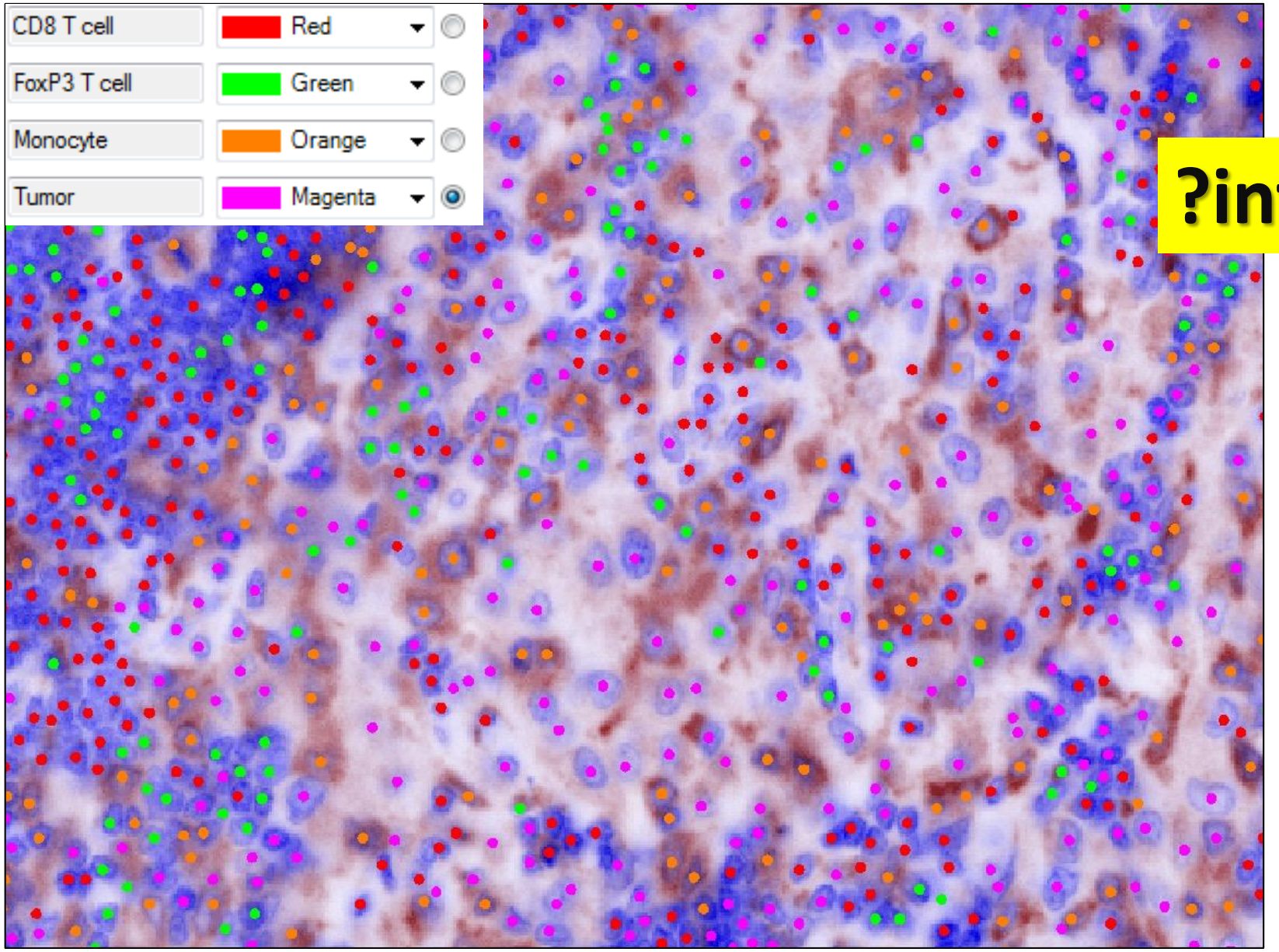
Sloan Digital Sky Survey: "The Cosmic Genome Project"

- Public database and spectrograph built at JHU (SkyServer, images with spatial statistics, and catalogues)
- Started in 1992, finished in 2008: The world's most used astronomy facility today (2.8B web hits in 16 years)
- *We have modified the SkyServer structure to host our tumor-immune maps*

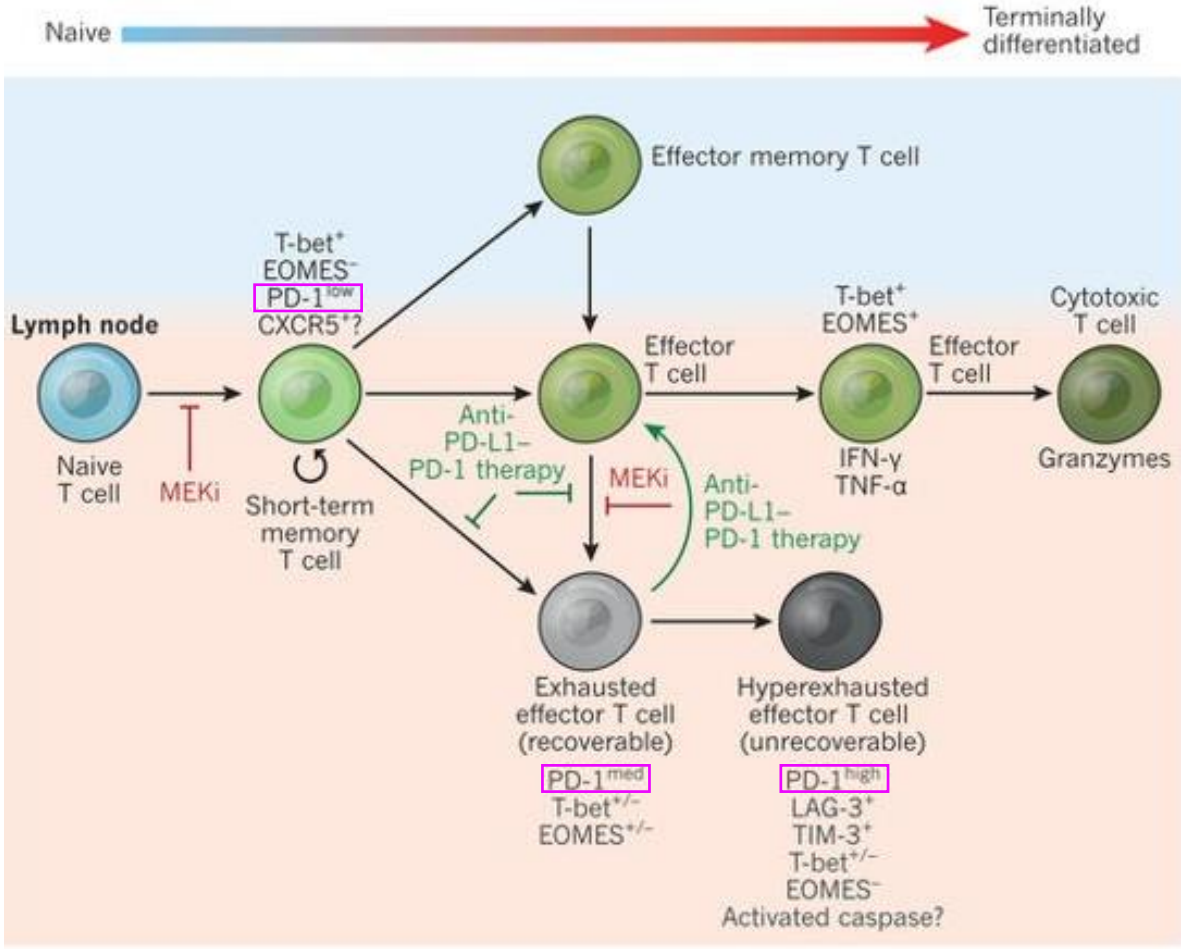




Multiplex IF panel (cell quantification, distance metrics)



