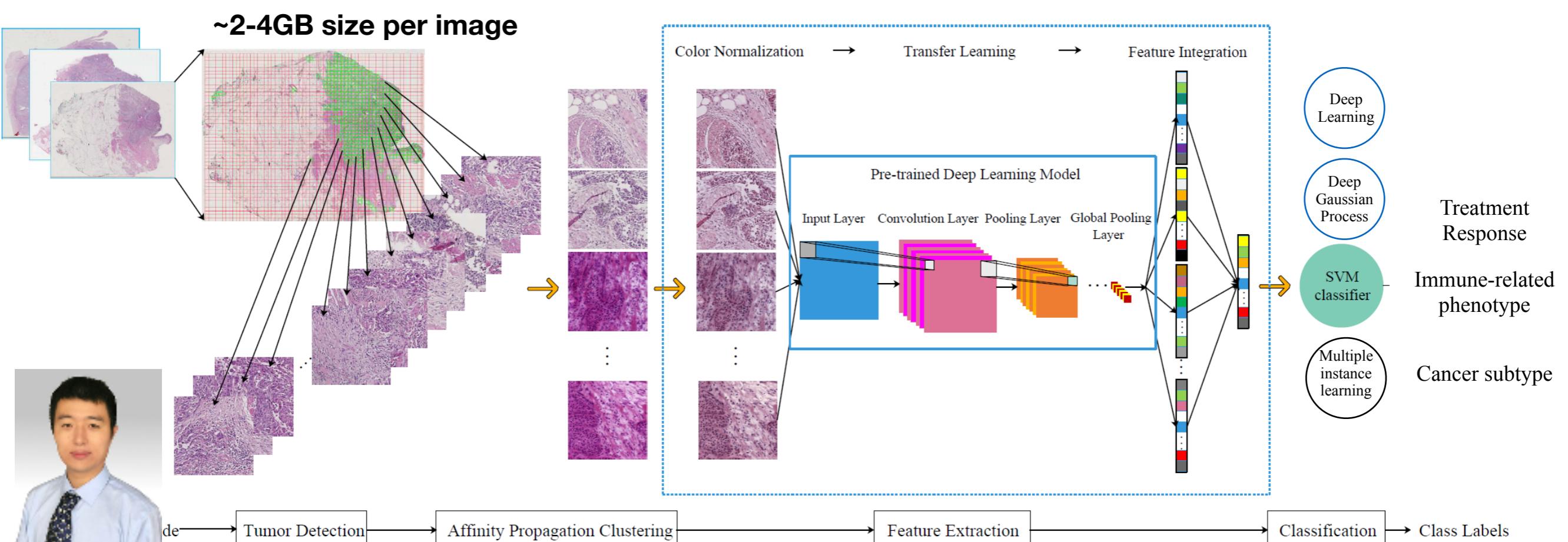


Machine Learning and AI driven Image-based biomarker discovery and predictive modeling to predict immunotherapy response

- **Motivation:** Develop predictive biomarkers based on whole slide histopathological image data which is universally available for the most of patients to predict response to available chemotherapy, targeted therapy and/or immunotherapy
- **Objective:** Develop an algorithm based on whole slide histopathology image to predict immunotherapy response.
 - Input: Whole slide histopathological image
 - Output: a) Immunotherapy response/phenotype (e.g., TMB, MSI, TILs status), 2) image-based biomarkers



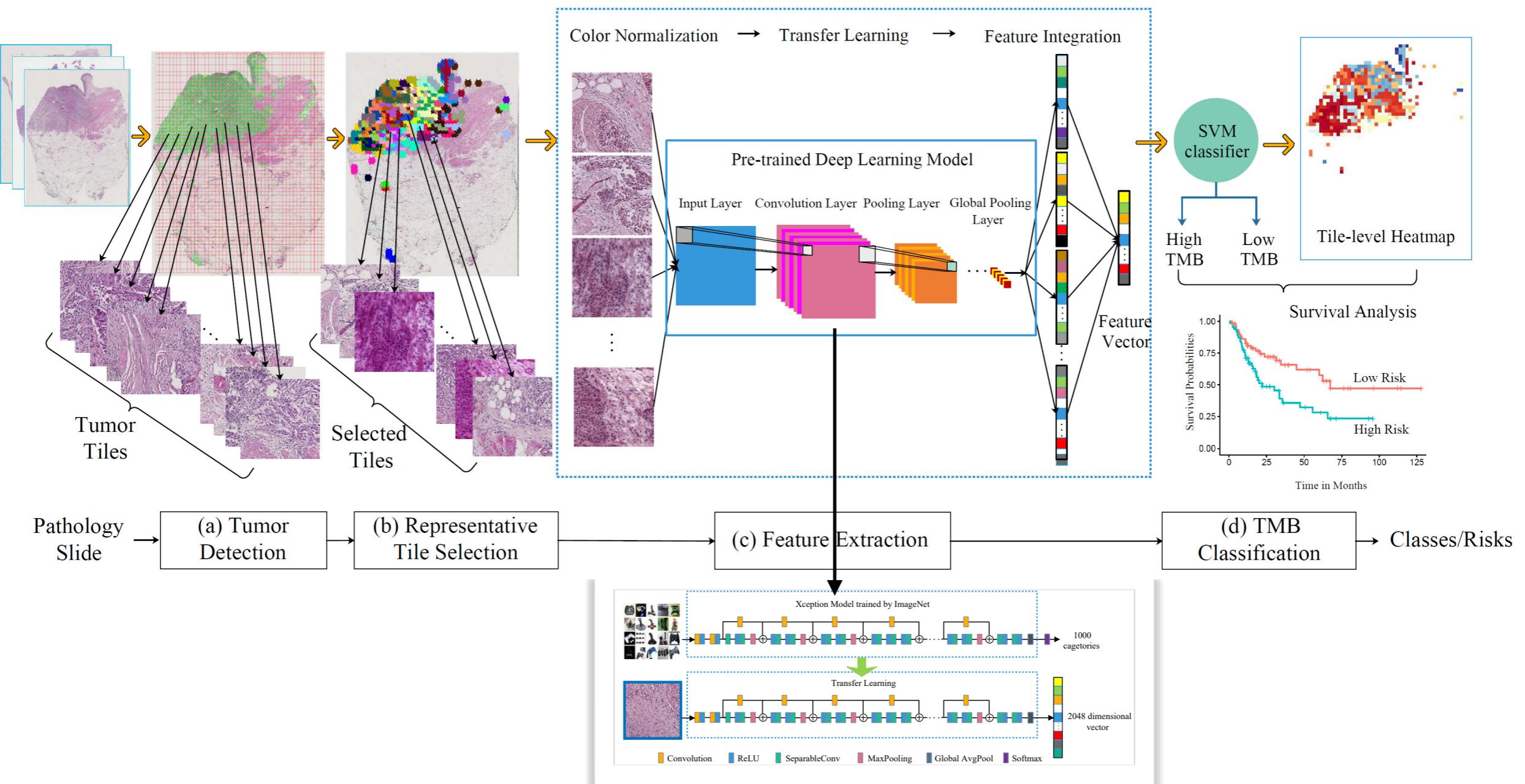
Our approach

• Input

- Whole slide histopathological image from TCGA bladder cancer
- Tumor Mutation Burden (TMB) status (Galsky et. al., Clinical Cancer Research 2020 showing TMB and PDL1 status is associated with anti-PD1 response in bladder cancer)

• Output

- TMB status (e.g., TMB high or low)
- Spatial heterogeneity of TMB status within a tumor



Unpublished results

Carefully designed Deep Learning/AI models can accurately predict TMB status using whole slide image

- Our proposed method could achieve the overall best accuracy and AUC scores compared to the state of the art methods to predict TMB status.

Table 1: TMB prediction comparative results

Cohorts	Methods	ACC (%)	SPE (%)	SEN (%)	AUC (95% CI)
TCGA-BLCA	LBP+SVM	60.47	64.52	56.59	0.623 (0.550-0.689)
	Designed CNN	61.66	62.10	61.24	0.651 (0.581-0.741)
	VGG16-TL2	65.22	66.94	63.57	0.707 (0.639-0.766)
	MIL [46]	58.89	58.87	58.91	0.647 (0.577-0.710)
	Resnet18 [45]	66.80	65.32	68.22	0.701 (0.638-0.765)
	Proposed	73.12	75.81	70.54	0.752 (0.694-0.810)
TCGA-LUAD	LBP+SVM	66.67	70.00	63.69	0.706 (0.645-0.763)
	Designed CNN	63.82	67.02	60.95	0.667 (0.583-0.741)
	VGG16-TL2	69.85	62.77	76.19	0.703 (0.621-0.766)
	MIL [46]	60.27	60.00	60.51	0.643 (0.578-0.698)
	Resnet18 [45]	67.00	65.00	68.79	0.727 (0.666-0.779)
	Proposed	70.37	67.86	72.61	0.742 (0.679-0.795)

Spatial Heterogeneity of TMB status within a tumor

- We divided the WSI into a small tile and predicted the status of TMB for each tile and measured spatial heterogeneity status using the entropy of TMB-high/low within the WSI.

