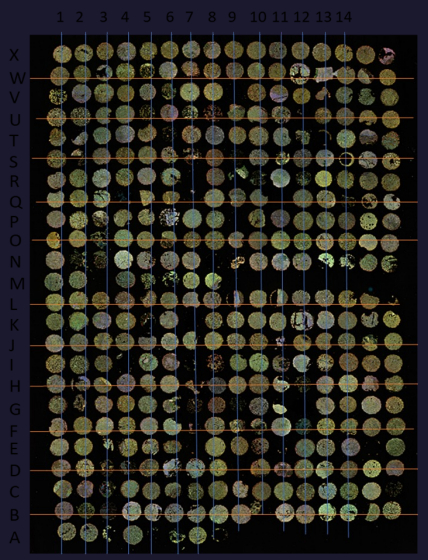
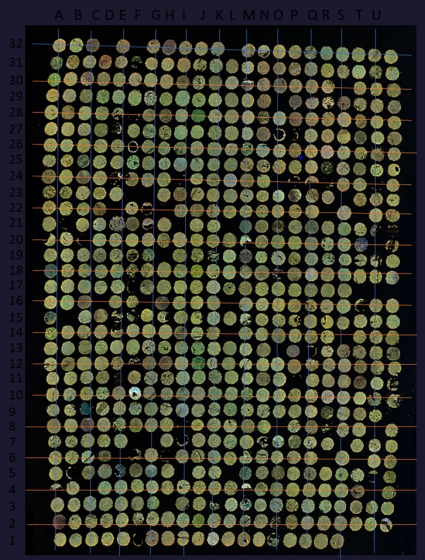
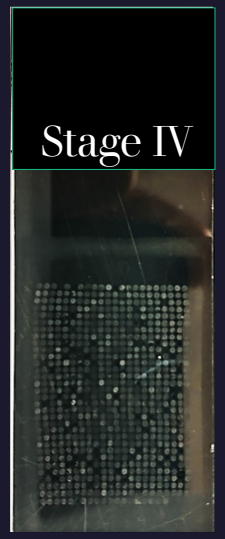
Current Cohorts to Establish Signatures

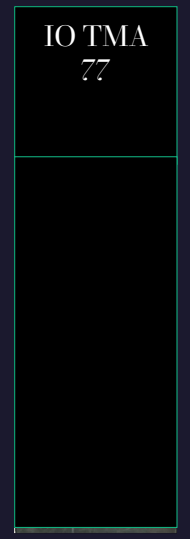
Slide	Stage III		Stage IV	
Antibody Conjugation	Fluorescent	Metal	Fluorescent	Metal
# of Core (ROI)	384	301	704	640
# of Plex	7	36	7	37
Type of file	.qptiff	.mcd & .txt	.qptiff	.mcd & .txt