CD8+ T-cells expressing PD-1^{low} vs. PD-1^{med} vs. PD-1^{high} indicate distinct populations with different biomarker potential



Chen and Mellman, Nature 2017

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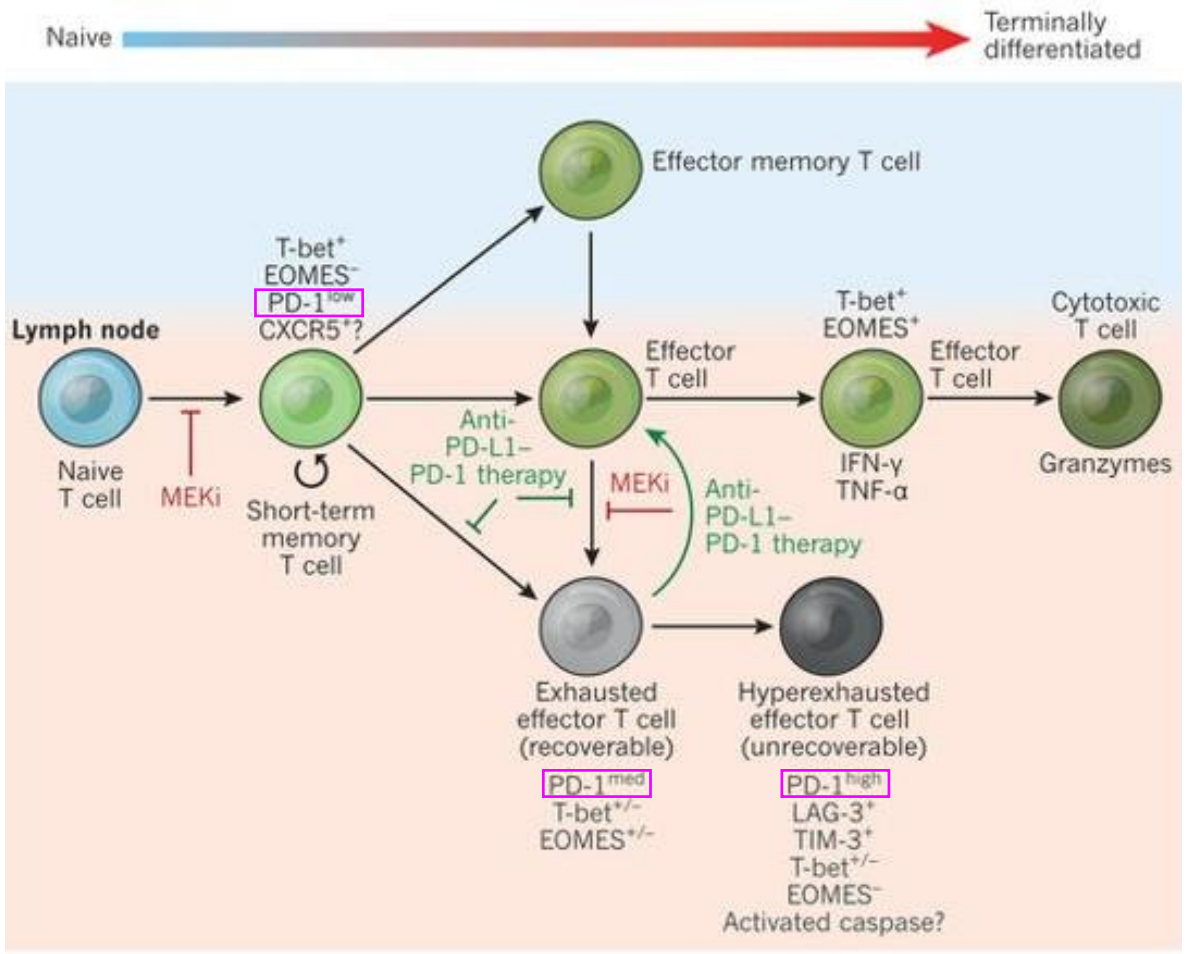
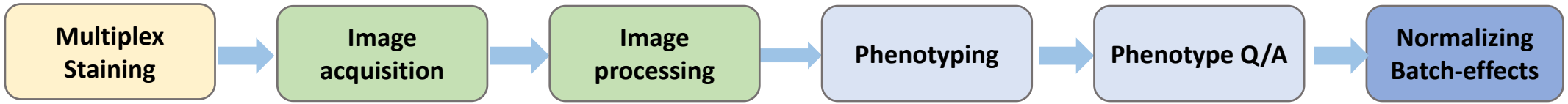


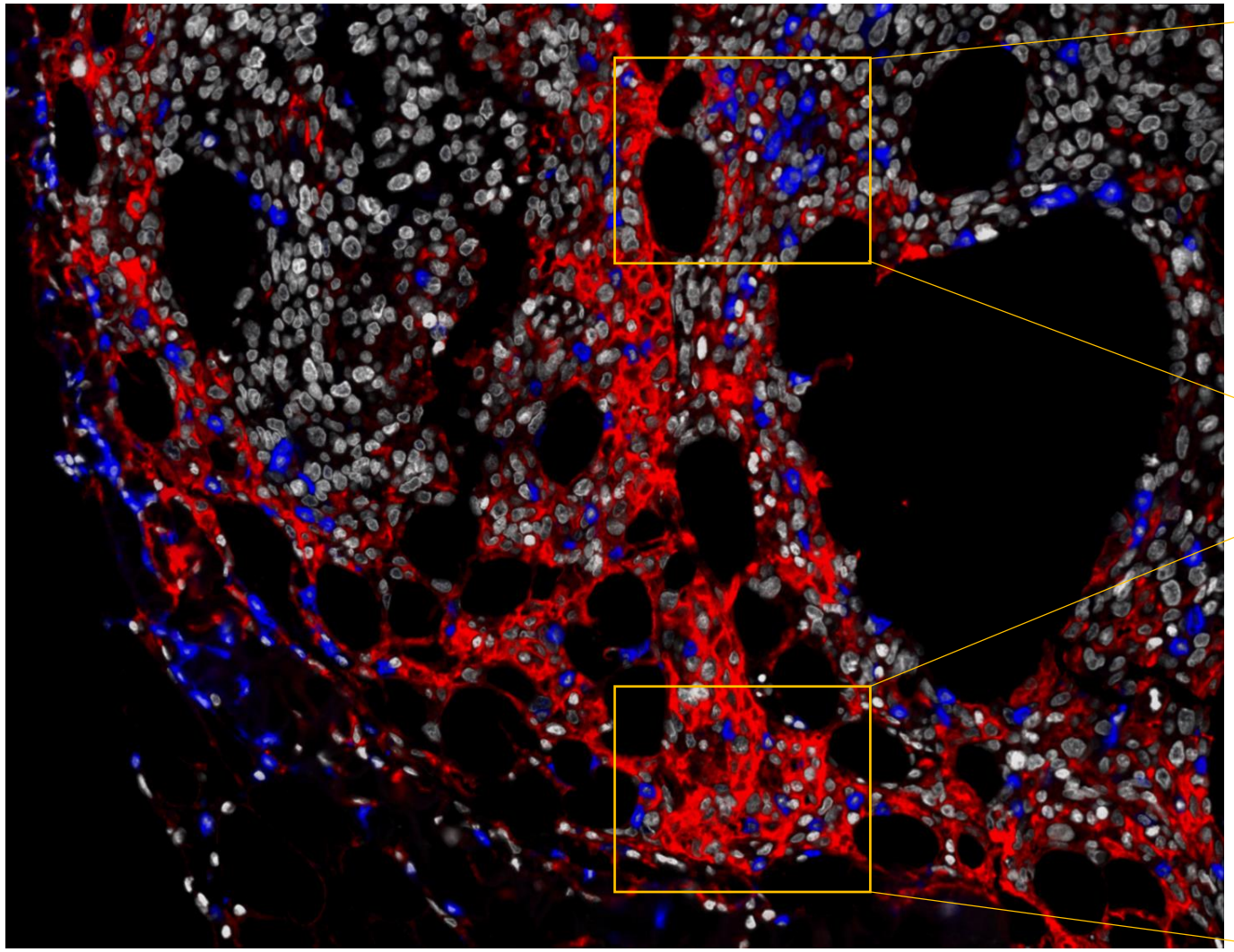
Goal: robust assessment of marker intensity *in situ*