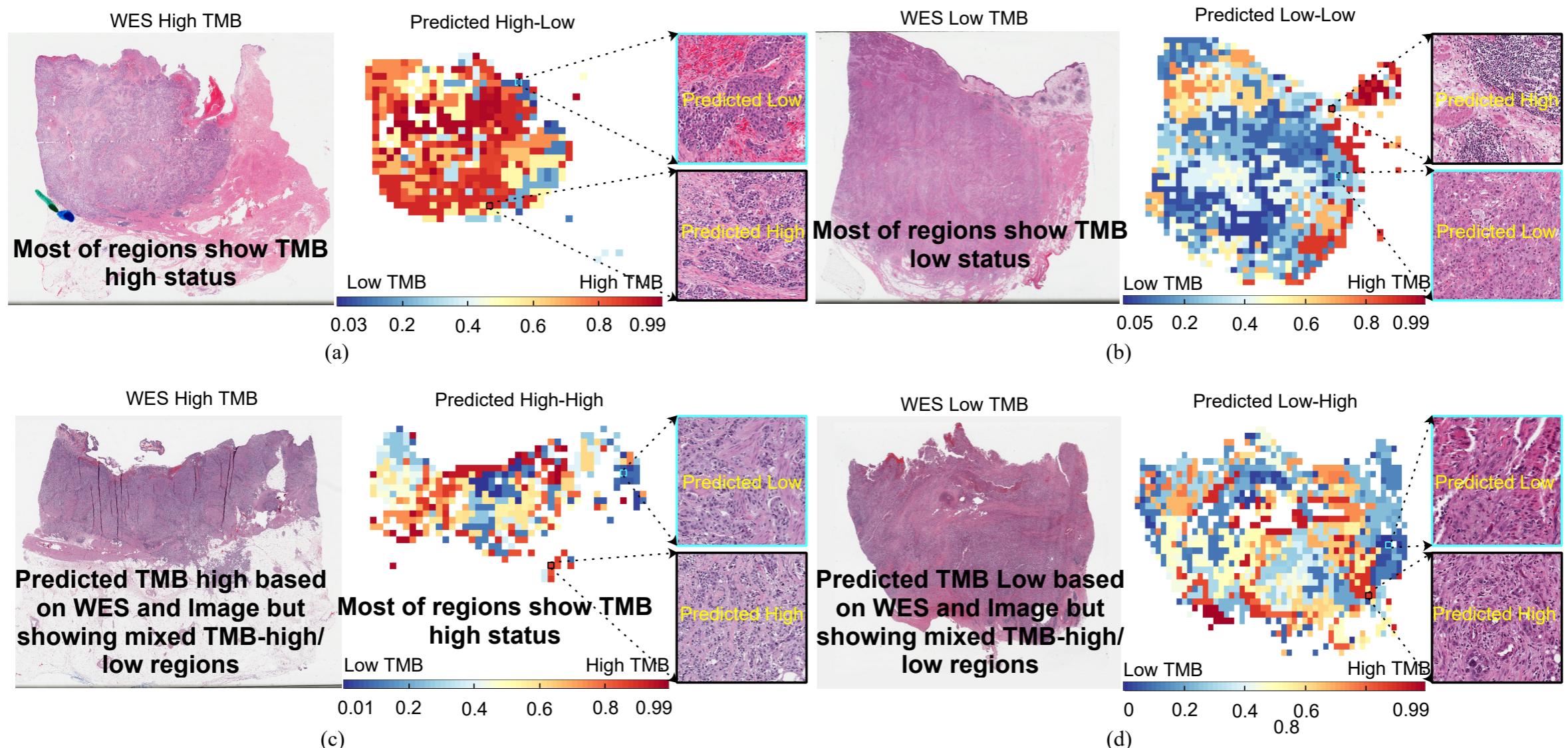
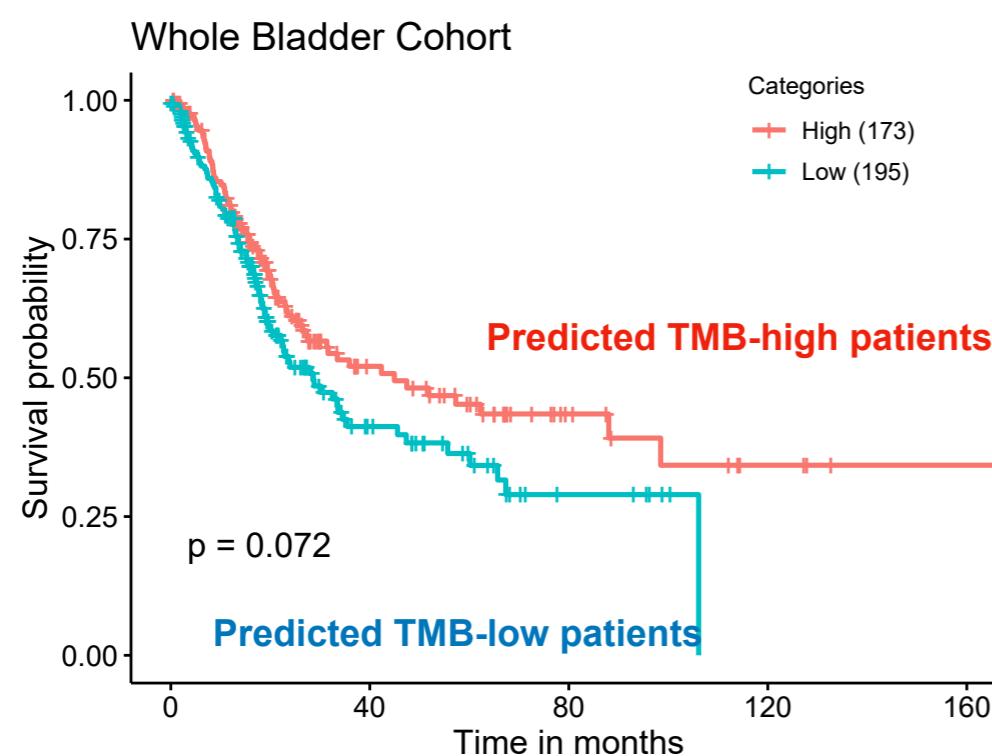


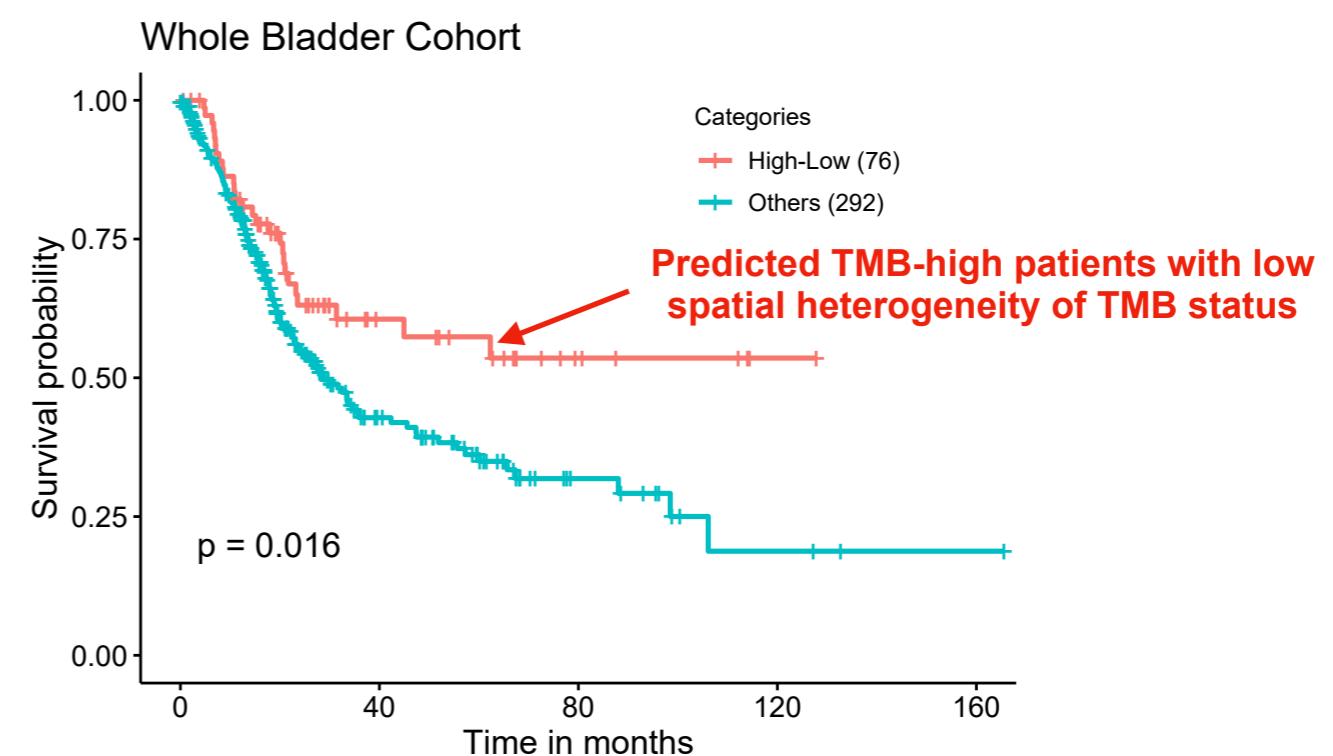
Figure 3: TMB prediction heatmaps. (a) High TMB patient (TCGA-UY-A9PD) predicted as High-Low (i.e., high TMB and low entropy). (b) Low TMB patient (TCGA-FD-A43S) predicted as Low-Low. (c) High TMB patient (TCGA-DK-A3IT) predicted as High-High. (d) Low TMB patient (TCGA-FD-A3B7) predicted as Low-High. High entropy indicates more severe heterogeneity of TMB prediction heatmaps.

Spatial Heterogeneity of TMB status associated with overall survival

- We predicted TMB high/low status for each TCGA BLCA patient using diagnostic WSI.
 - We further divided predicted TMB high patients into TMB-high & spatial heterogeneity low and other subgroups.
 - Predicted TMB-high with low spatial heterogeneity subgroup showed better survival outcome.

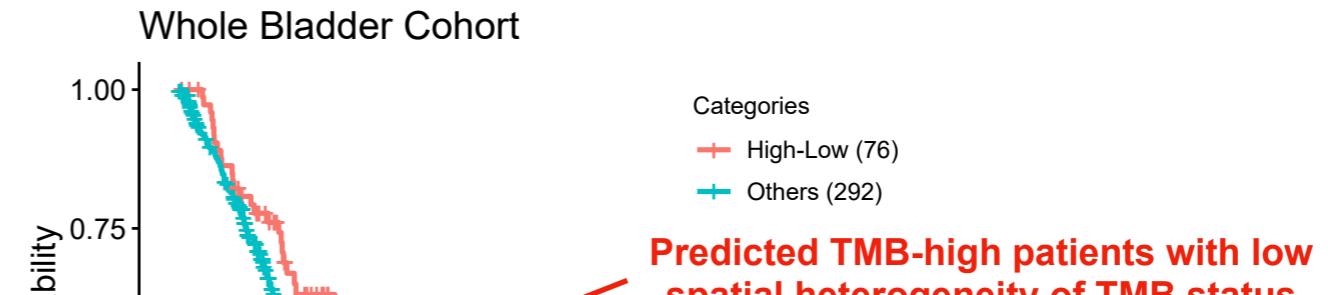
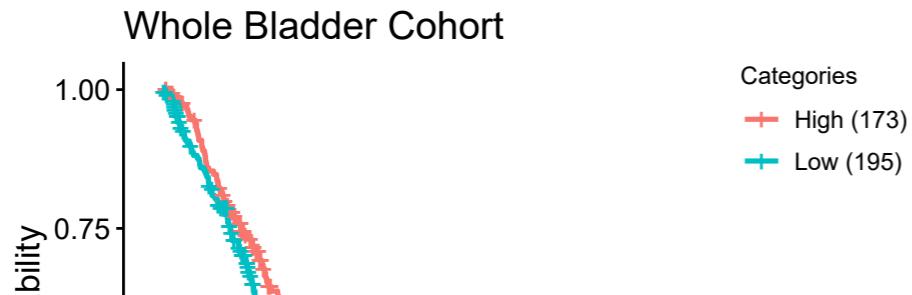


(a)

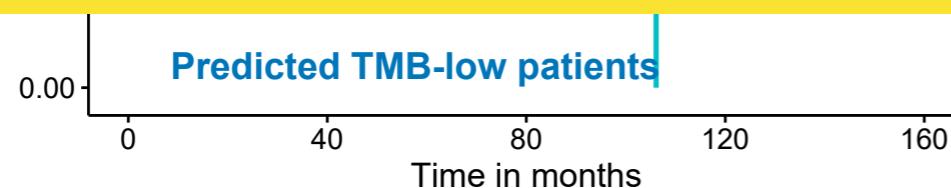


(b)

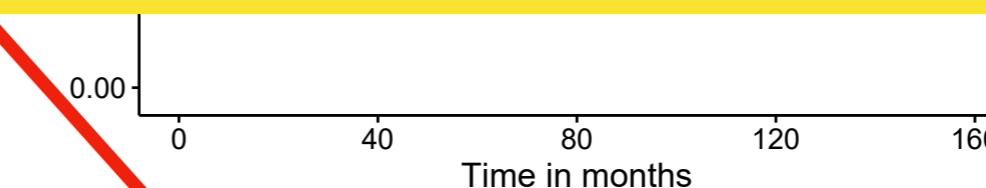
Spatial Heterogeneity of TMB status associated with overall survival



