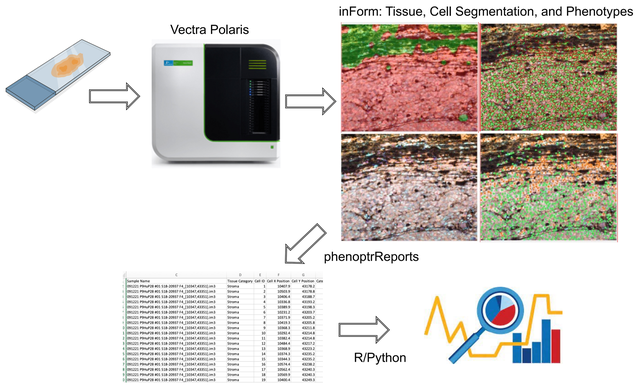
Multiplex Imaging of Melanoma FFPE Specimens to Interrogate Phospho-STAT Signaling as Biomarkers to Checkpoint Immunotherapy Response/Resistance.

Ann Strange, Carol Amato, Emily Monk, Brian Thompson, Paulo Burke, David Woods
University of Colorado Anschutz Medical Campus

Background

Multiplex immunohistochemistry (mIF) is a useful approach to interrogating the tumor microenvironment through simultaneously interrogating multiple markers in relatively abundant banked FFPE specimens. Additionally, spatial information obtained in mIF uniquely allows questions to be addressed such as: localization of immune cells in the TME (e.g. intra-tumoral, tumor-border), co-localization of proteins (e.g. STAT heterodimers), and the cellular location of proteins (i.e. surface vs. cytoplasmic vs. nuclear). We previously identified expression of several phosphorylated STAT protein in peripheral blood CD4+ and CD8+ T-cells of patients with advanced melanoma as significantly associated with response to immune checkpoint inhibitors (e.g. increases in pSTAT3(S727) associated with response and increase in pSTAT5(Y694) associated with relapse). To build upon these finding, we leveraged mIF using the Vectra platform to examine the TME of FFPE samples obtained from melanoma patients receiving immunotherapy. A nine-parameter immunofluorescence panel was optimized and applied to samples. Regions of tumor border in FFPE metastatic melanoma tumor samples were identified for 40x imaging. Images were spectrally unmixed and machine learning applied to identify tumor vs stroma in ~500 images. Cell segmentation and phenotyping enabled a detailed itemization of cell metrics such as area, diameter, and location, enabling a host of possible visualizations and exploration in the single cell, spatial domain.

Methods



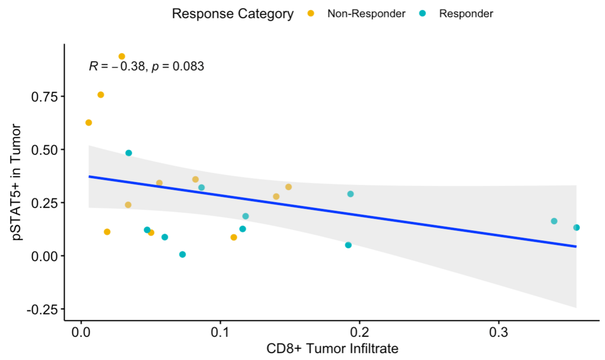
Samples

Pre-Treatment for Ipi and/or Nivo

Non-Responders			Responders		
Slide	MB	Location	Slide	MB	Location
8	3558	Left Foot Primary	22	4790	Left Forearm Primary
9	5072	Right Chest Primary	23	3546	Scalp
10	4686	Upper Left Arm	24	2778	Soft Tissue (Neck)
11	S17-29240	Groin Lymph Node	25	3781	Pelvic Lymph Node
19	4259	Ear Canal Primary	26	3549	Right Ear
20	2421	Brain	27	5068	Back Primary
21	3555	Illiac Lymph Nodes	28	4794	Right flank
29	5073	Medial Right Knee	33	3839	Lung
30	3552	Left Calf	34	2492	Brain
31	4799	Maxillary Sinus Left	35	4801	Left Preauricular Primary
32	5066	Brain	36	4797	Right Cheek primary