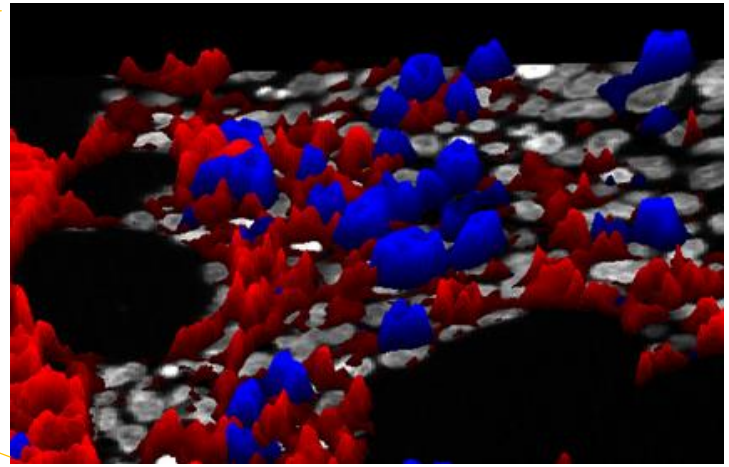
Chen and Mellman, Nature 2017

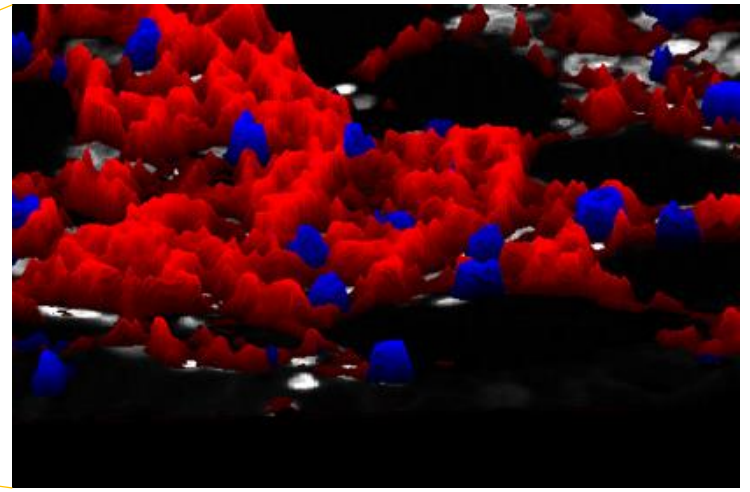
Multiplex IF analysis pipeline



Robust measurements of marker intensity *in situ*



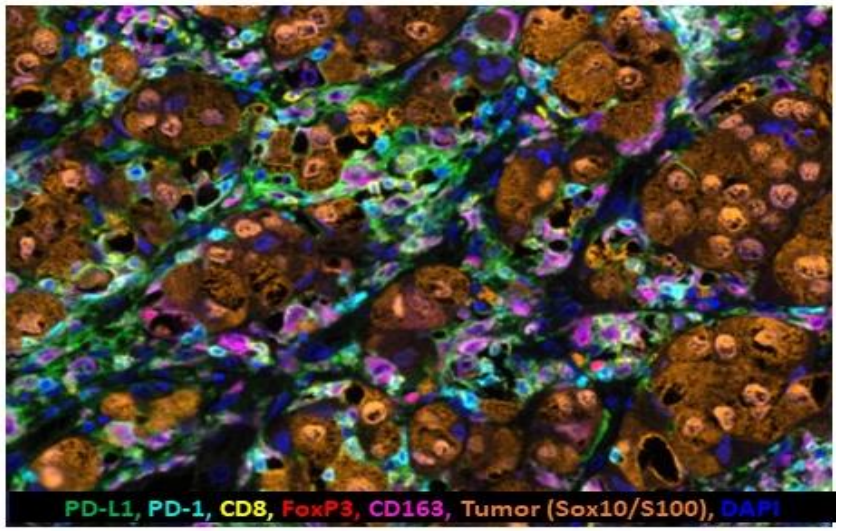




PD-1

PD-L1

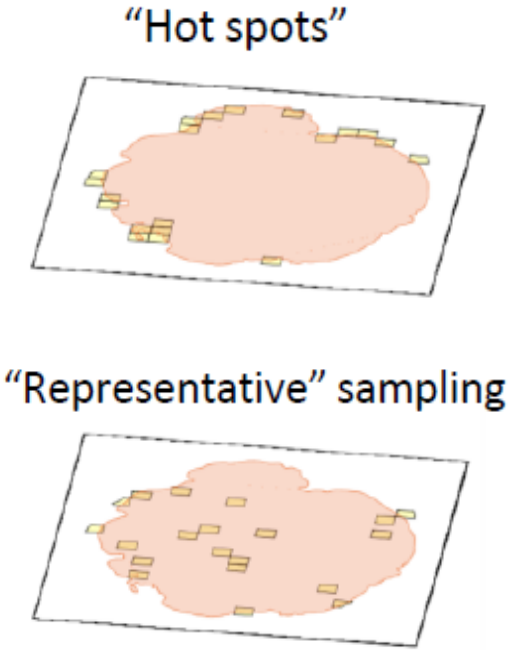
Analysis of pre-treatment specimens from 53 patients with advanced melanoma receiving anti-PD-1



Methods: Acquired tiled images across entire TME in an unbiased fashion, and then either:

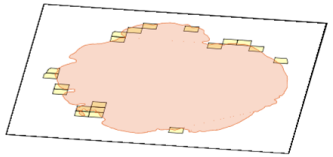
1) Ranked them by CD8+ cell density in decreasing order

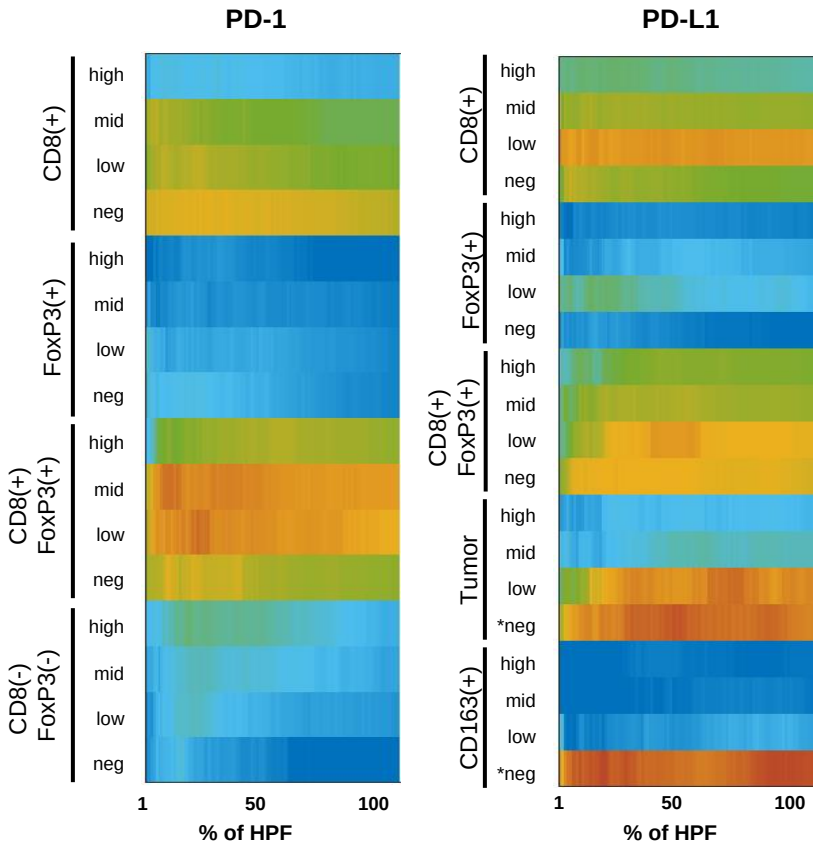
2) Assessed “representative” fields, mixed between inflamed vs. non-inflamed and central vs. peripheral areas of tumor