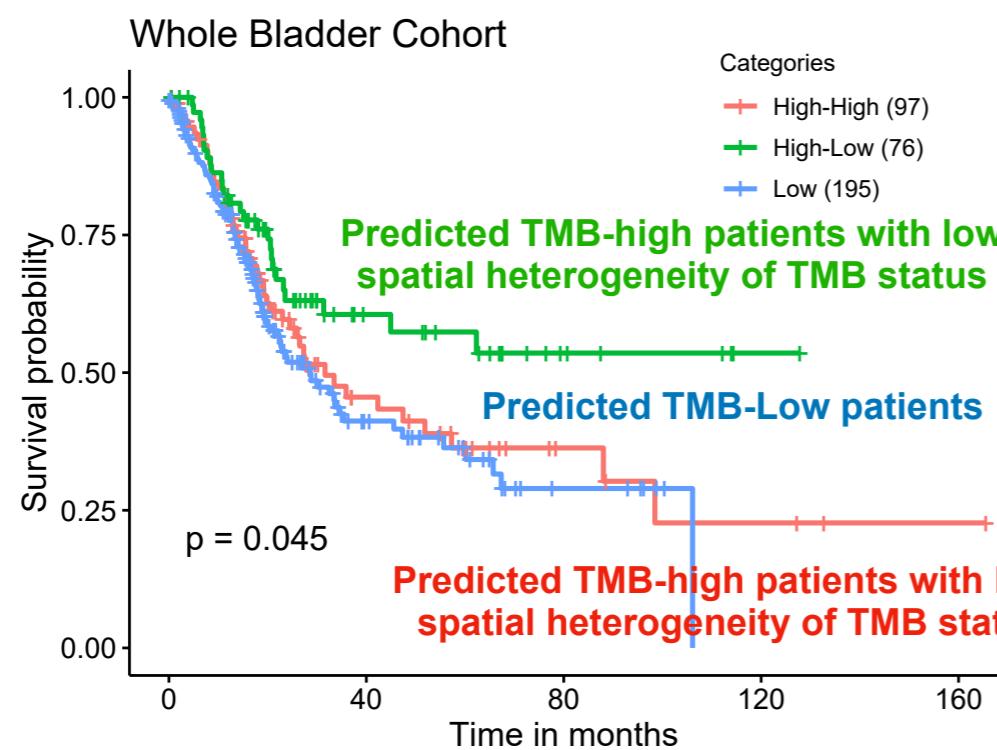
- Incorporating TMB high and spatial heterogeneity status predicted by Deep Learning algorithm with WES-based TMB status could improve patient stratification!



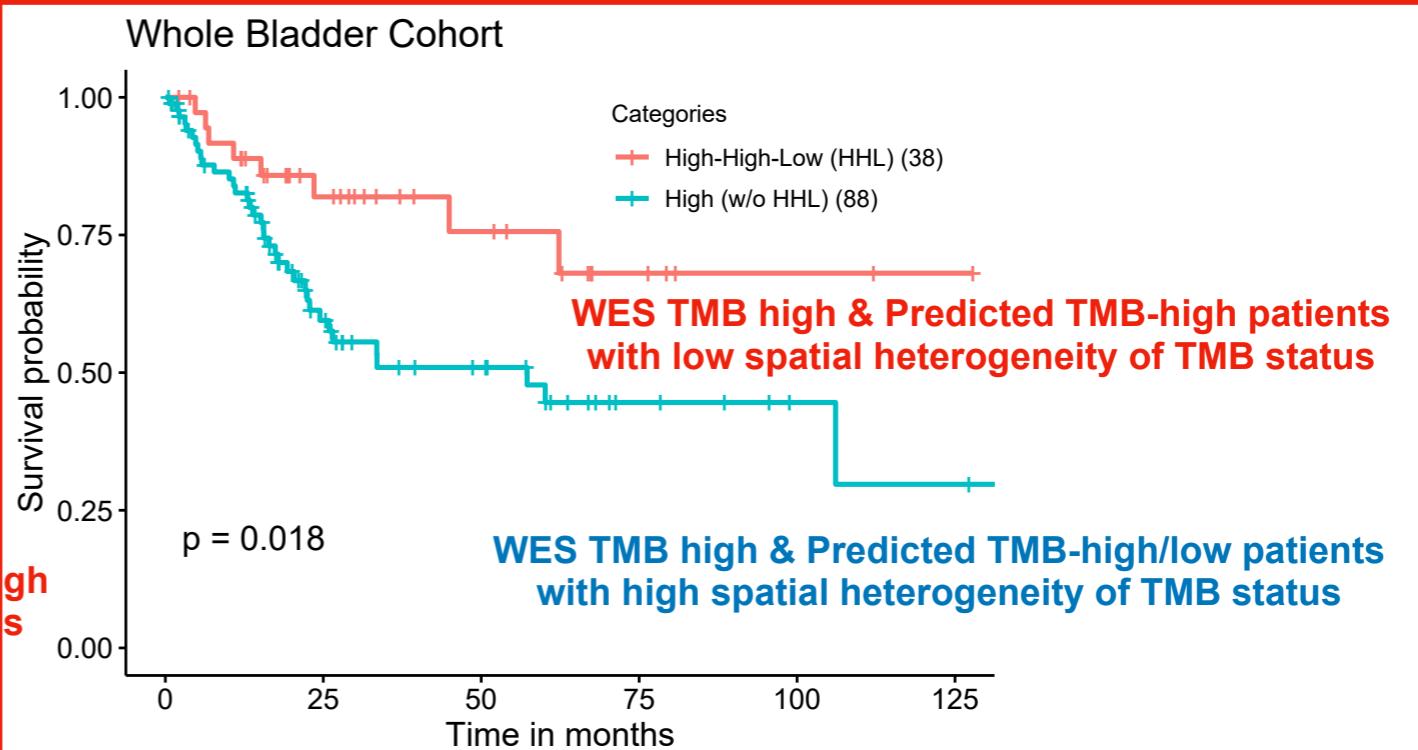
(a)



(b)



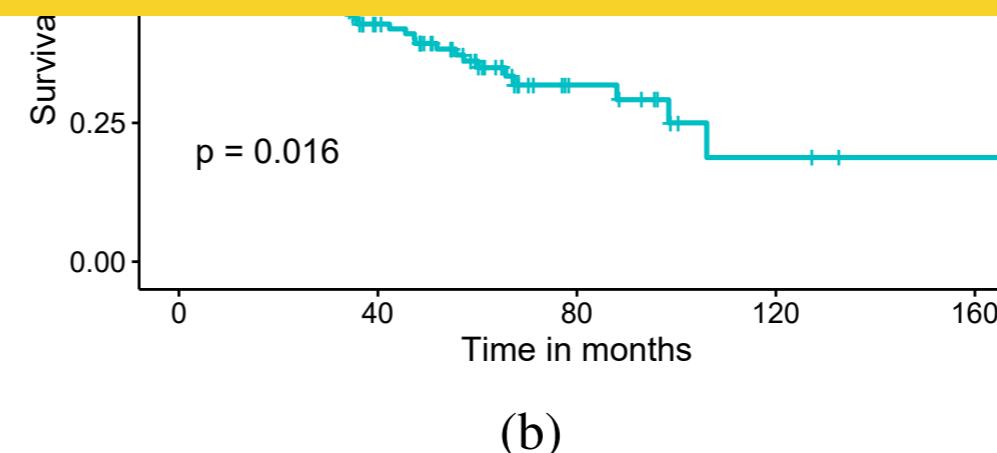
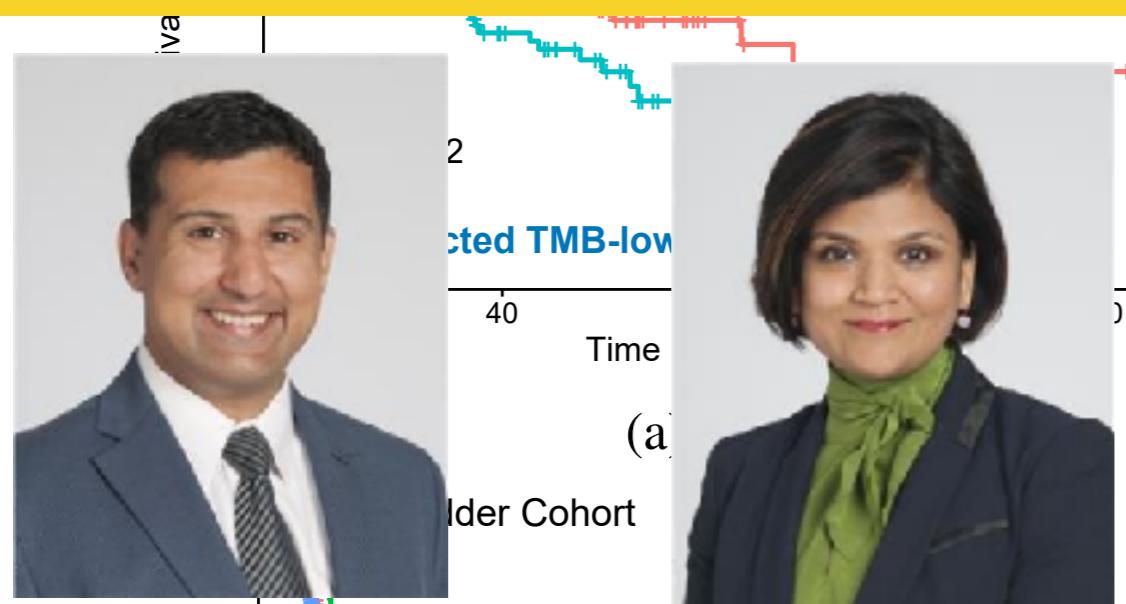
(c)