Results

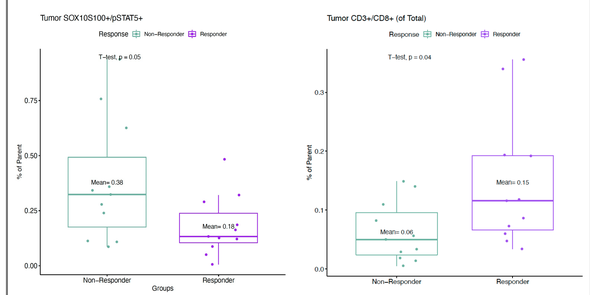
As pSTAT5 increases in Tumor cells, the less we see CD8+ cytotoxic T-cell infiltration



We developed a Vectra Polaris panel with the HIMSR Core to assess phosphorylates of STAT proteins (STAT1, STAT3, STAT5) and additional serine residue phosphorylations on STAT3, in relation to Tumor Infiltrating Lymphocytes (TILs). Panels were optimized for staining. InForm and phenoptrRpts software were used for tissue and cell segmentation and phenotype, with further statistical analyses performed in R.

Tumor pSTAT5+ decreases in Responders

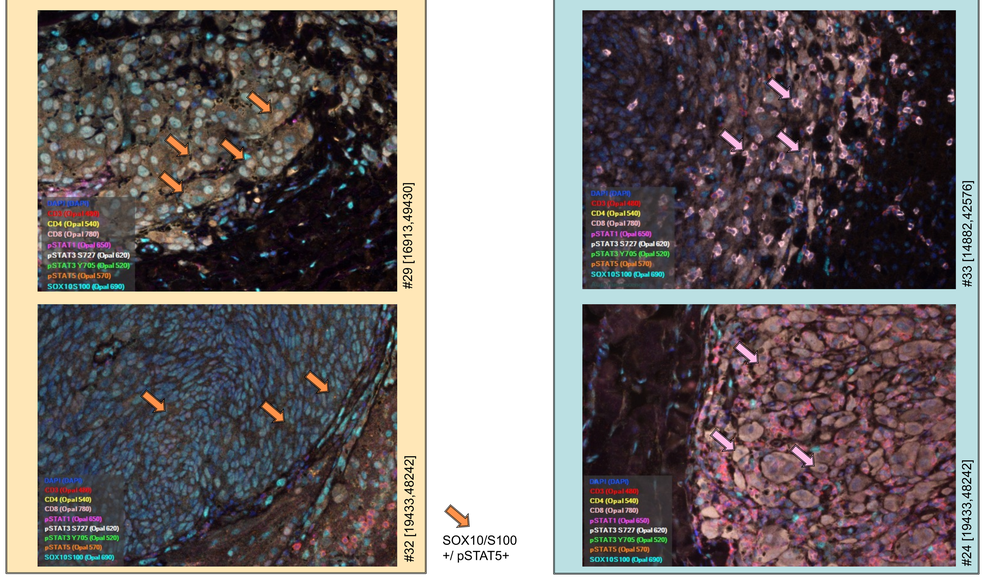
CD3+/CD8+ Infiltrate increases in Responders



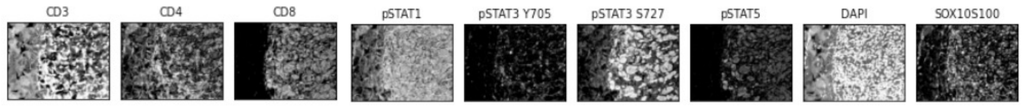
Examples

Non Responders with SOX10/S100+/pSTAT5+ & Low TIL

Responders with High CD3+/CD8+ (TILs) & Low pSTAT5



Phenotype Intensities, spectrally unmixed (Brightness & Contrast Adjusted):



Next Steps

- More images/study are needed to power the statistical tests and further illustrate the dynamics.
- Additional Colocalization Analysis is possible from this data. We can look at nearest neighbor and adjacency data.
- Explore automated thresholding algorithms
- Apply PhenoComb R Package to find additional exploratory avenues.

Conclusions

- As pSTAT5 increases in Tumor cells, the less we observe CD3+/CD8+ cytotoxic T-cell infiltration:
- pSTAT5 in Tumor correlates with Non-Responders
- The relationship between pSTAT5+ within Tumor and TILs supports the roles of STAT signaling in health and disease.
- mIF and Spatial analytics offers a powerful tool in understanding the heterogeneity in the variety of tissue types

Supporting Information

Prior Study ex:
https://pubmed.ncbi.nlm.nih.gov/29485532/

ann.strange@cuanschutz.edu