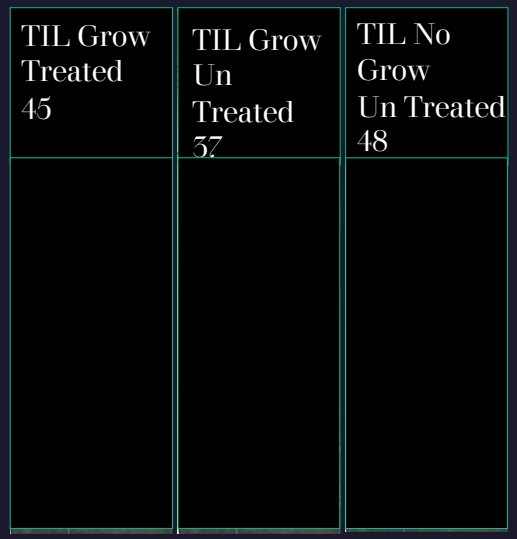




IO TMA	
Fluorescent	Metal
77	77
7	36
.qptiff	.mcd & .txt

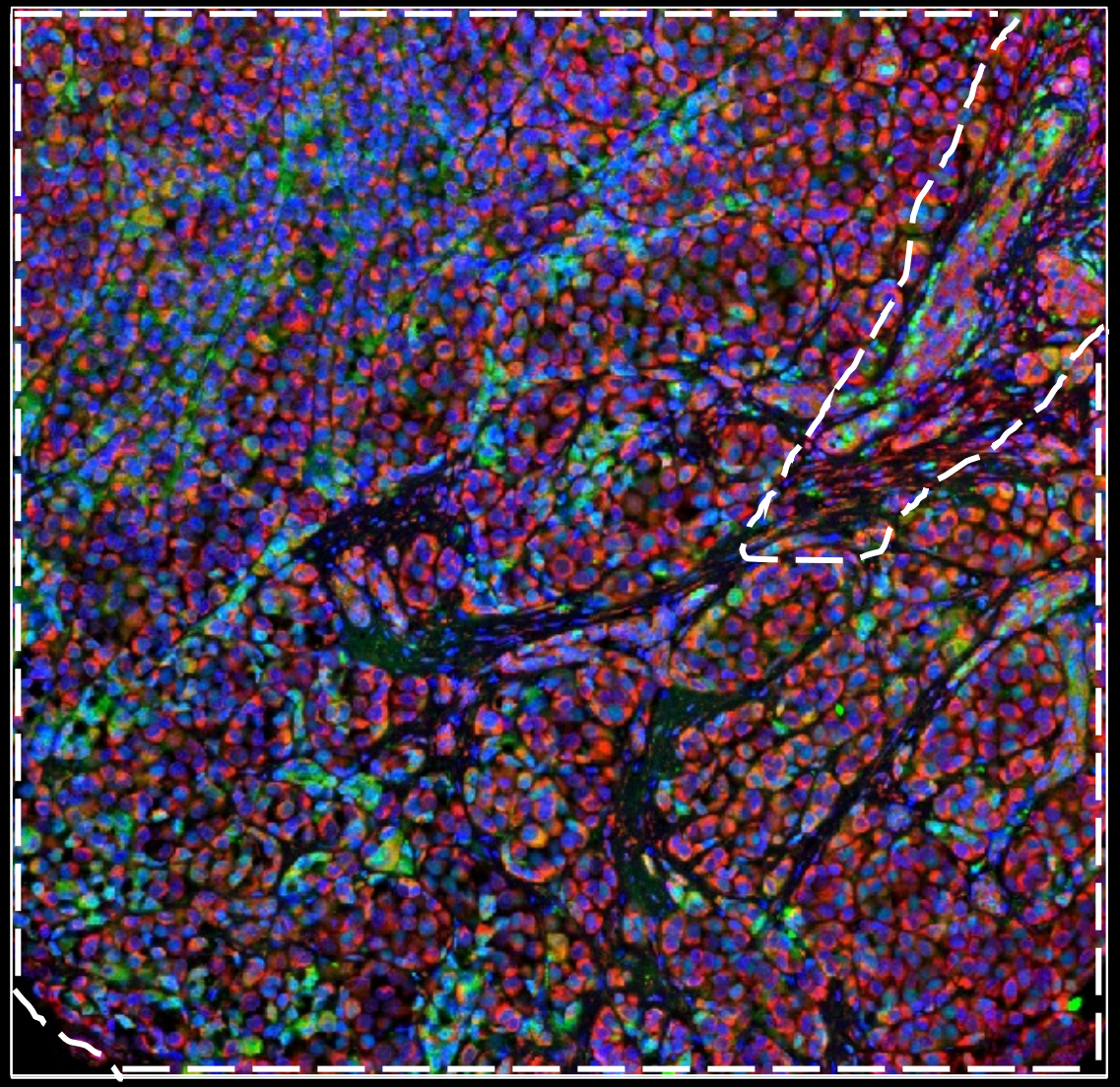


TIL Samples	
Fluorescent	Metal
130	45
7	36
.qptiff	.mcd & .txt

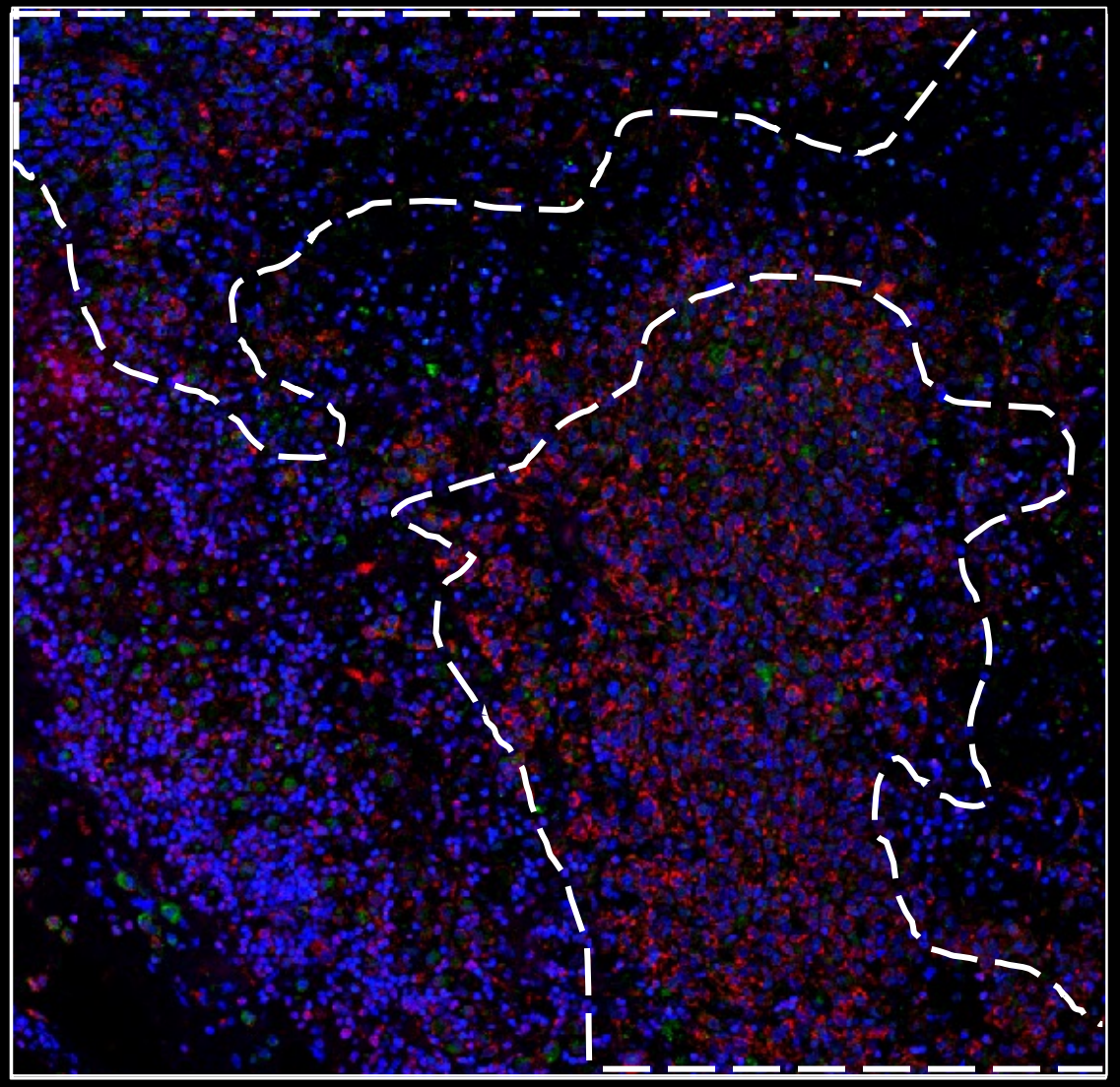


iNOS; mPGES1

Low TIL



High TIL



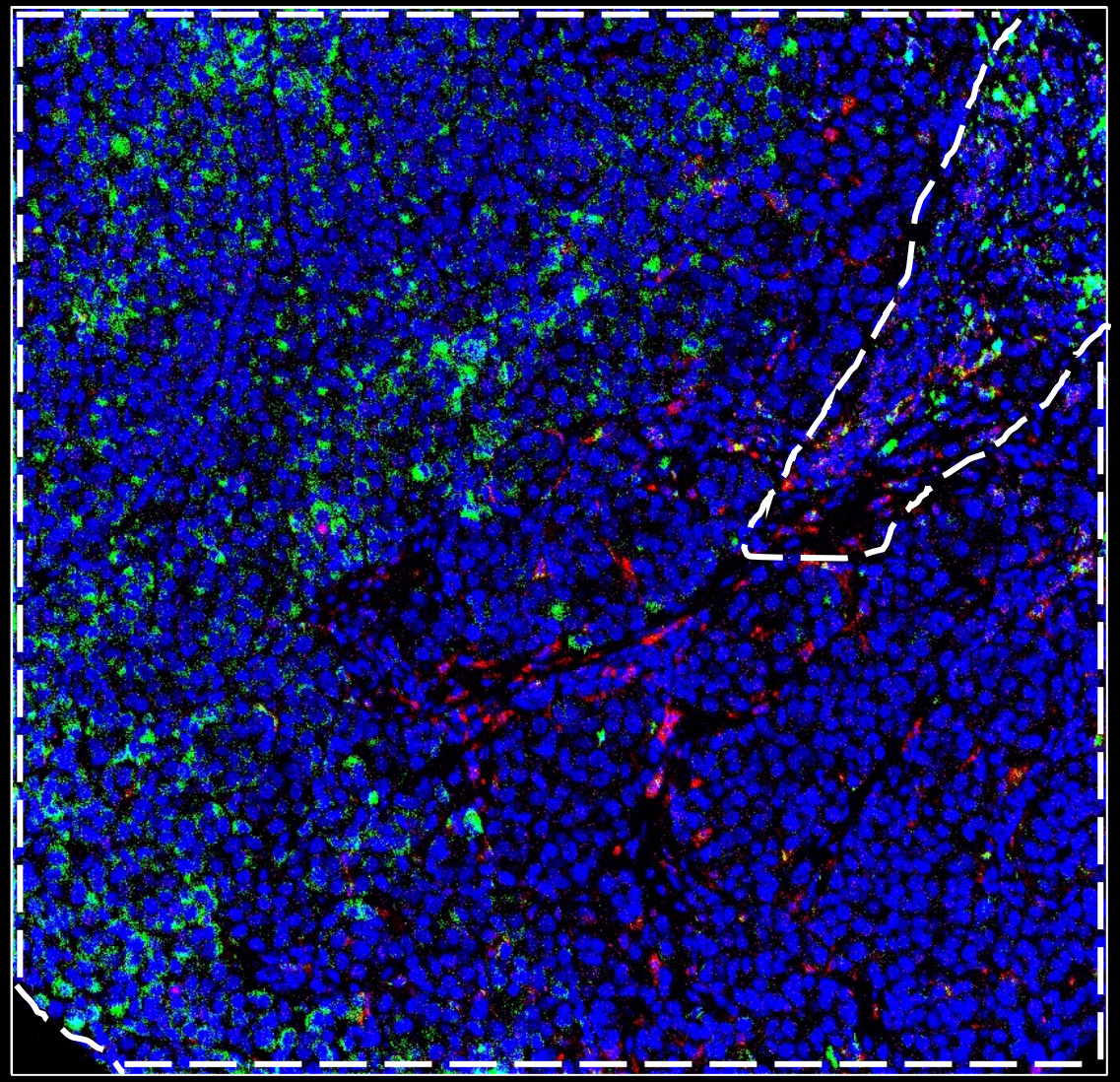
Opal panel