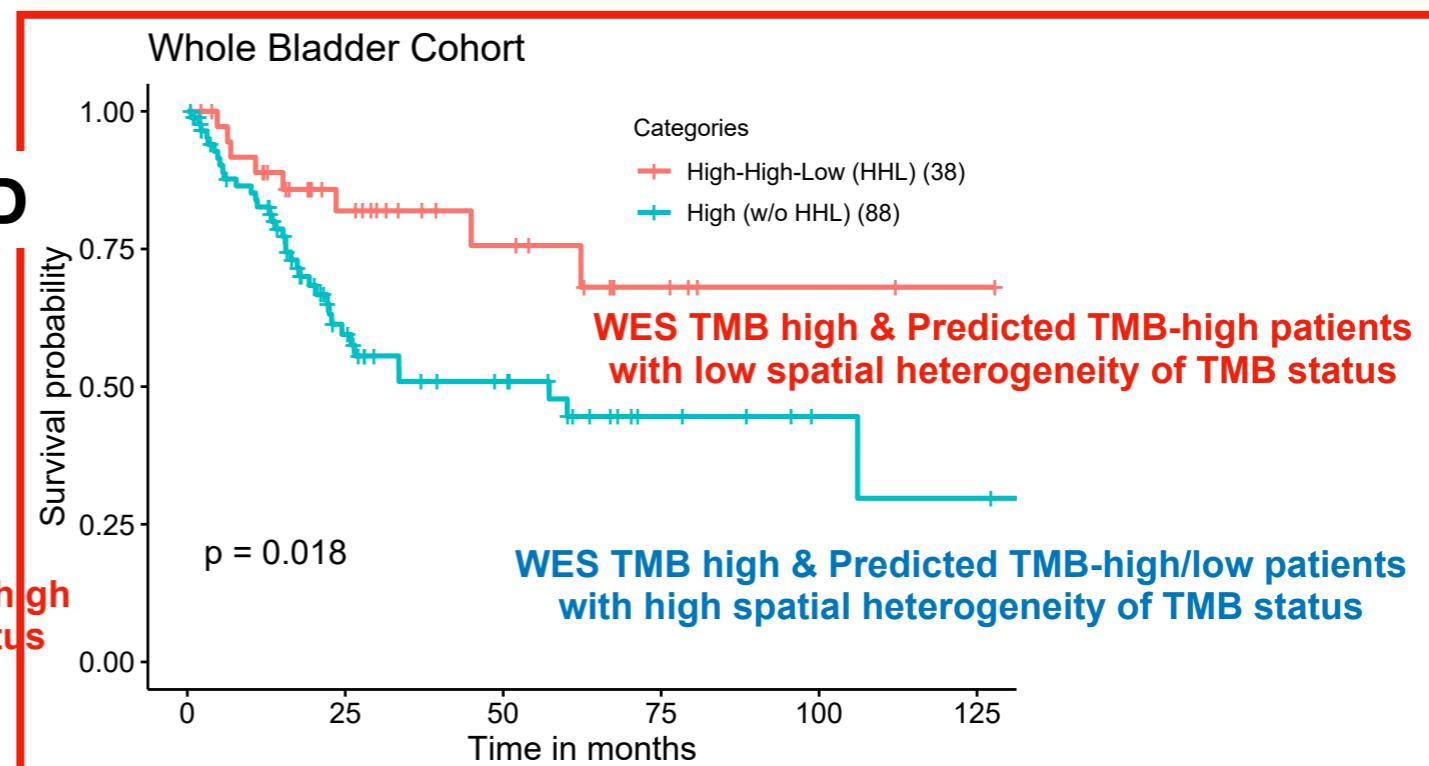
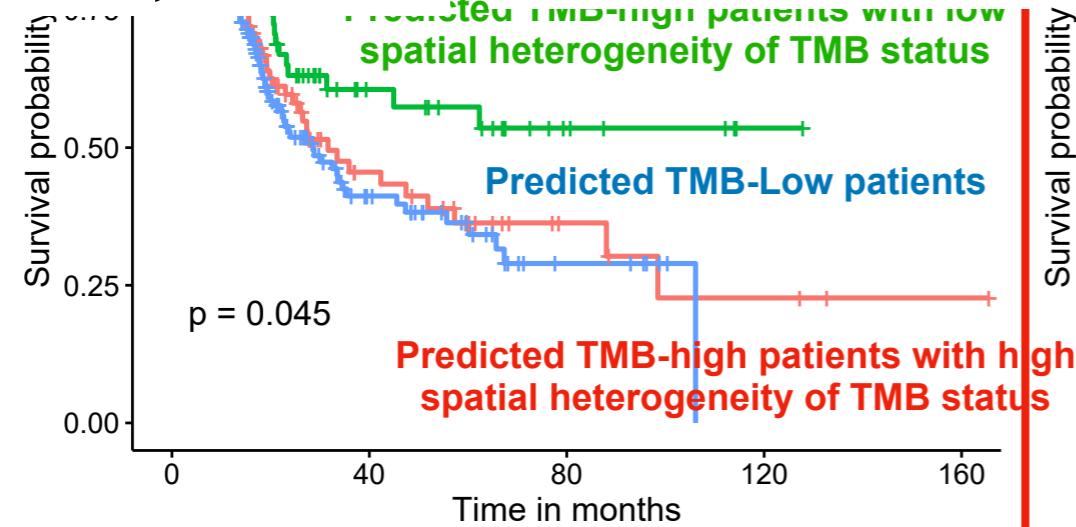
(d)

Spatial Heterogeneity of TMB status associated with overall survival

Currently analyzing Phase II trial Nivolumab with cisplatin and gemcitabine in the neoadjuvant management of MIBC (BLASST-1 Bladder Cancer Signal Seeking Trial-NCT03294304) and open randomized studies of Immune check inhibitor in BCG refractory NMIBC (NCT03711032) and advanced/metastatic BC (NCT01295827, NCT01772004) with Drs. Gupta and Mian with two DOD Translational Team Science and Idea Development awards.



Omar Mian, MD PhD Shilpa Gupta, MD

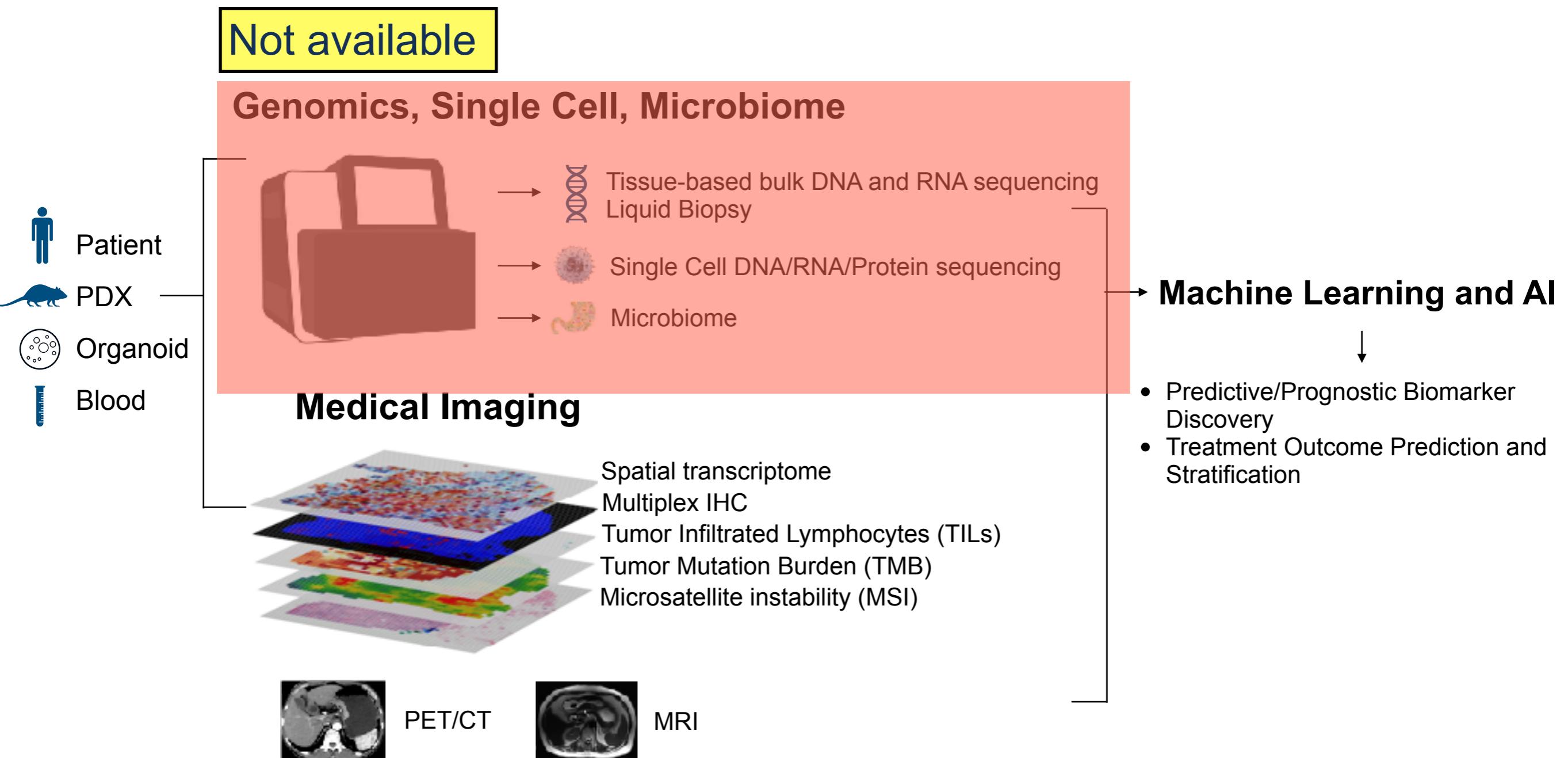


Unpublished results (c)

(d)

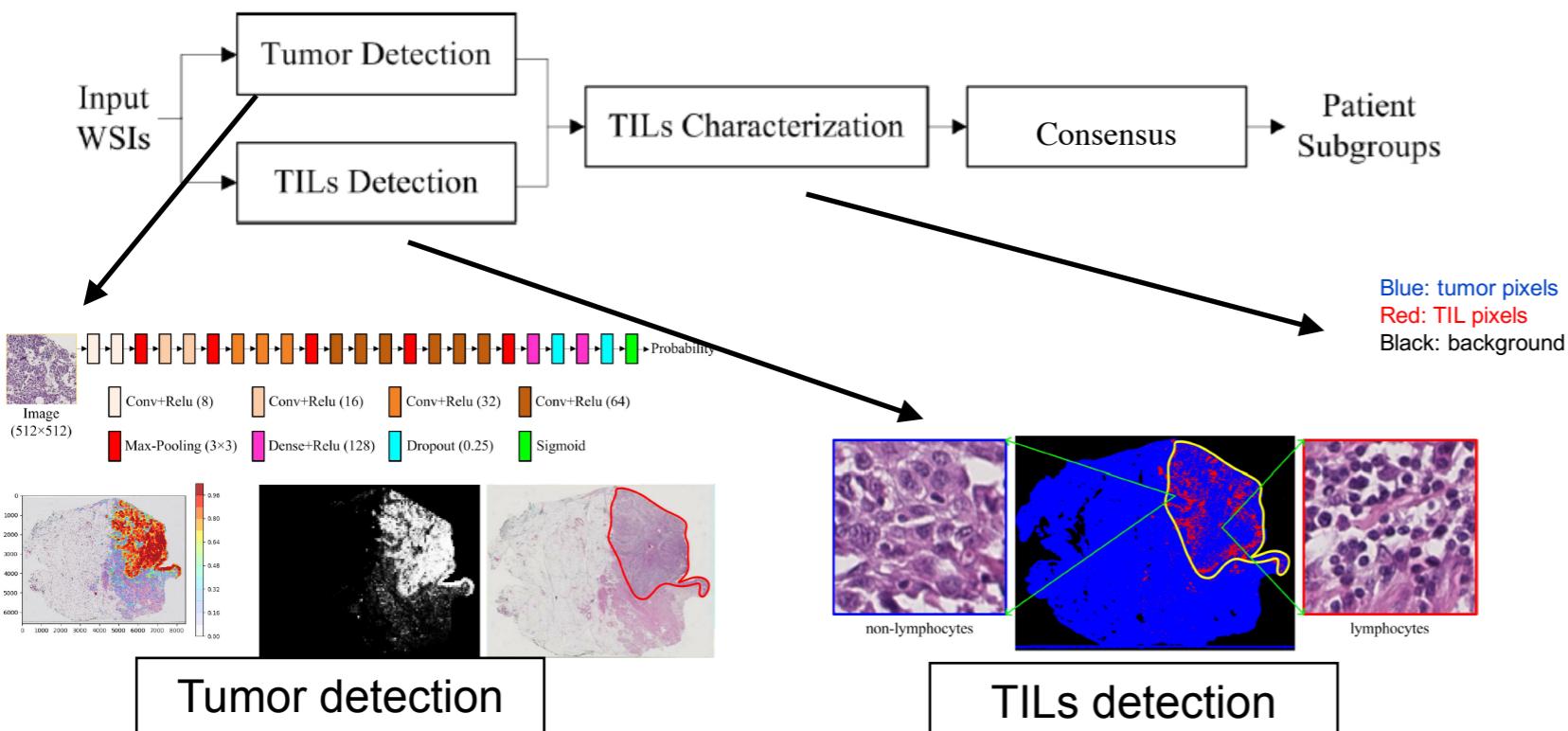
Image data can provide clinically useful genomic information

- Recent studies show that whole slide images can be used to predict MSI, TILs, and other genomic information without the molecular data.