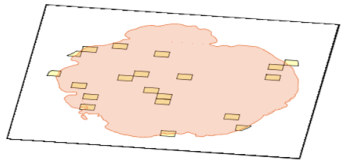
Analysis of pre-treatment specimens from 53 patients with advanced melanoma receiving anti-PD-1

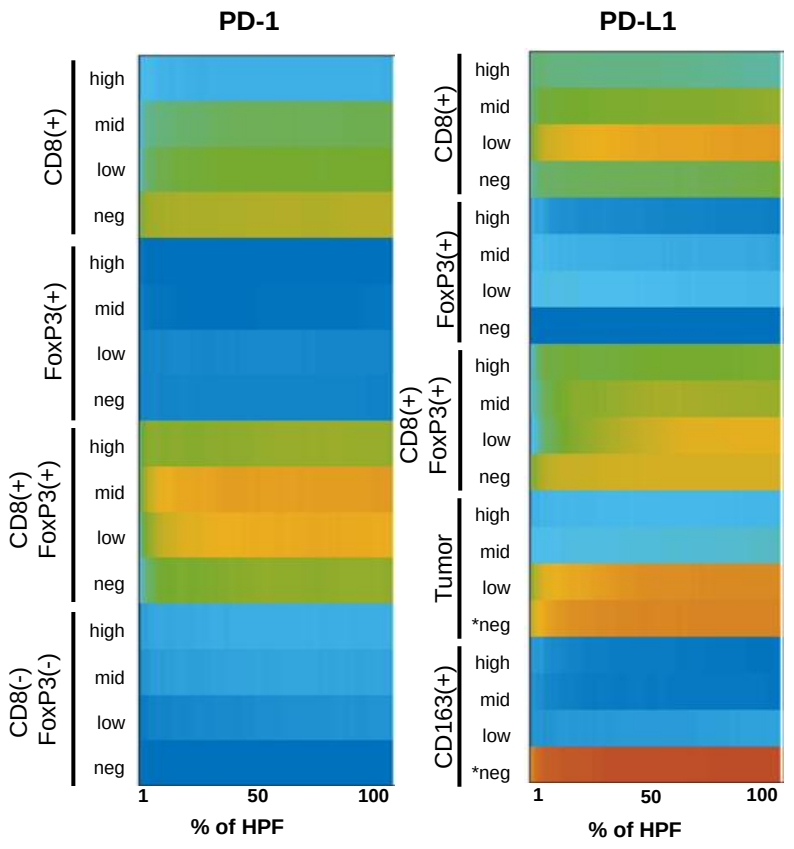
‘Hot-spot’ sampling

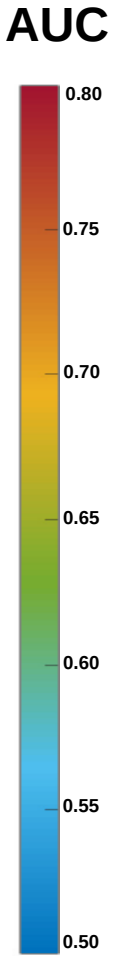




‘Representative’ sampling







Berry, et al. *Science*, 2021

Complete quantitative analysis

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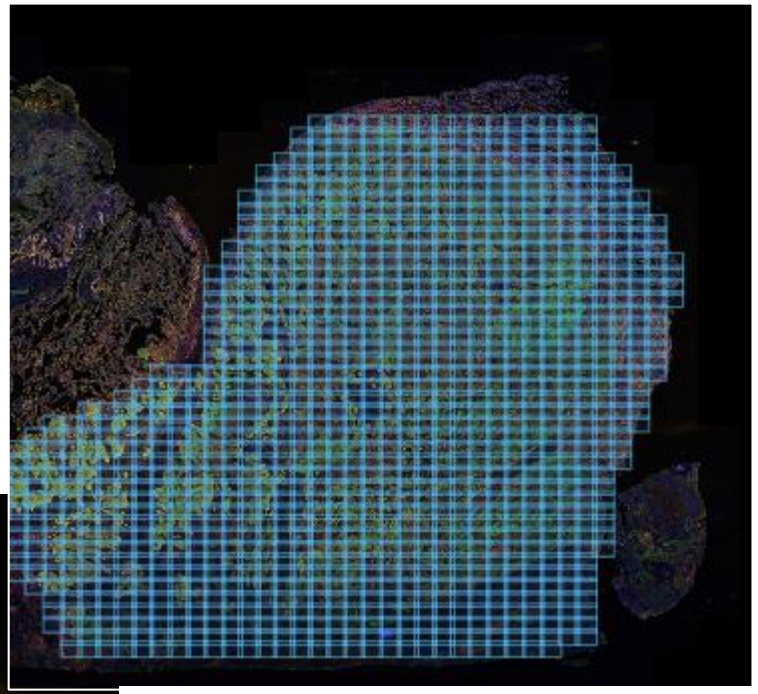
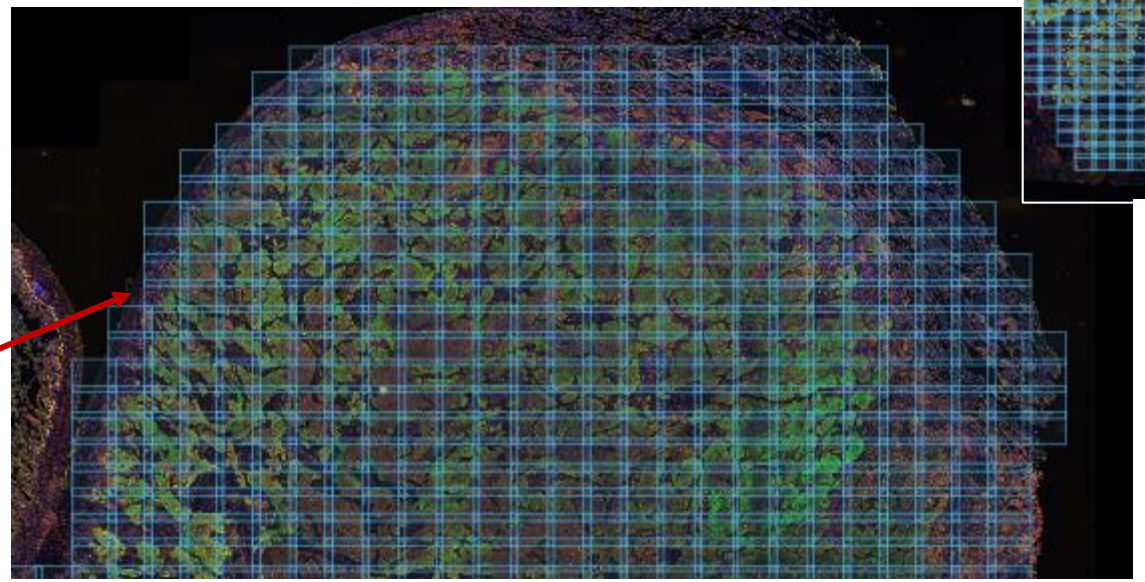
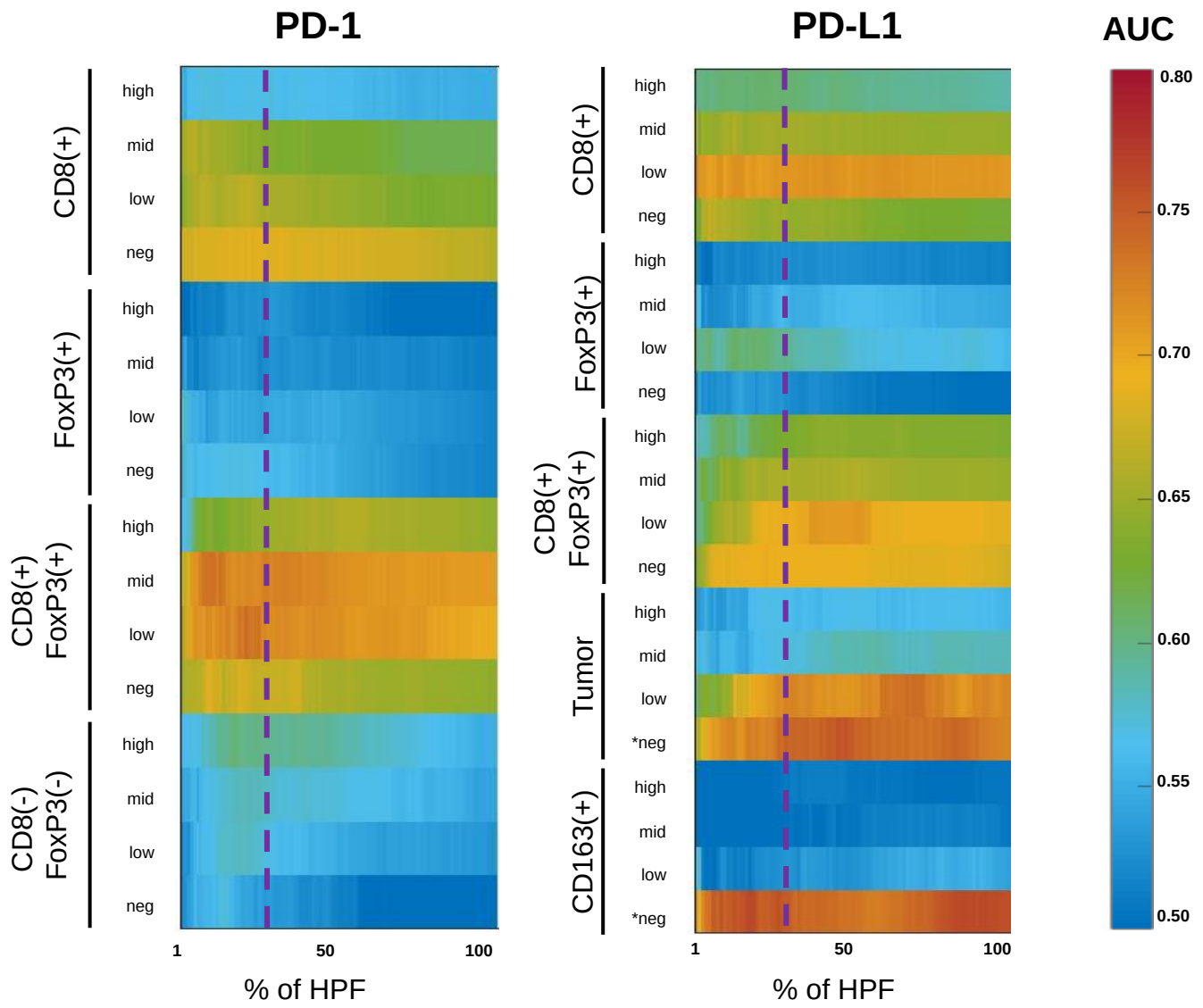