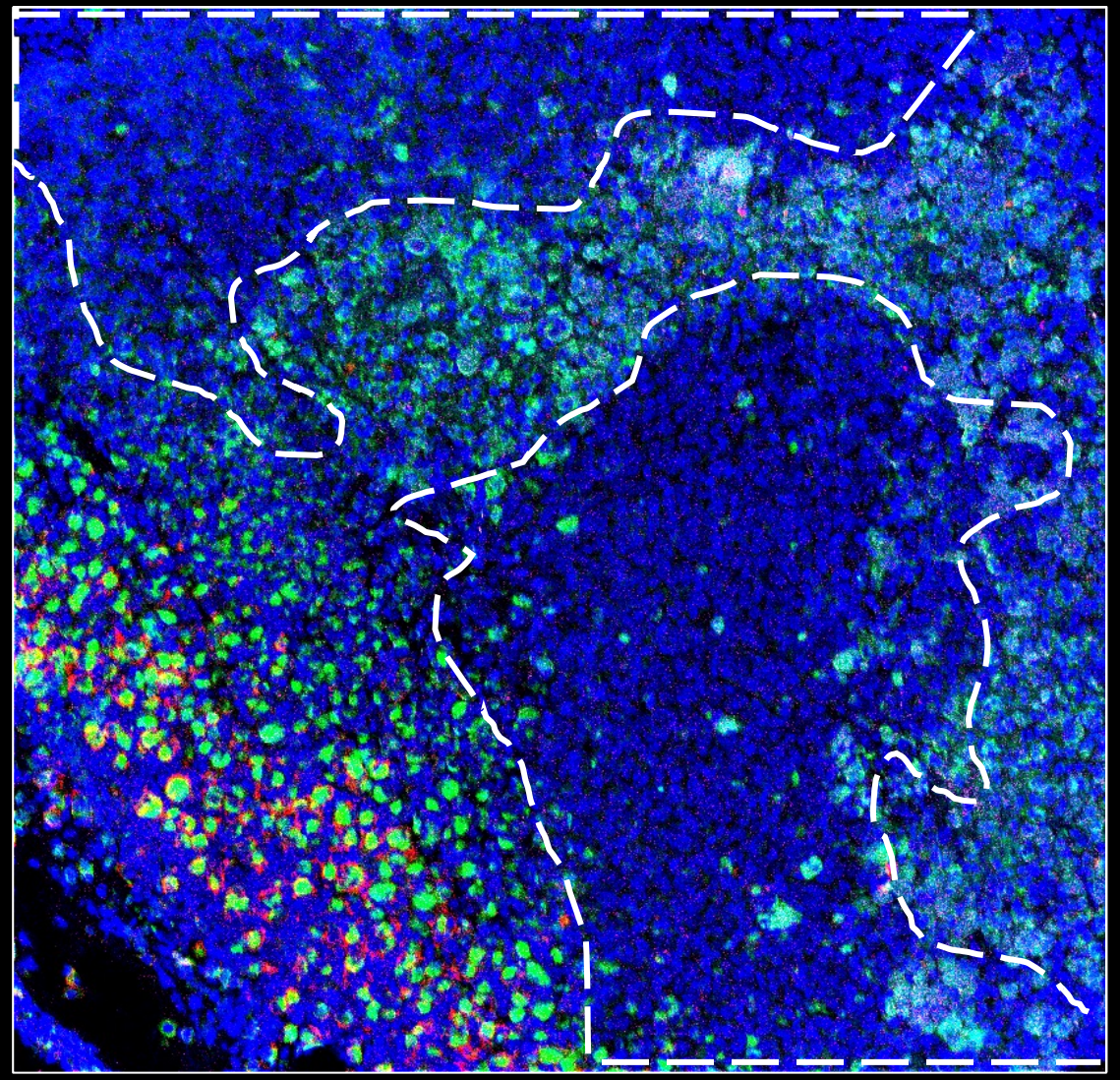
: Melanoma

CD68; CD163; Arg1

Low TIL

High TIL





IMC panel

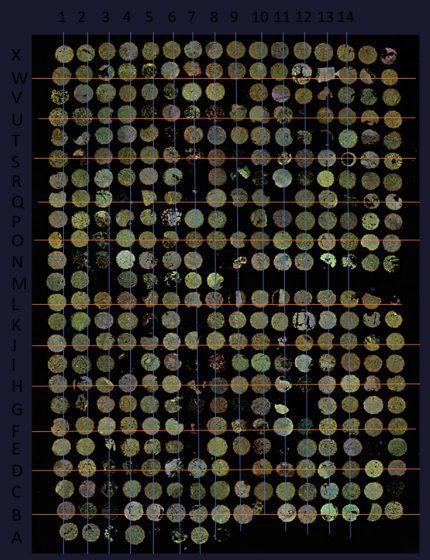
: Melanoma

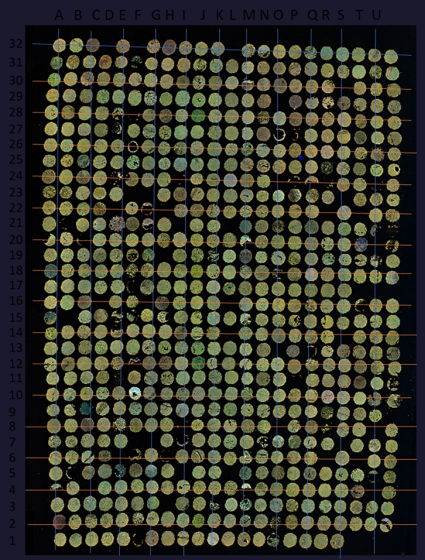
Current Cohorts to Establish Signatures

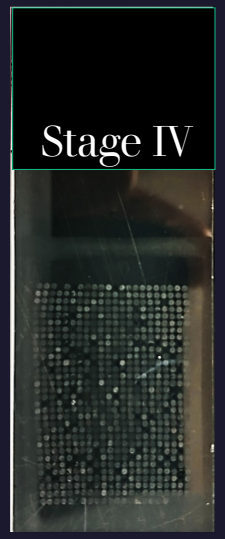
Slide	Stage III		Stage IV	
Antibody Conjugation	Fluorescent	Metal	Fluorescent	Metal