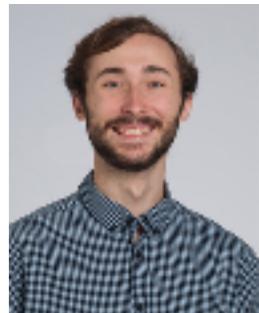


Our group actively developing AI algorithms to analyze histopathological image data to predict clinically relevant information (e.g., MSI, TILs, etc.)

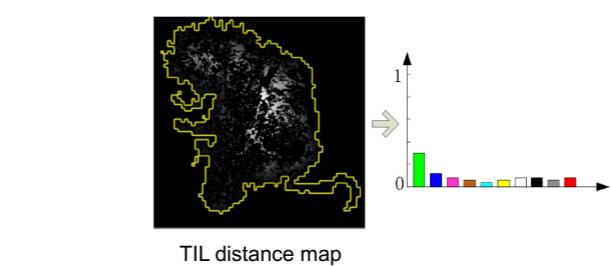
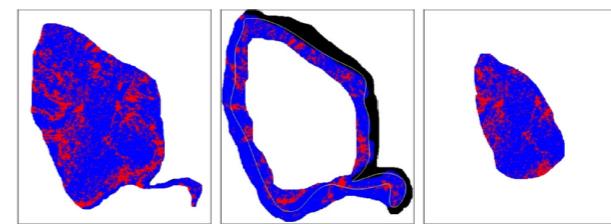
- **Motivation:** Analyze fraction and spatial patterns of MSI and Tumor-Infiltrating Lymphocytes (TILs) correlated with patient survival outcome and/or immunotherapy response
- **Objective:** Develop/apply deep learning algorithms based on whole slide histopathology image to analyze MSI/TILs to predict clinical outcome (e.g., survival/immunotherapy response)
 - Input: Whole slide histopathological image
 - Output: Clinical outcome prediction, MSI status, TILs detection



Hongming Xu, PhD



Isaiah Pressman

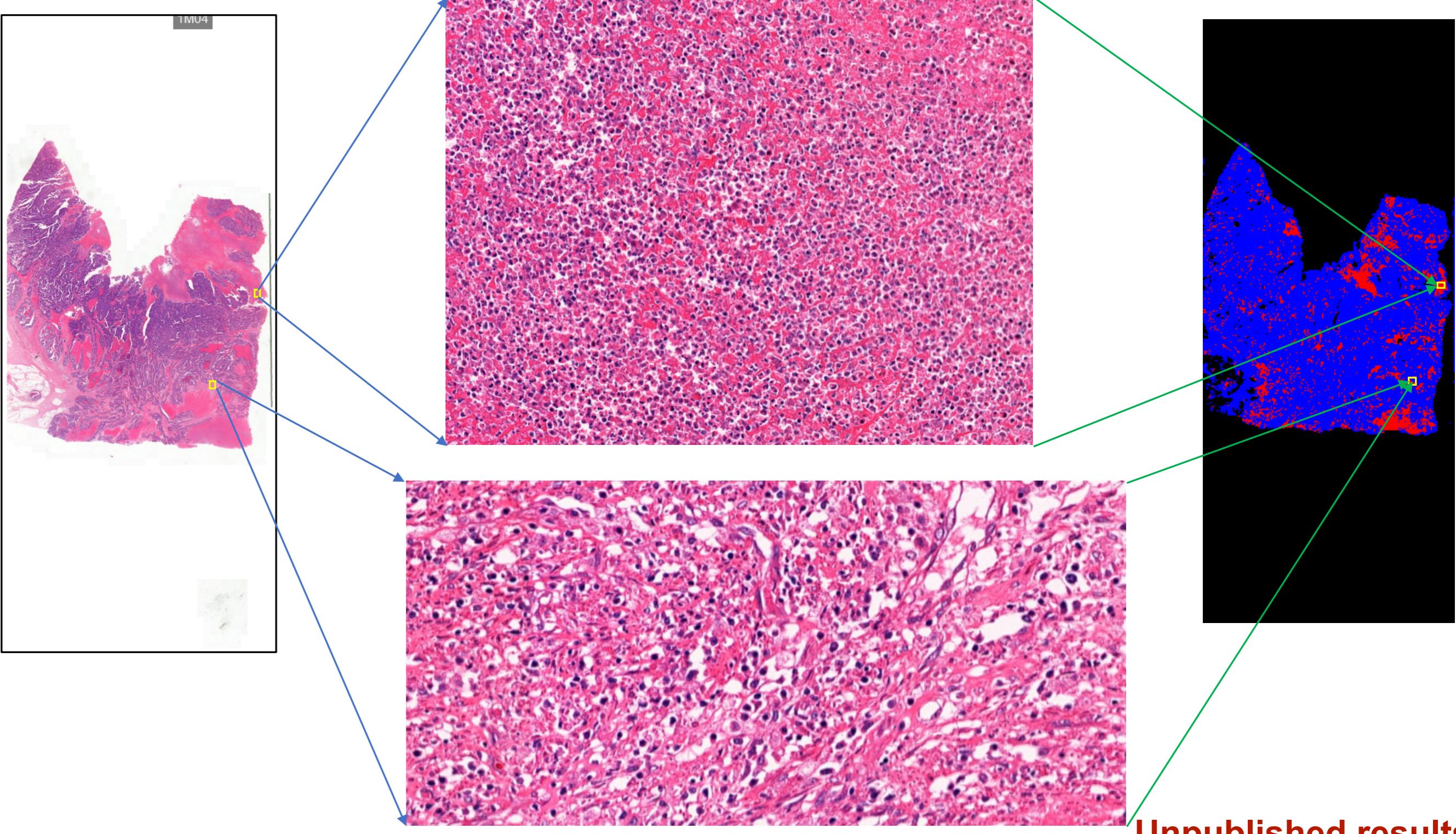


Unpublished results

TILs characterization

Predicted TILs regions in the WSI (1/2)

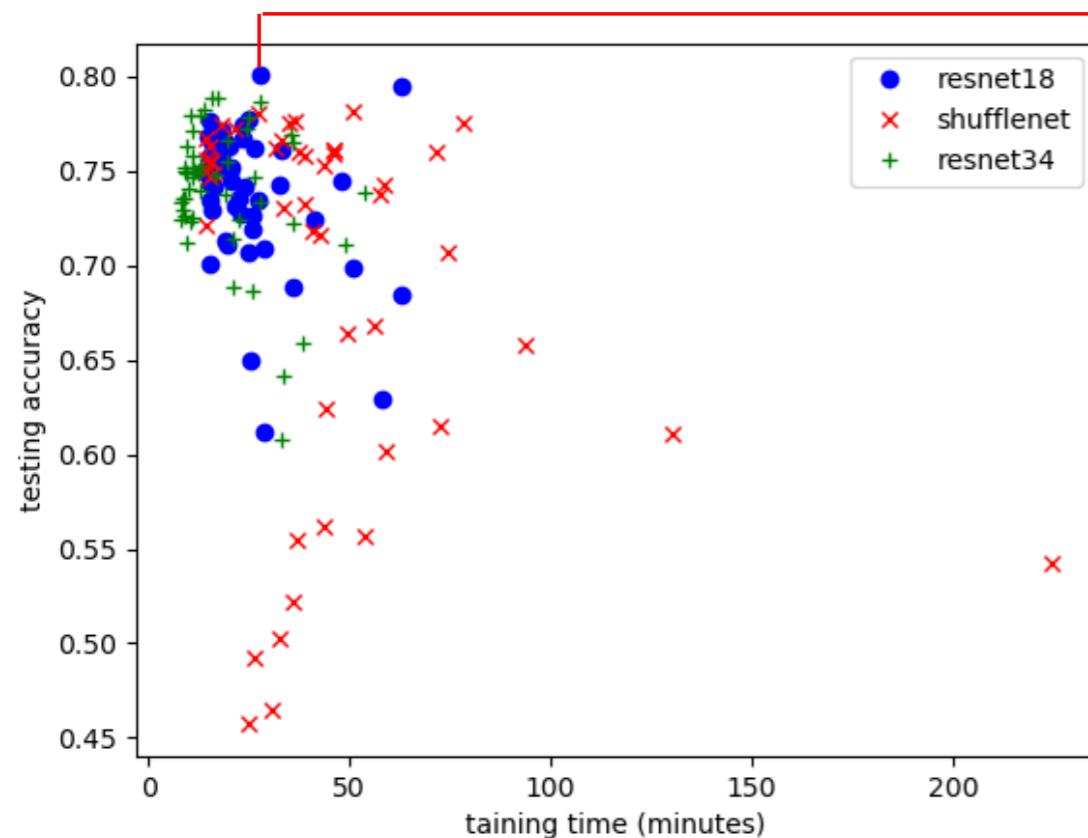
Identified TILs Regions



Predicted TILs regions in the WSI (2/2)

Deep Learning algorithm (Resent18) provides overall best accuracies for TILs detection

- Test accuracies of 144 different models on the testing dataset are:



The best testing accuracy is: **80.06%**

The best model is: *resent18_0_adam_0.0001_4*

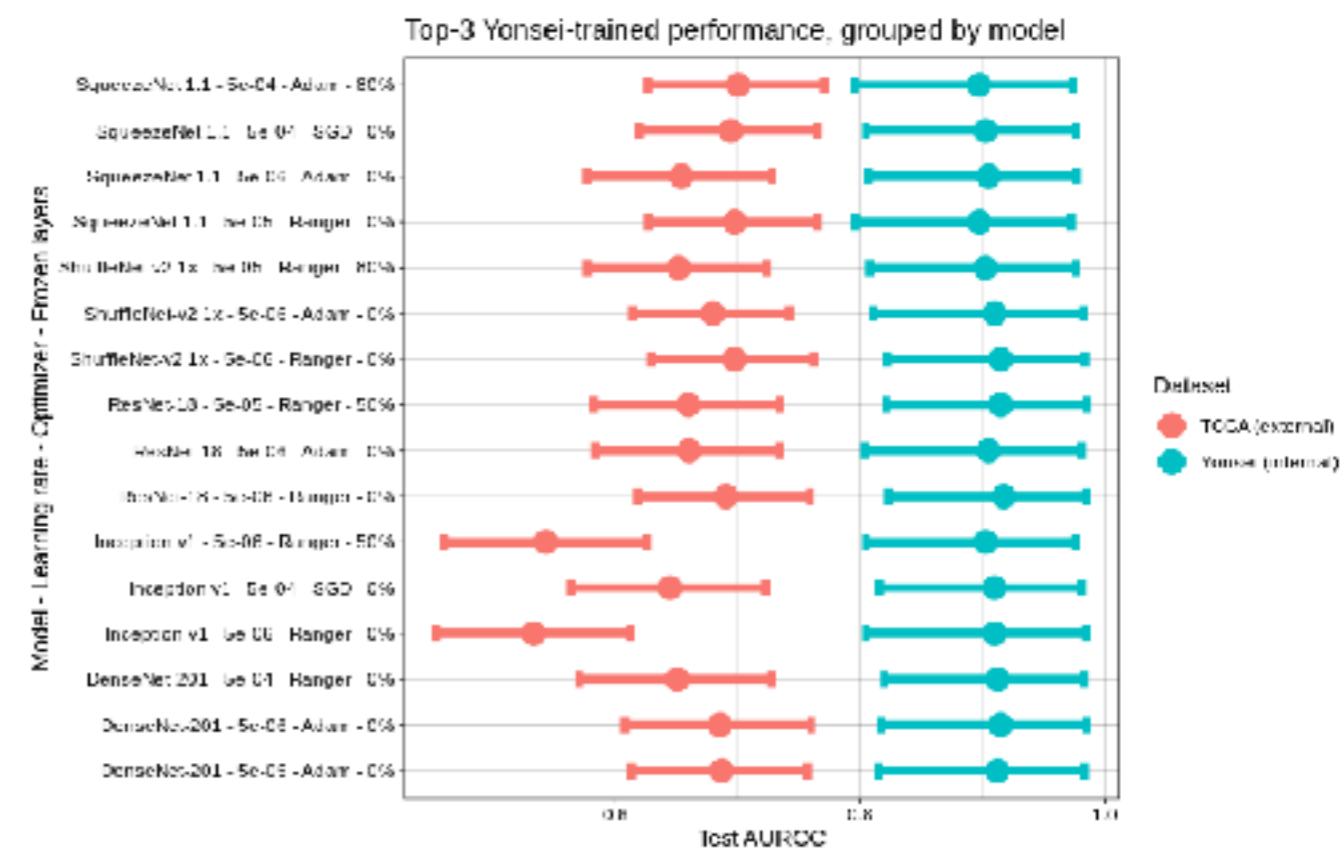
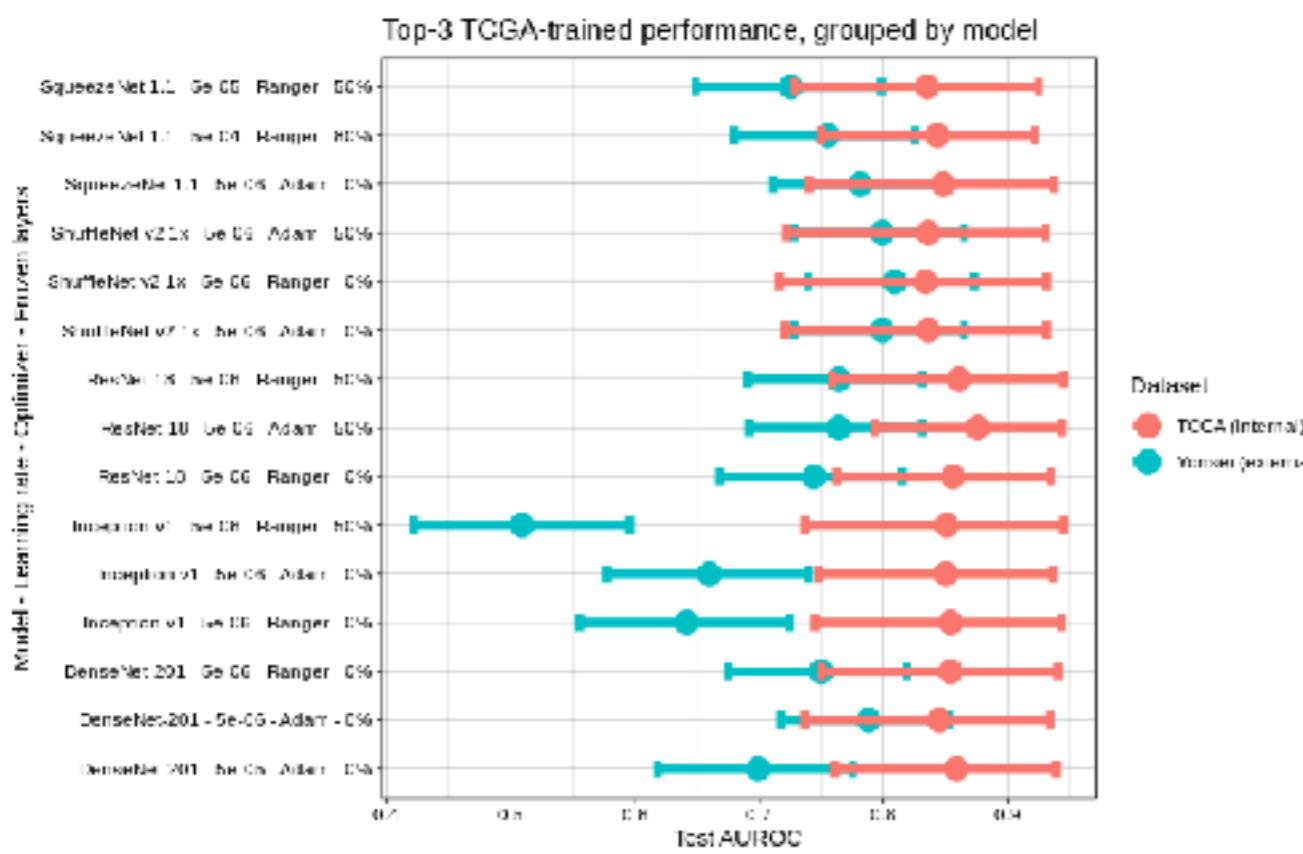
- i. Model: resent18
- ii. Frozen: 0%
- iii. Optimizer: adam
- iv. Learning rate: 0.0001
- v. Batch size: 4

Horizontal: training time

Vertical: classification accuracy

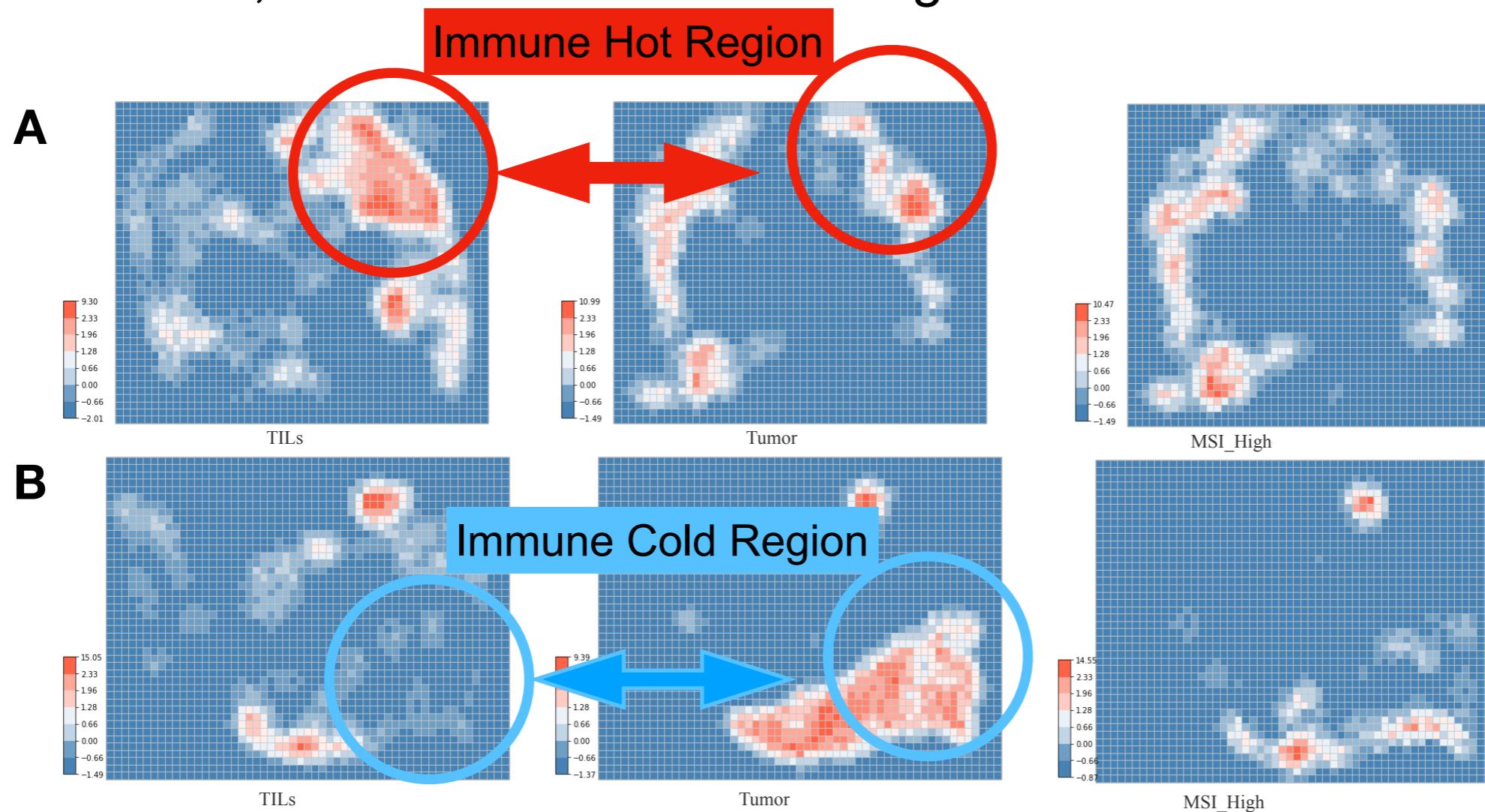
Deep Learning algorithms can accurately predict MSI-H status given a whole slide histopathological image in gastric cancer

- We trained and evaluated various state-of-the art deep learning algorithms to predict MSI-H status using whole slide images
- Carefully trained deep learning algorithms could achieve 0.7 to 0.9 AUROC across different gastrointestinal cancer datasets