Each square= 1 HPF*

AUC heat maps for response to therapy



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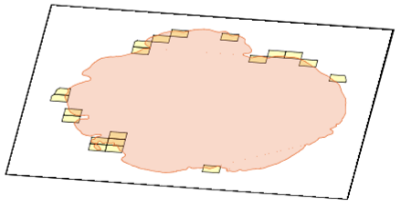
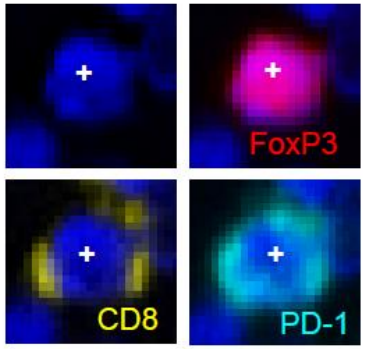
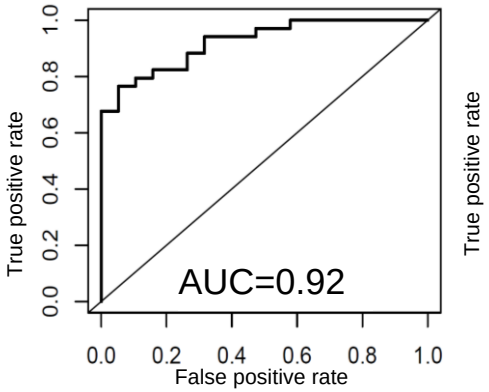


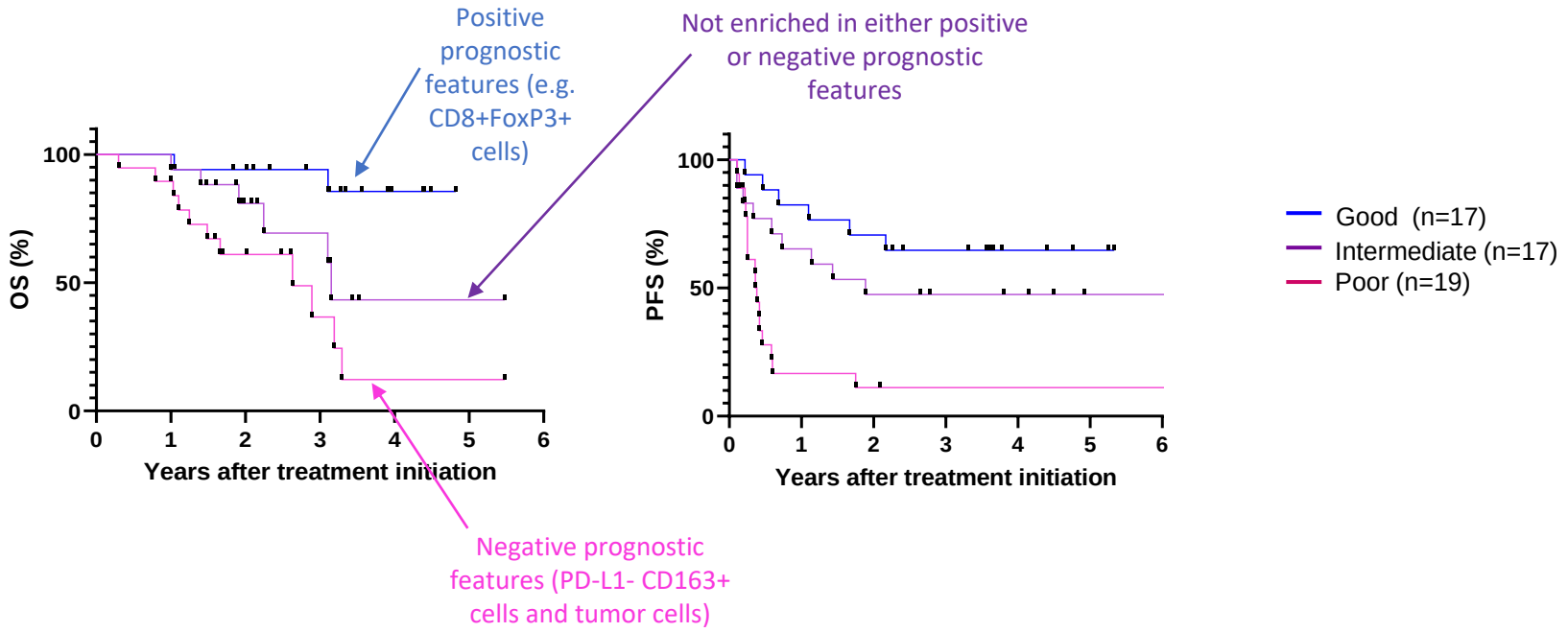


- 30% of TME sampled
- Could consider optimizing different sampling strategies or % sampled for different markers