# of Core (ROI)	384	301	704	640
# of Plex	7	36	7	37
Type of file	.qptiff	.mcd & .txt	.qptiff	.mcd & .txt

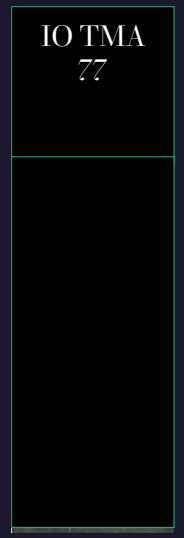
IO TMA	
Fluorescent	Metal
77	77
7	36
.qptiff	.mcd & .txt

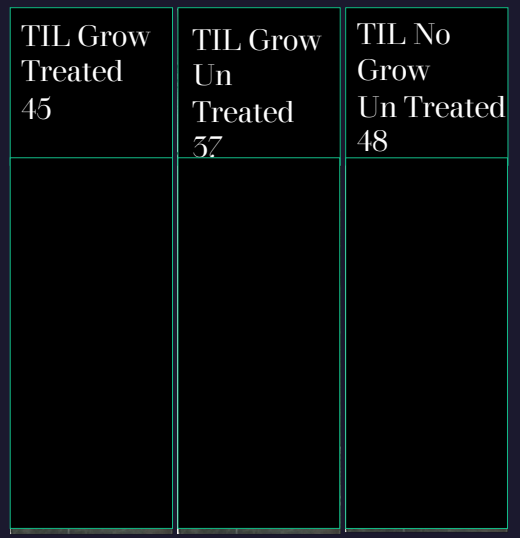
TIL Samples	
Fluorescent	Metal
130	45
7	36
.qptiff	.mcd & .txt