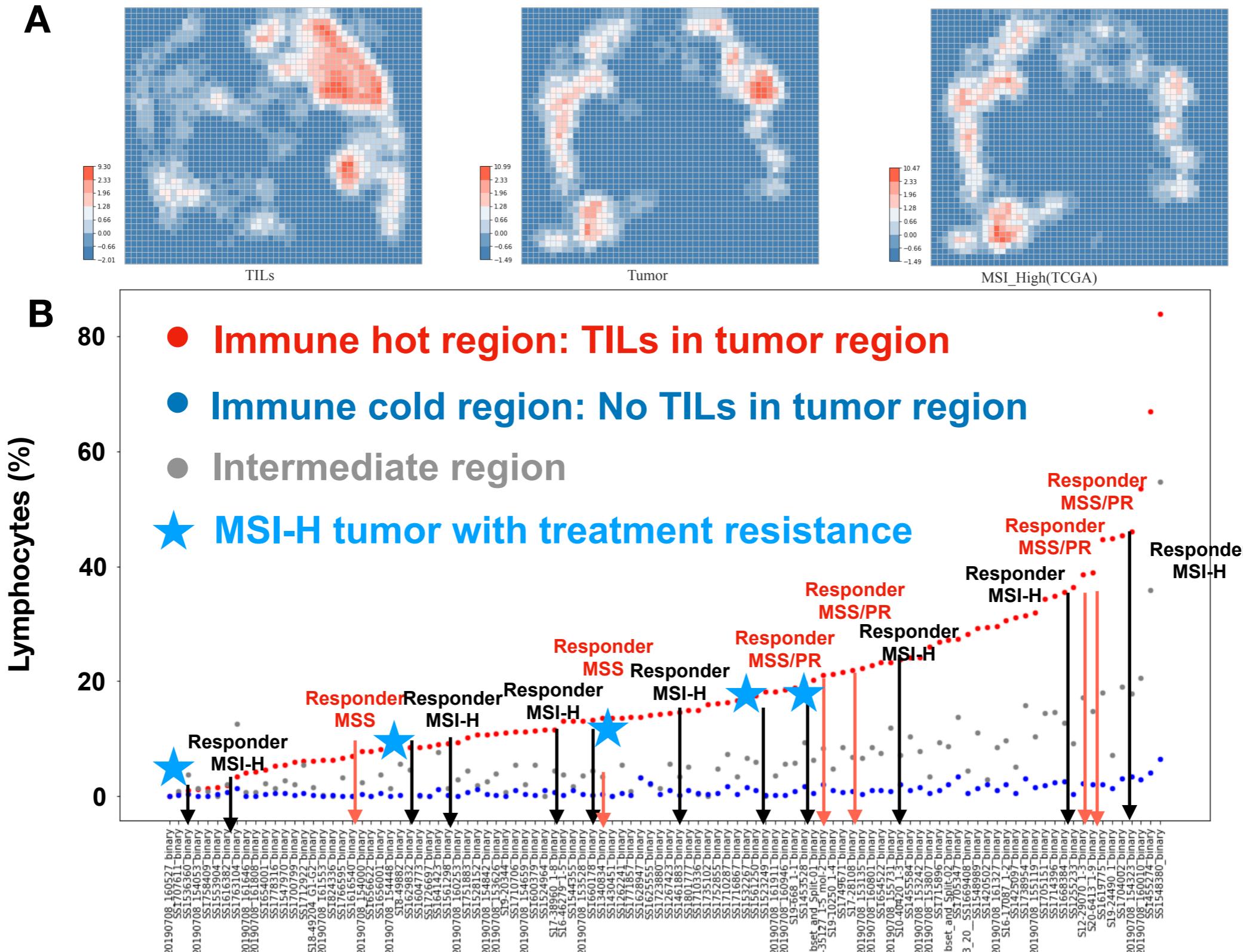
Integrative Tumor, MSI/TMB, and TILs analysis could provide novel insights of spatial organizations of **tumor and immune microenvironment** in gastric cancer

- We collected WSIs for 81 chemo-naive gastric cancer patients who received anti-PD1 inhibitor as the first line therapy.
- Given a WSI, we can detect multi-level genomic/immune characteristics.



Two gastric cancer patients' whole slide H&E images. (A) MSI-H patient, (B) MSS patient

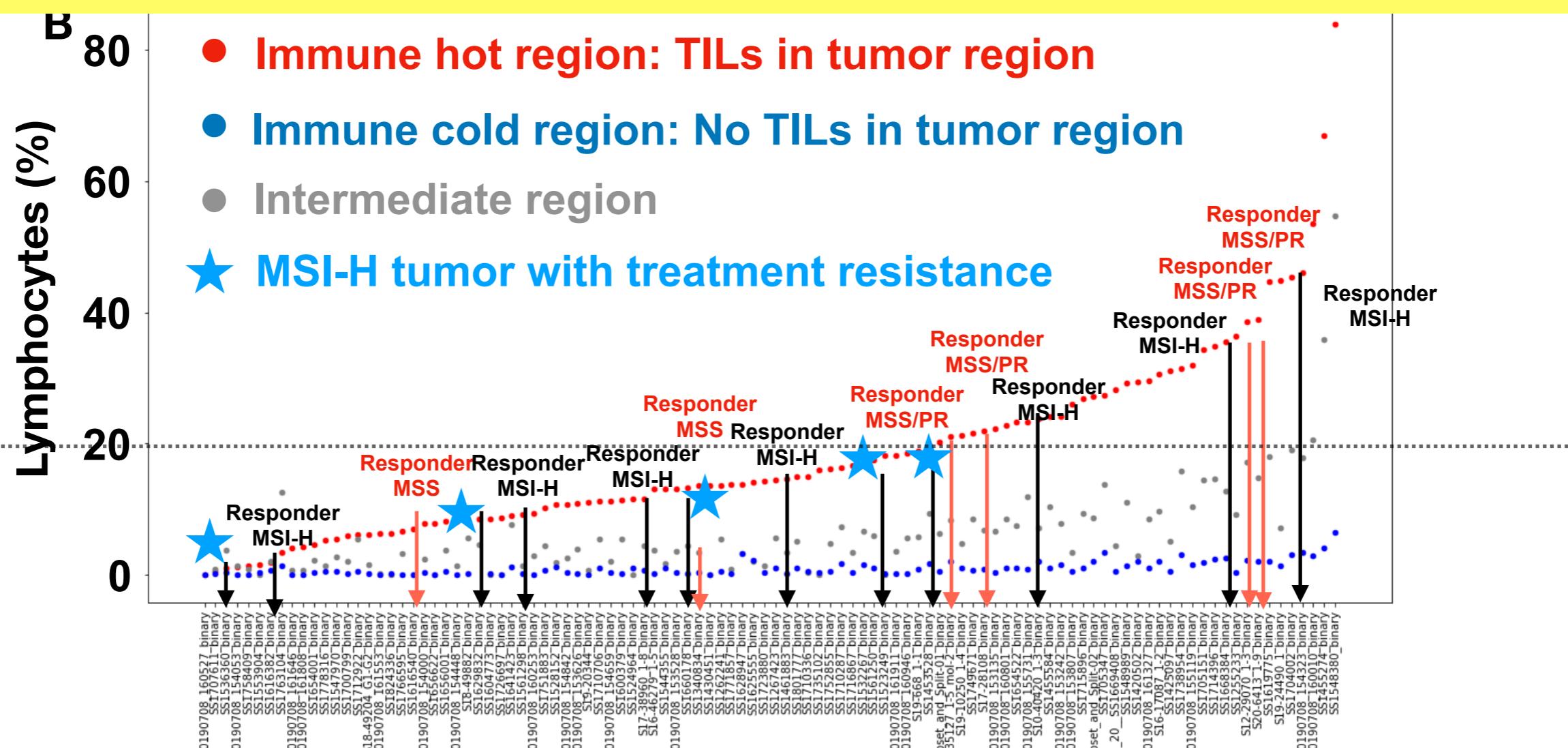
Various spectrums of Immune hot and cold tumors exist in GC



(A) Predicted Tumor, TILs, and MSI-H regions. (B) TILs percentages in the predicted regions. The x axis represent samples, and the y axis represents the percentage of TILs in each immune hot (red dot), immune cold (blue dot), and intermediate spot (gray dot) regions. Immune hot and cold regions contain higher and lower proportions of TILs in the tumor region, respectively

MSI-H tumors containing less than 20% Immune hot regions did not respond to anti-PD-1 inhibitor

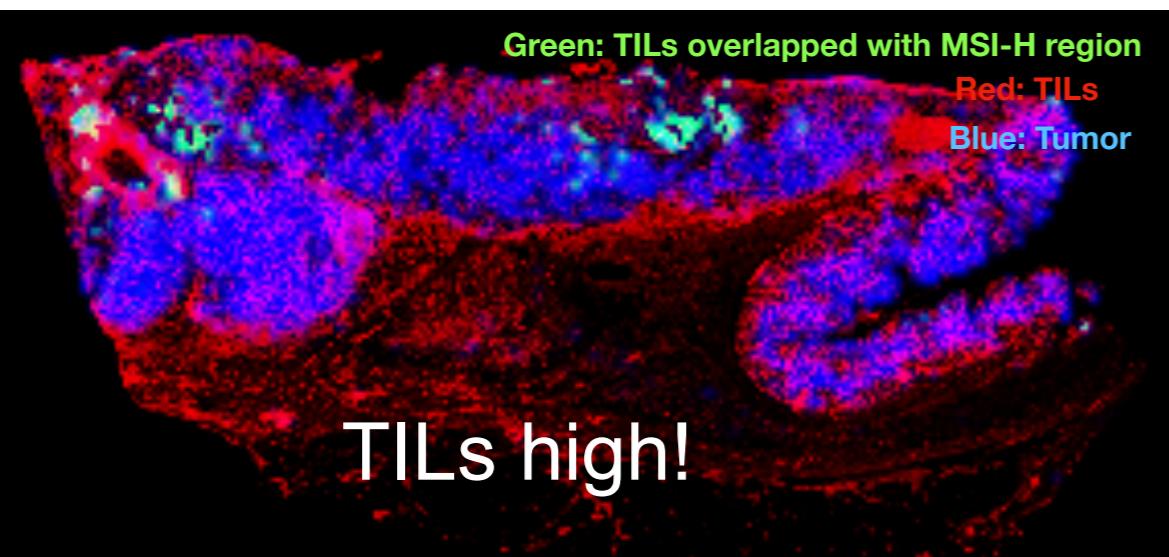
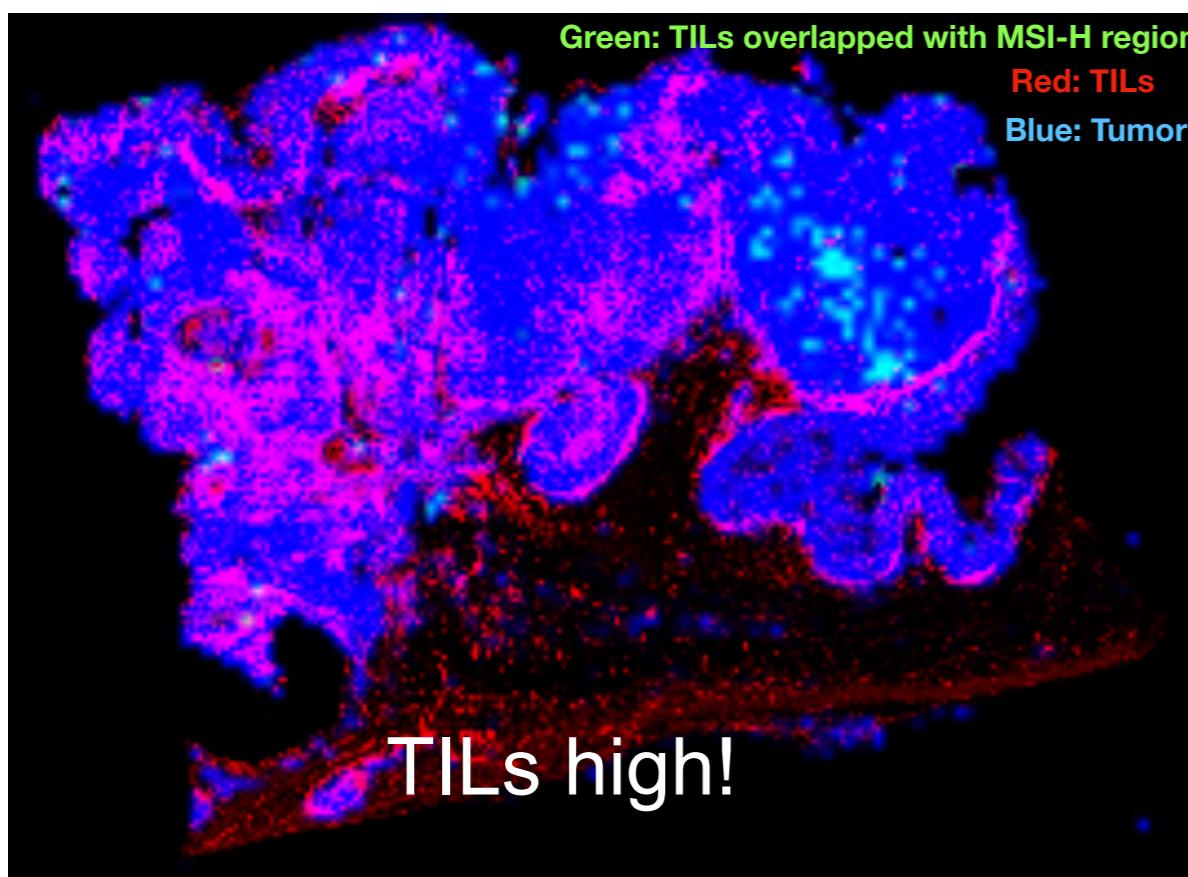
- 5 out of 9 MSI-H tumors (44% response rate) containing less than 20% immune hot regions did not respond to anti-PD-1 inhibitor.
 - 3 out of 3 MSI-H tumors (100% response rate) containing more than 20% immune hot regions responded to anti-PD-1 inhibitor.
 - 4 out of 34 MSS tumors (12% response rate) containing more than 20 % immune hot regions responded to anti-PD-1 inhibitor.
 - 2 out of 35 MSS tumors (6% response rate) containing less than 20% immune hot regions did not respond to anti-PD-1 inhibitor.