A combination of key TME features predicts objective response and survival after anti-PD-1 therapy

Discovery Cohort

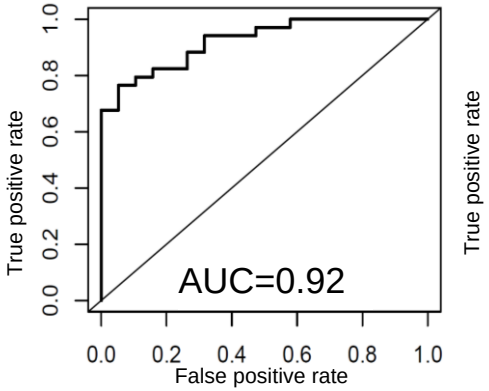


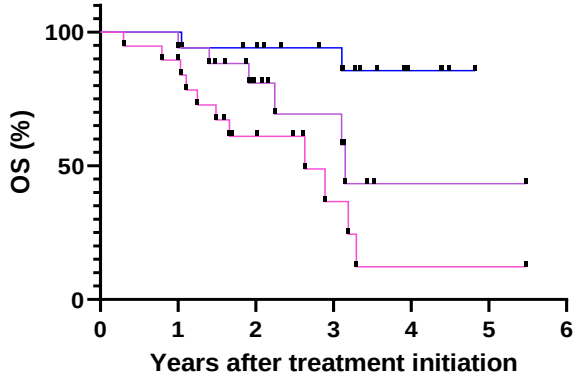


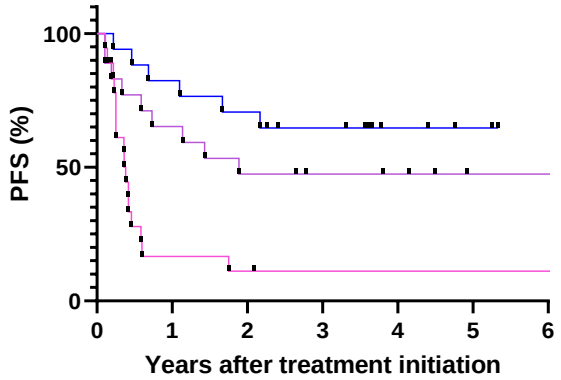
Berry, et al. *Science*, 2021

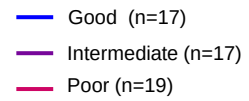
ROC curves for objective response and long-term outcomes after anti-PD1

Discovery Cohort

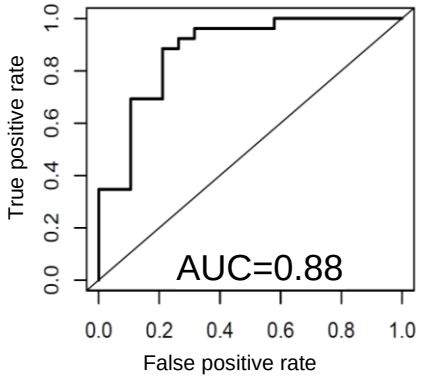


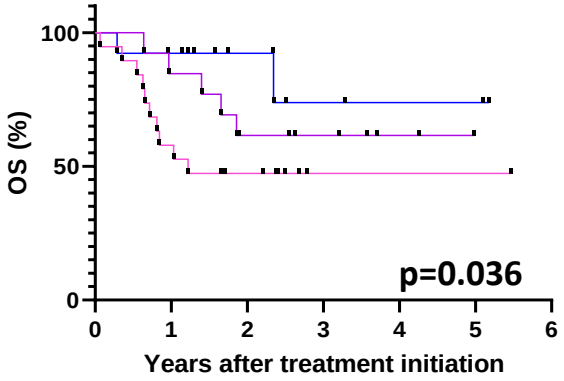


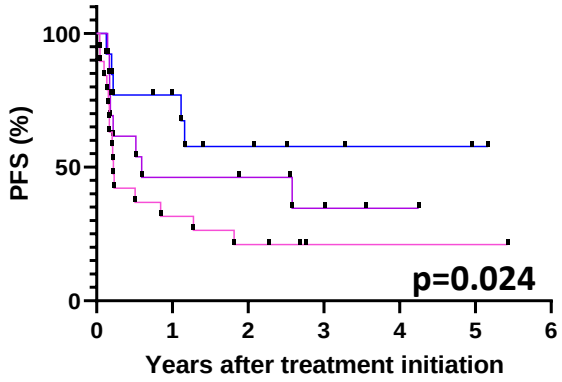


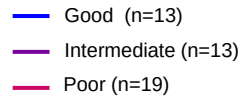


Validation Cohort







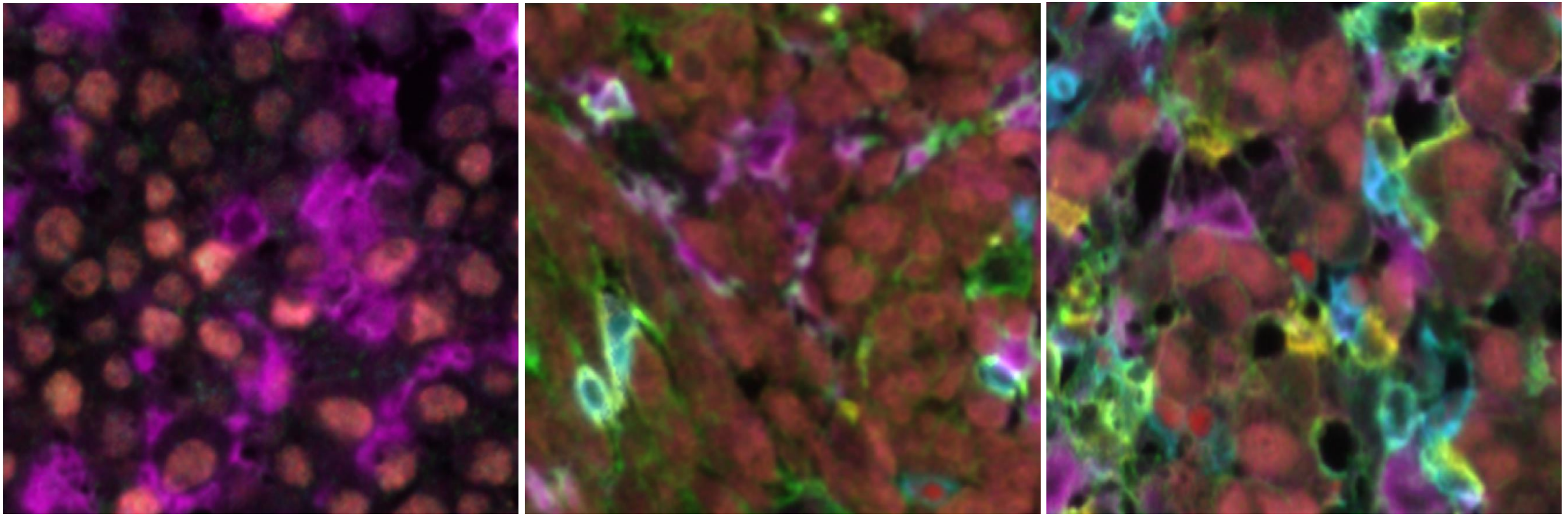


Berry, et al. *Science*, 2021

Poor prognosis

Intermediate prognosis

Good prognosis



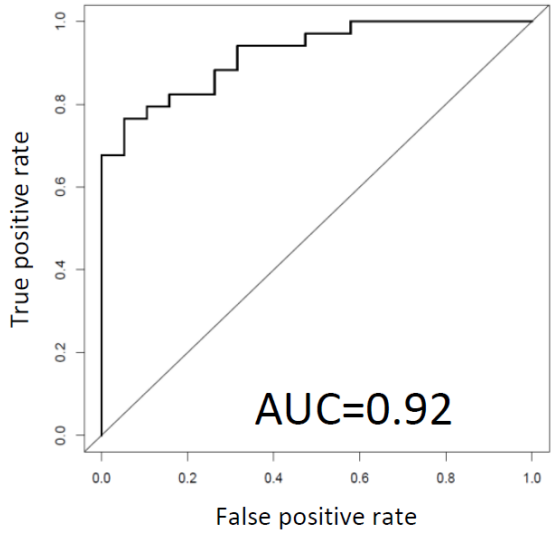
PD-L1, CD8, FoxP3, Tumor, PD-1, CD163

Pre-treatment predictors of checkpoint blockade response and resistance in advanced melanoma