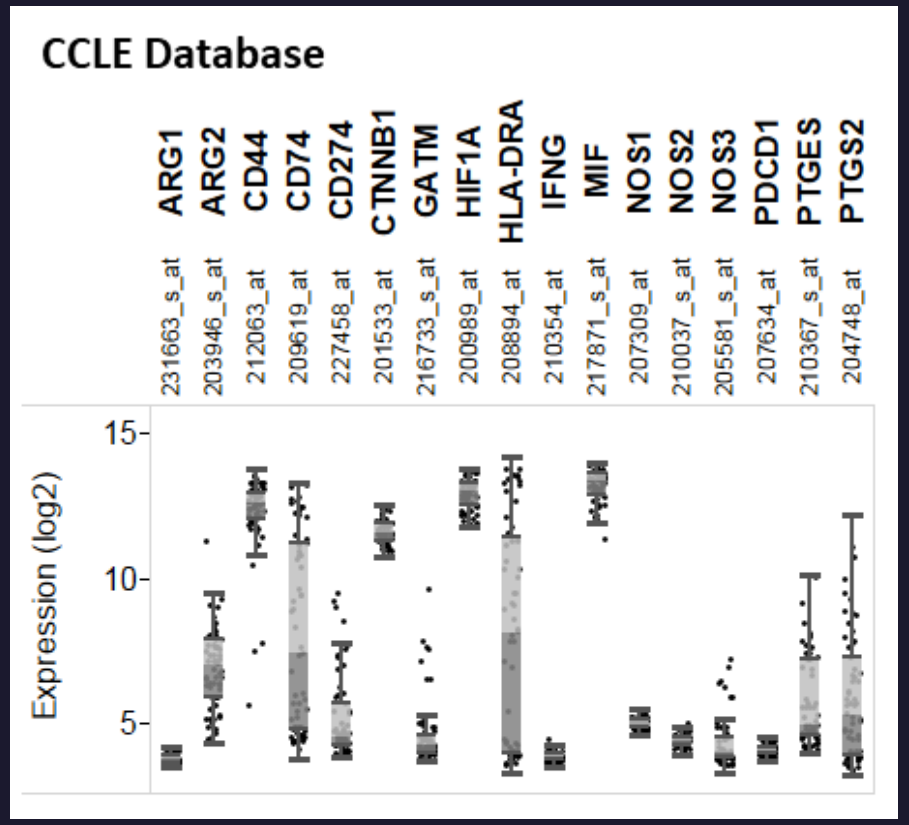




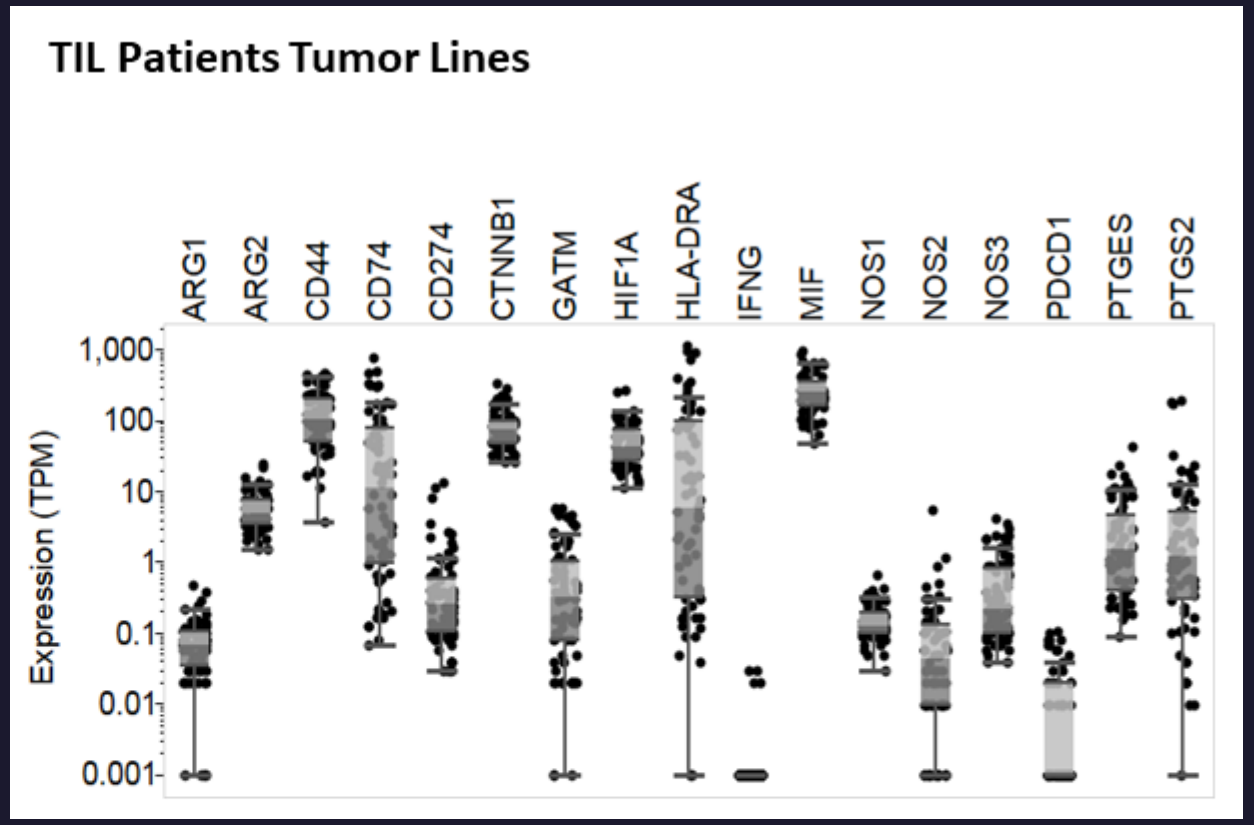


Initial Findings w/Individual Markers in TIL Treated Patients

Gene Expression of CD74 Node Markers (CD44, MIF, NOS2, mPGESl) in CCLE and TIL Cohorts



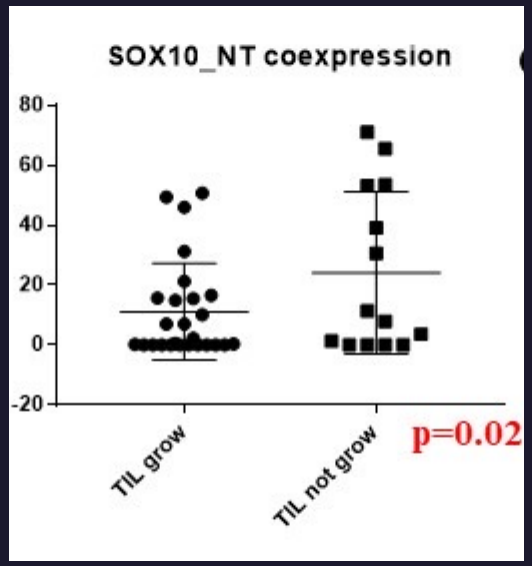
CD74 related genes mRNA expression (based on affymetrix mRNA arrays) in melanoma cell lines from the Cancer Cell Line Encyclopedia (CCLE). The *y* axis represents the log2 of the robust multi-array average.



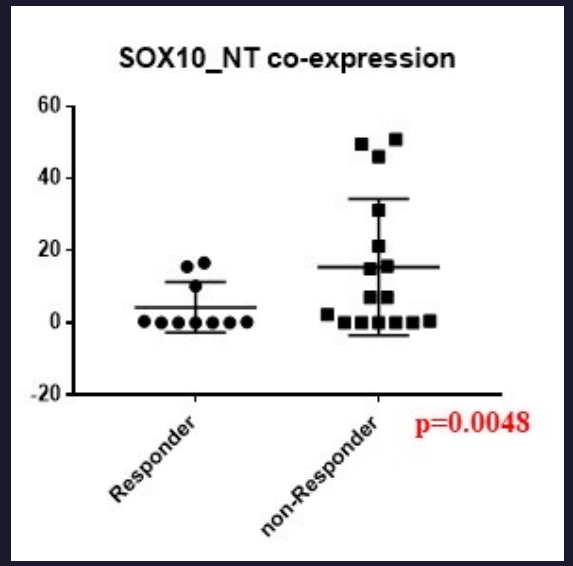
The expression of CD74 related genes mRNA [in transcripts per million (TPM) in MDACC TIL patients' tumor lines dataset.

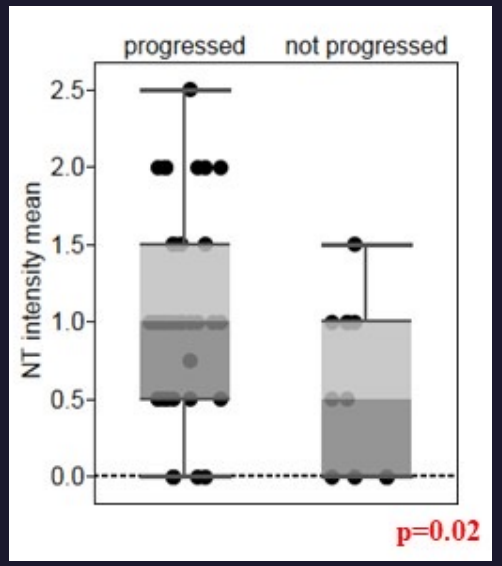
Initial Findings w/Individual Markers in TIL Treated Patients

Tumor Cell NT Expression Correlates with Poor or No TIL Growth from Tumor Samples



NT expression in tumor cells associated with poor TIL growth. NT expression in tumor cells in successful TIL growth group, compared to TIL not grow and the significance of this association.