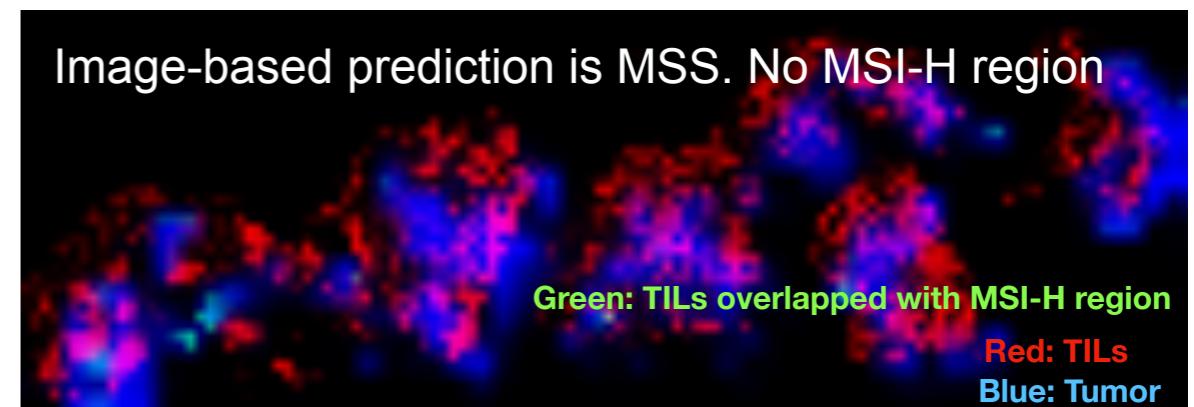
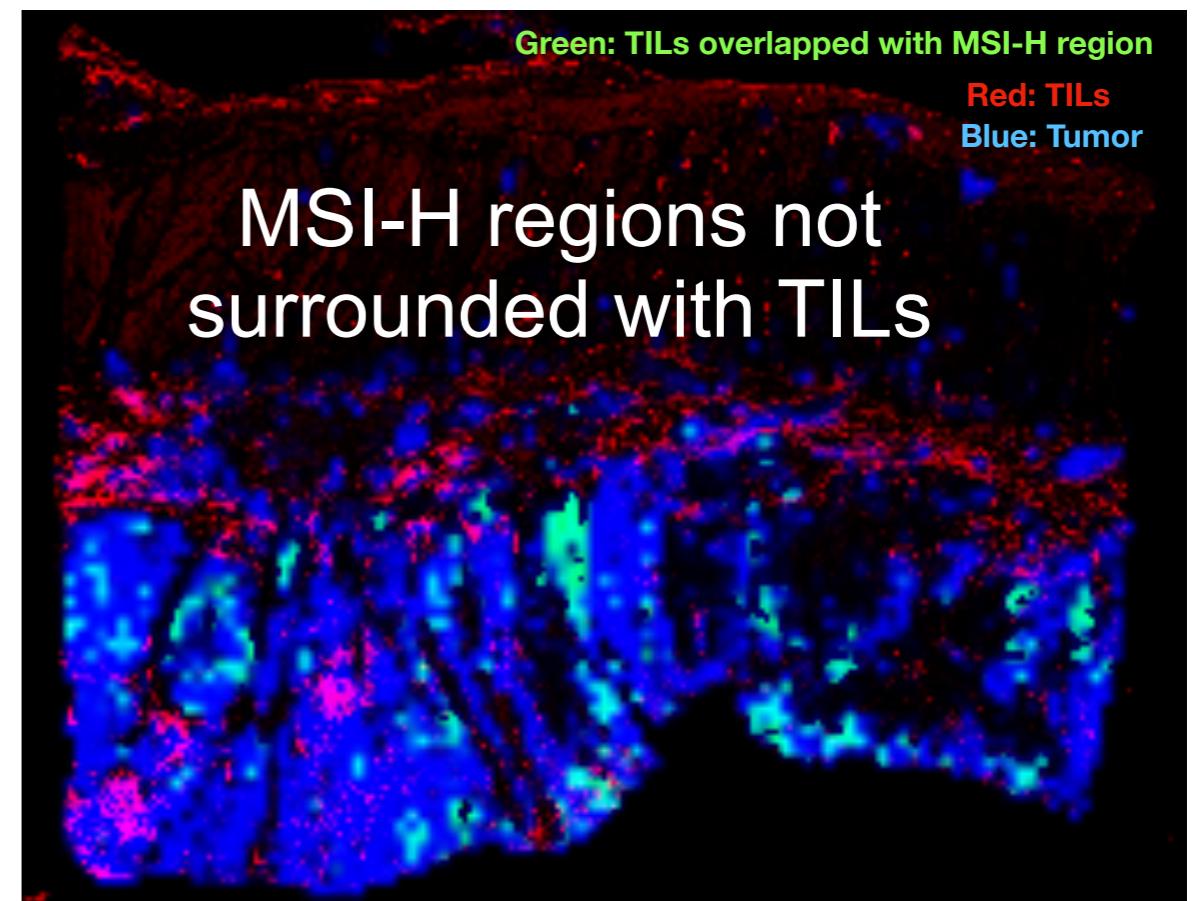
(A) Predicted Tumor, TILs, and MSI-H regions. (B) TILs percentages in the predicted regions. The x axis represent samples, and the y axis represents the percentage of TILs in each immune hot (red dot), immune cold (blue dot), and intermediate spot (gray dot) regions. Immune hot and cold regions contain higher and lower proportions of TILs in the tumor region, respectively

Incorporating spatial co-organization of MSI-H regions with immune cold/hot environment

MSS with responder



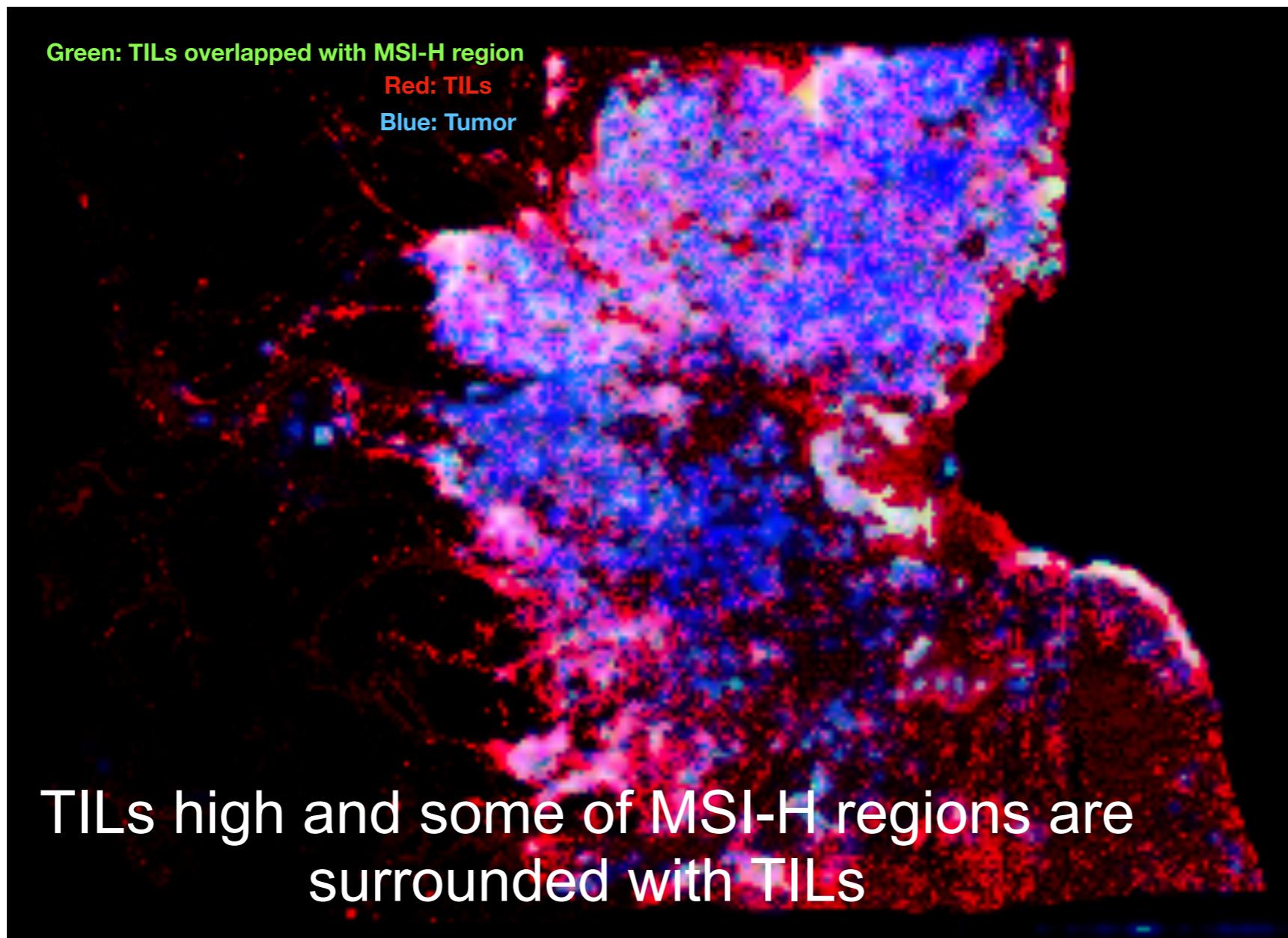
MSI-H with non-responder



Incorporating molecular data with spatial co-organization of MSI-H regions and image-based immune cold/hot environment

- We performed bulk RNAseq deconvolution to determine cell type abundance and expression using CIBERSORTx (Newman. et. al., Nature Biotech 2019)

T-Reg high/ MSS



Incorporating molecular data with spatial co-organization of MSI-H regions and image-based immune cold/hot environment

- We performed bulk RNAseq deconvolution to determine cell type abundance and expression using CIBERSORTx (Newman. et. al., Nature Biotech 2019)

T cells CD8 and T cells CD4 memory high & B cell naive high/ MSI-H