Individual features

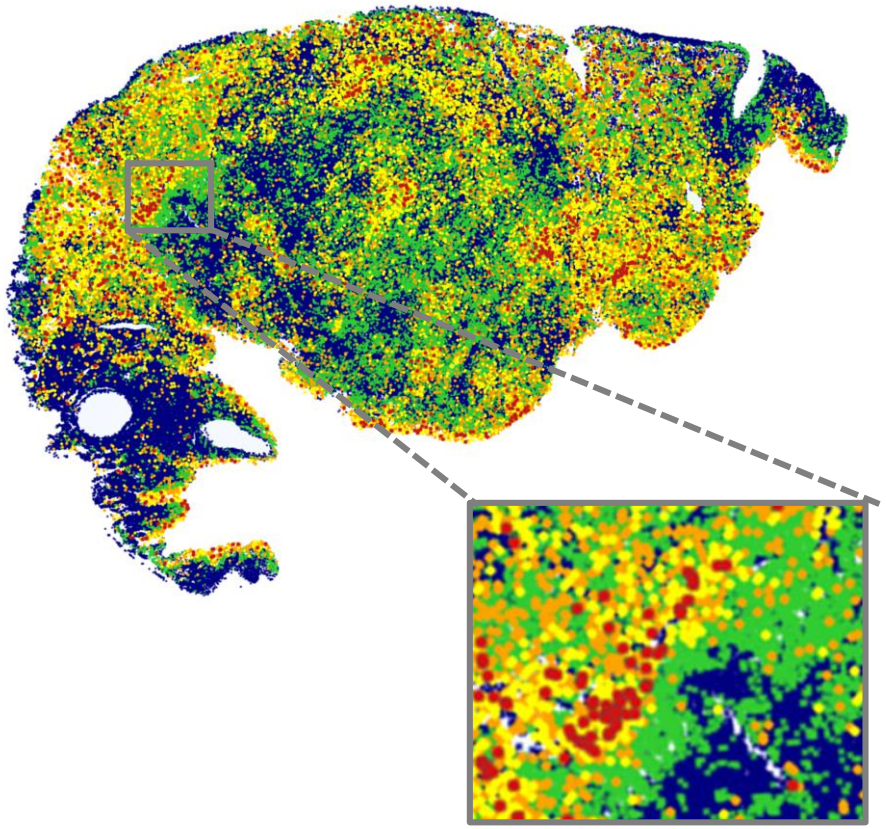
Feature ranking	Feature	Assoc. with outcome	AUCs
1	CD163+PD-L1^{neg}	-	0.75
2	Tumor PD-L1^{neg}	-	0.74
3	CD8+FoxP3+PD-1^{mid}	+	0.73
4	Tumor PD-L1^{low}	+	0.72
5	CD8+FoxP3+PD-1^{low}	+	0.72
6	CD8+PD-L1^{low}	+	0.71
7	CD8+FoxP3+	+	0.71
8	CD8+FoxP3+PD-L1^{low}	+	0.70
9	CD8+FoxP3+PD-L1^{neg}	+	0.69
10	Tumor	-	0.69

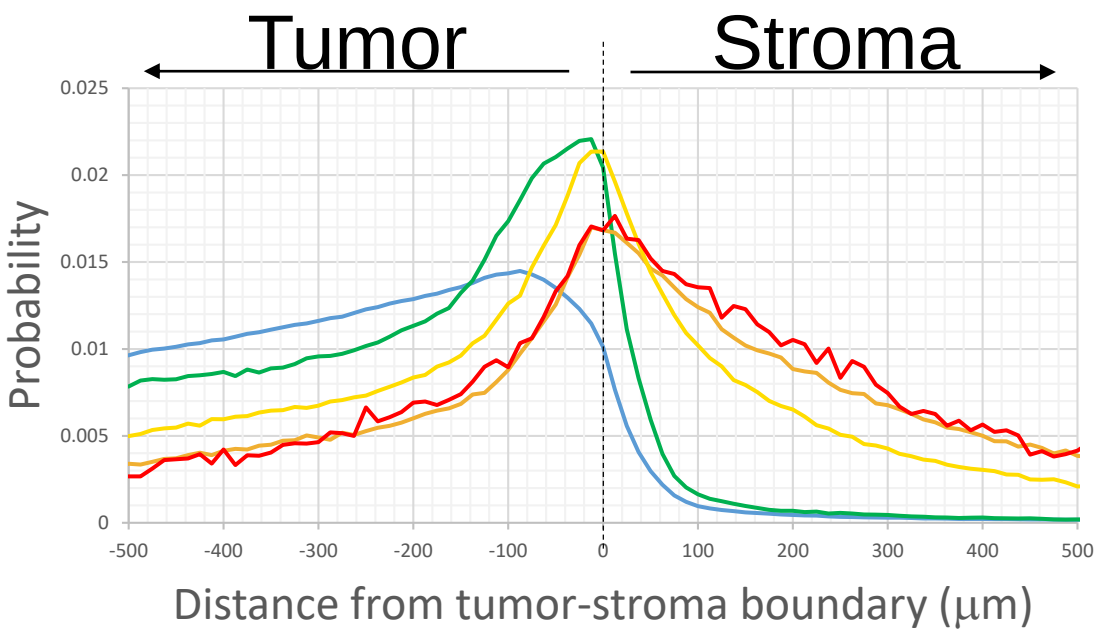
Combining the positive and negative features



S. Berry, *Science* 2021

Tumor-immune maps localizing CD8+FoxP3+ cells in pre-treatment melanoma specimens





- Tumor PD-L1-
- Tumor PD-L1+
- CD8+PD-1-
- CD8+PD-1+
- CD8+FoxP3+

<u>CD8+FoxP3+ cells</u>:

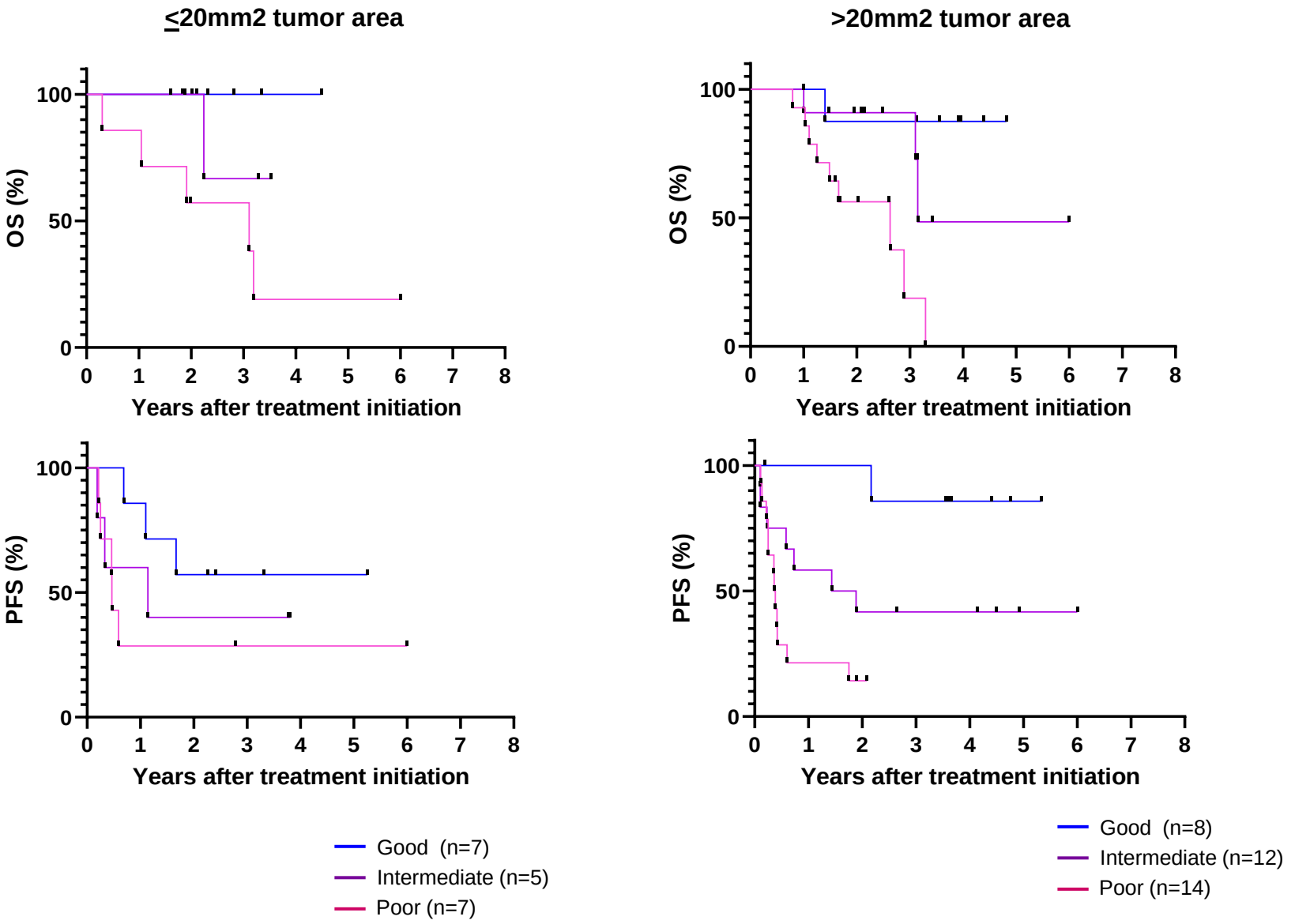
- ~2.5% of all CD8+ cells in the melanoma TME

S. Berry, *Science* 2021

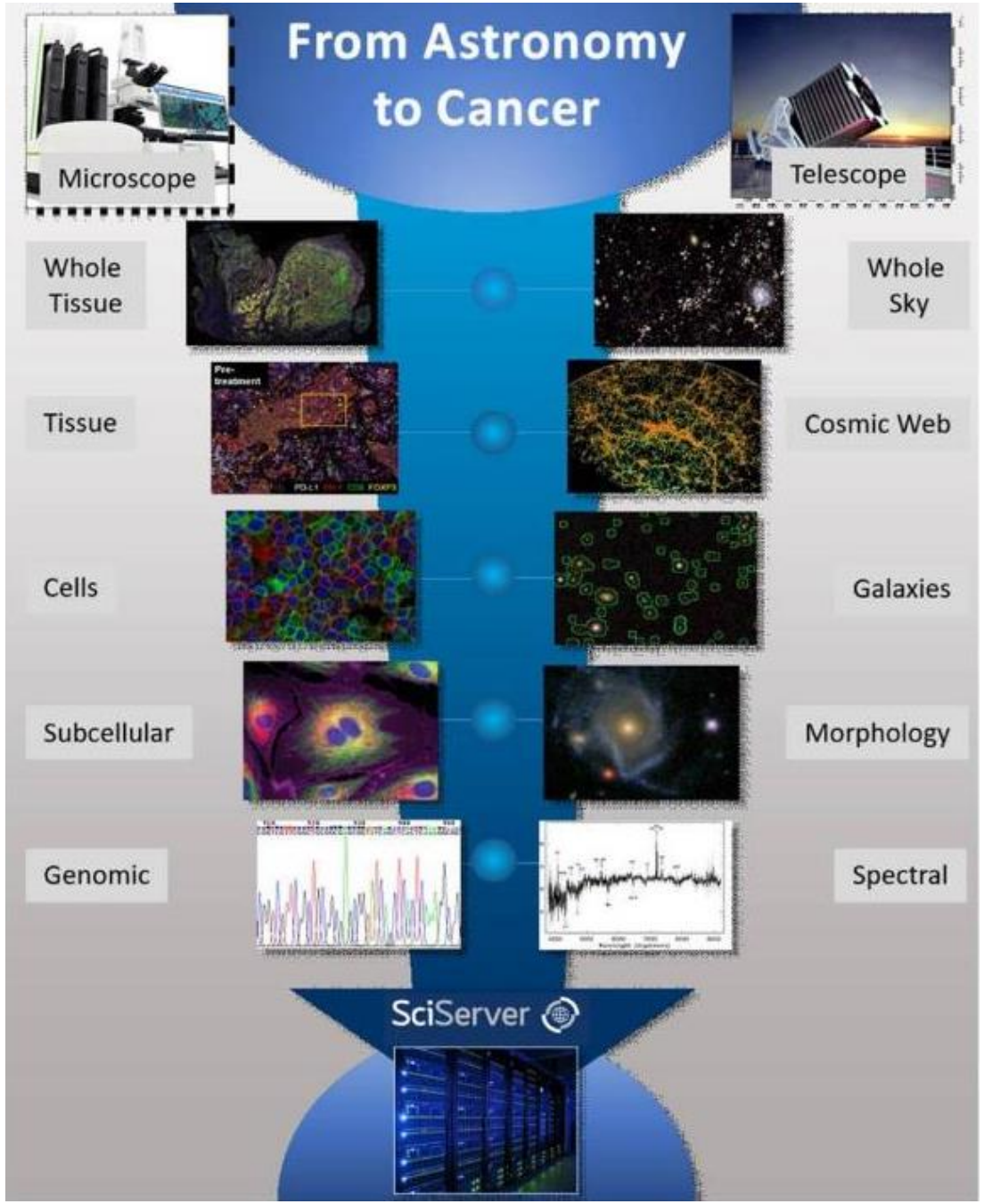
Comparative performance tested for:

- 100% TME sampling < 30% TME sampling
- 4-plex (CD8, PD-L1, CD163, tumor) < 6-plex
- Assessments of PD-1 and PD-L1 pos vs. neg < PD-1 or PD-L1$^{low, mid, high}$
- Smaller (<20 mm^2) = larger specimens

Small specimens are adequate for analysis



- Minimum 5 mm^2 tumor surface area for inclusion
- 20 mm^2 in surface area was chosen because it represents the size of 3 core biopsies (each 1 mm x 15 mm in size) with ~50% tumor in each core.
- Median size across both discovery and validation cohorts = 38 mm^2, average 68 mm^2



AstroPath platform can produce petabytes of tissue imaging data

- Developed a unique facility to produce petabytes of robust tissue imaging data (Discovery + Validation for melanoma specimens includes 131,892 HPF with over 72,847,963 distinct cells)
- Expanding to numerous pre- and on-treatment tumor types
- Adding in genomics and spatial transcriptomic data for treated samples
- Spatially mapping TCGA specimens and linking to TCGA

Acknowledgements

Taube Lab members

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