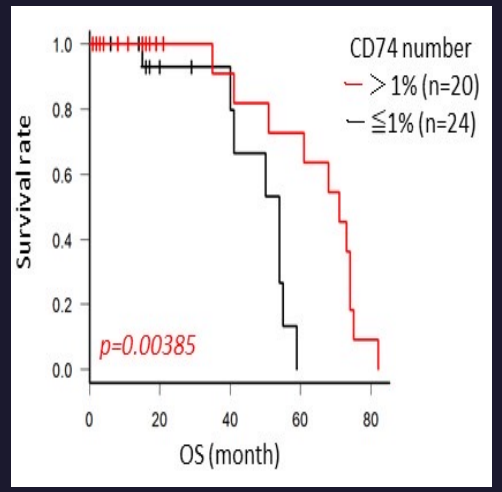


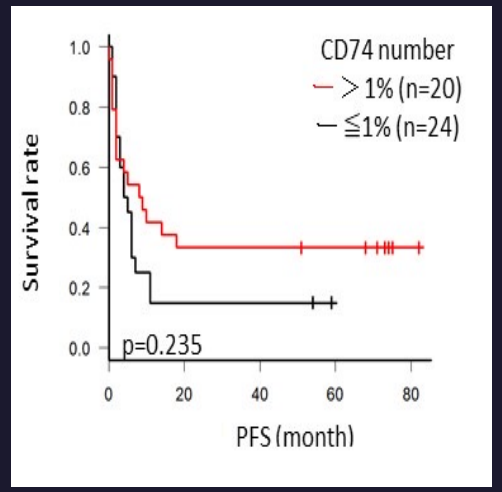


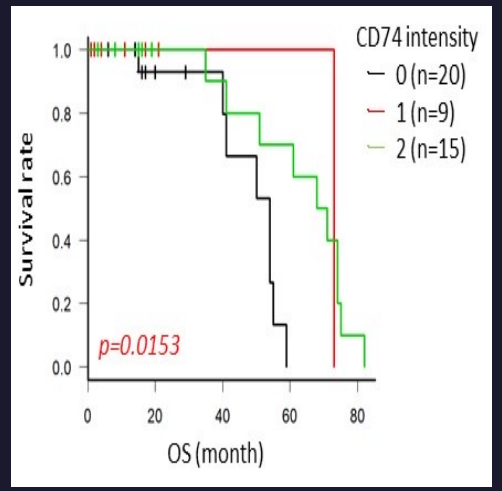
irRC of TIL Treated Patients and Progression of Disease Associate with NT Expression . NT expression in tumor cells in responders versus non-responders and their significance of this association. Mean staining intensity of NT expression in progressed patients is significantly high compared to non-progressed patients.

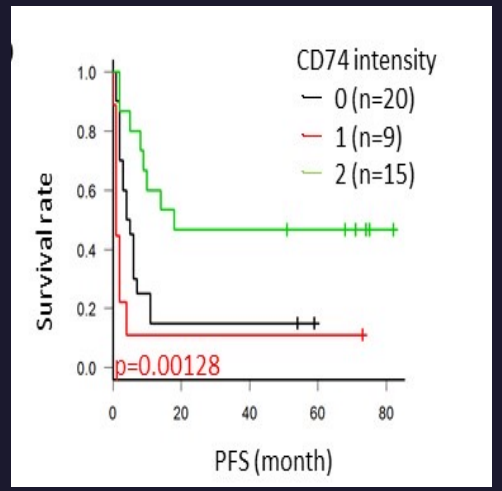
Initial Findings w/Individual Markers in TIL Treated Patients

Overall and Progression Free Survival by CD74 Number and Intensity

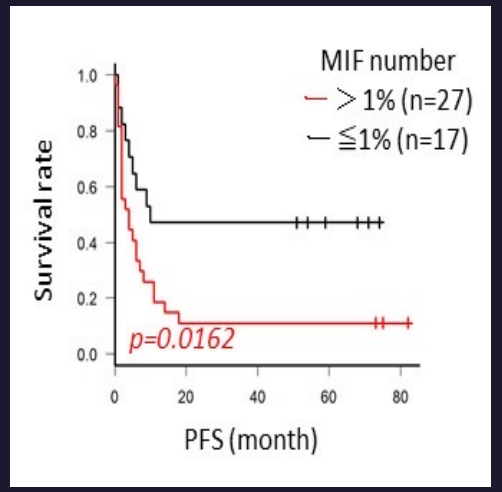


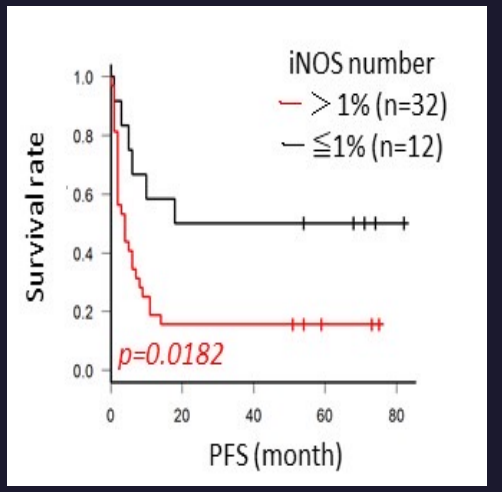


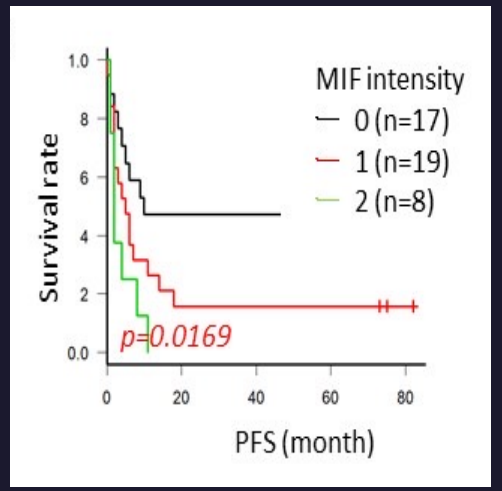


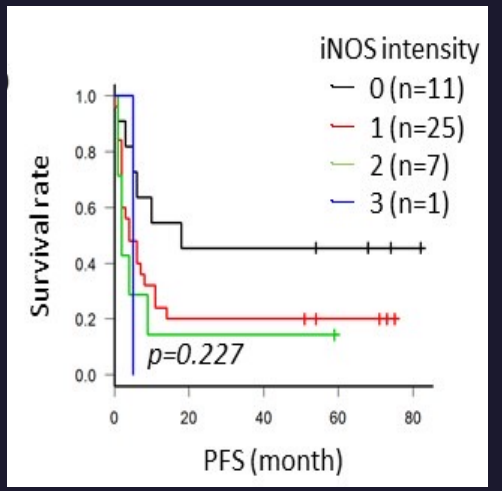


Progression Free Survival by MIF and iNOS Number and Intensity

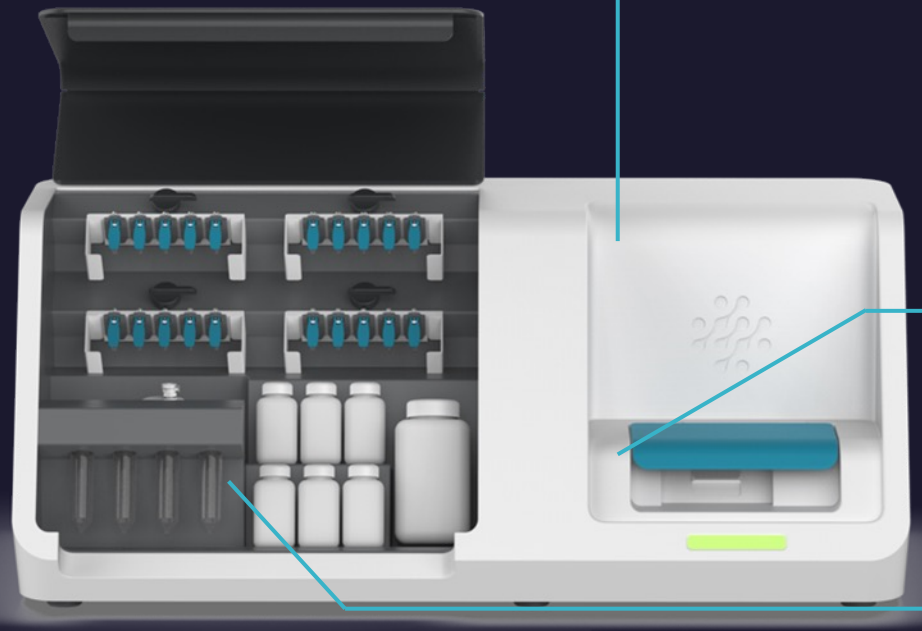








Lunaphore Comet



Microscope

- Fluorescent microscope TRITC, Cy5, DAPI
- 20X - 0.75 NA - 0.23 µm / pixel

Staining-Imaging Module

- 4 slides tray
- Works with standard histology slides
- Staining-imaging parallelization
- Temperature and pressure control

Reservoirs

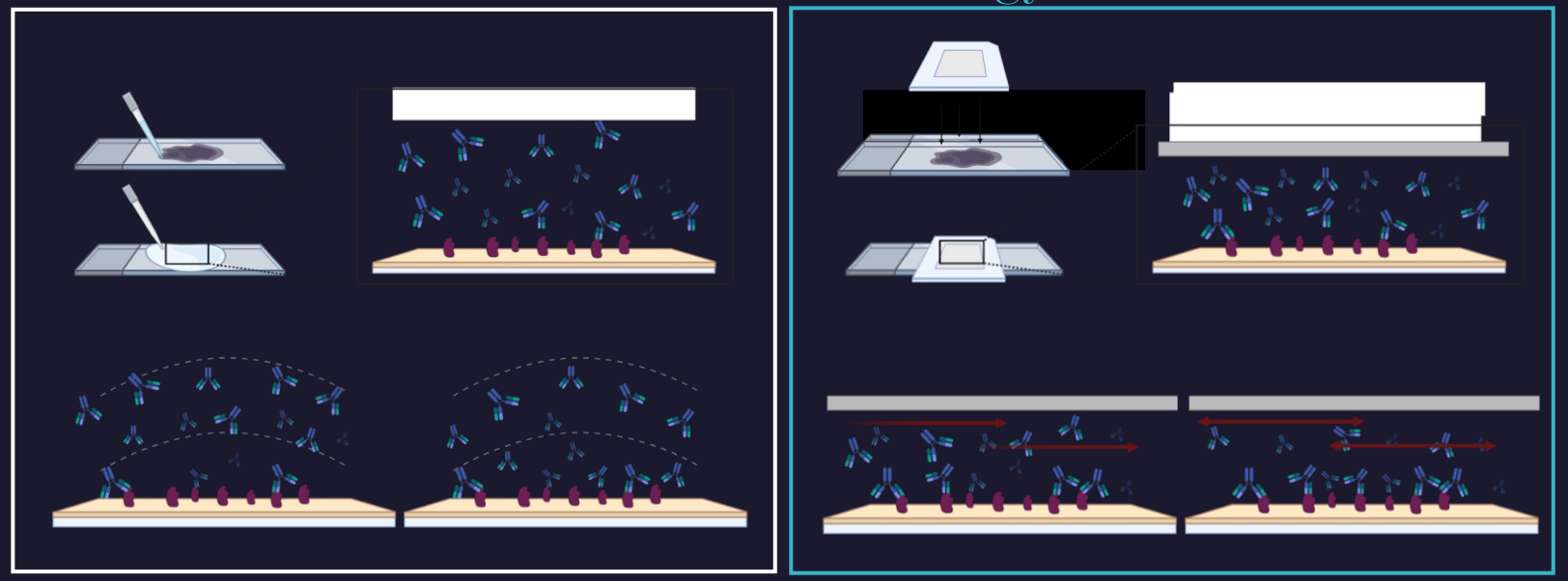
- 20 for Abl; 4 for Ab2; 7 for buffers
- Designed to process 4 slides without refill

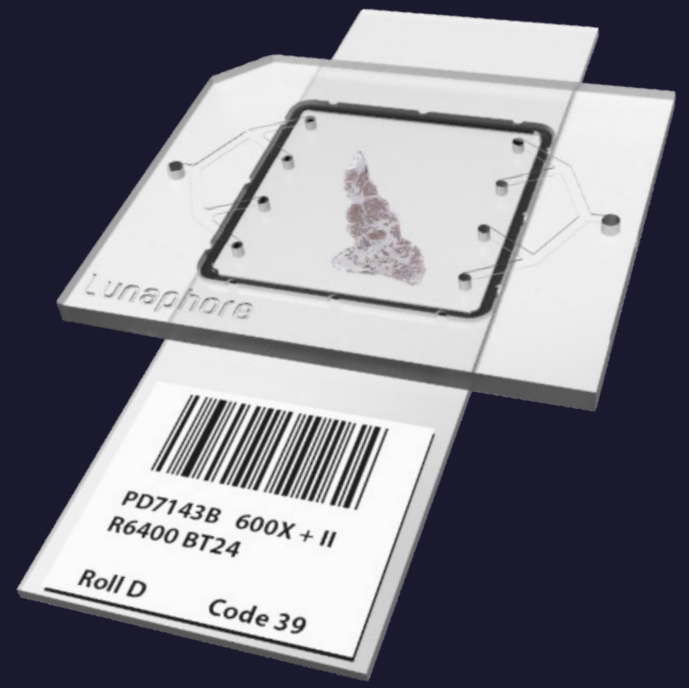
Slide Modified from Lunaphore

COMET – Core Chip Technology

Standard tissue incubation

Fast Fluidic Exchange (FFeX) Technology





- Microfluidic Imaging Chip enables staining & imaging
- Pressure-driven system allowing for fast and uniform delivery of reagents on a tissue section in a closed chamber
- Precisely controlled immuno-reaction within an extremely short incubation time enabled by the FFeX Technology

Slide Modified from Lunaphore

Protocol Times & Throughput

PREPARATION → AUTOMATED PROTOCOL

Slide Preparation
30 min – 2 hours

Staining (+ Elution) cycle <33 min
Imaging cycle ~20 min

Automated Protocol Times

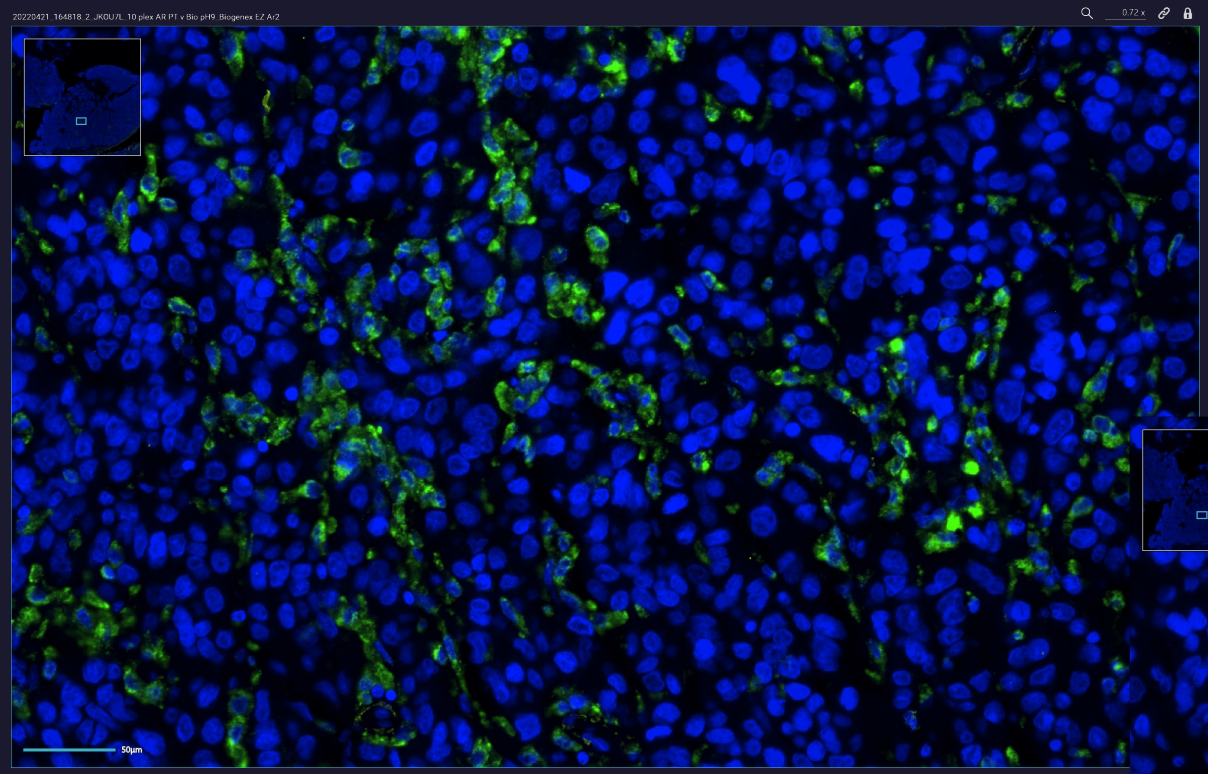
- 10-plex (Antibody Cocktail) on 4 slides: 10-15 hours
- 20-plex (Antibody Cocktail) on 4 slides: 24 hours

Throughput

- 30 slides / 5 days for 10-plex
- 20 slides / 5 days for 20-plex

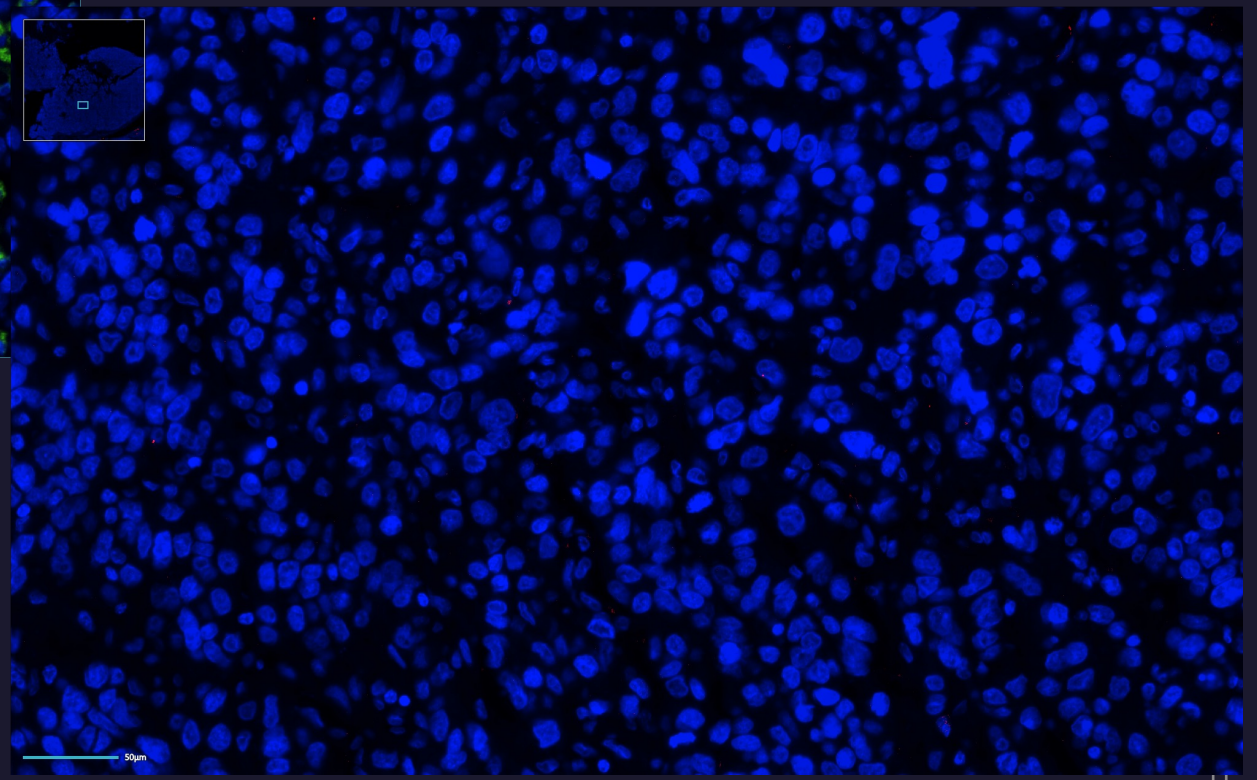
Slide Modified from Lunaphore

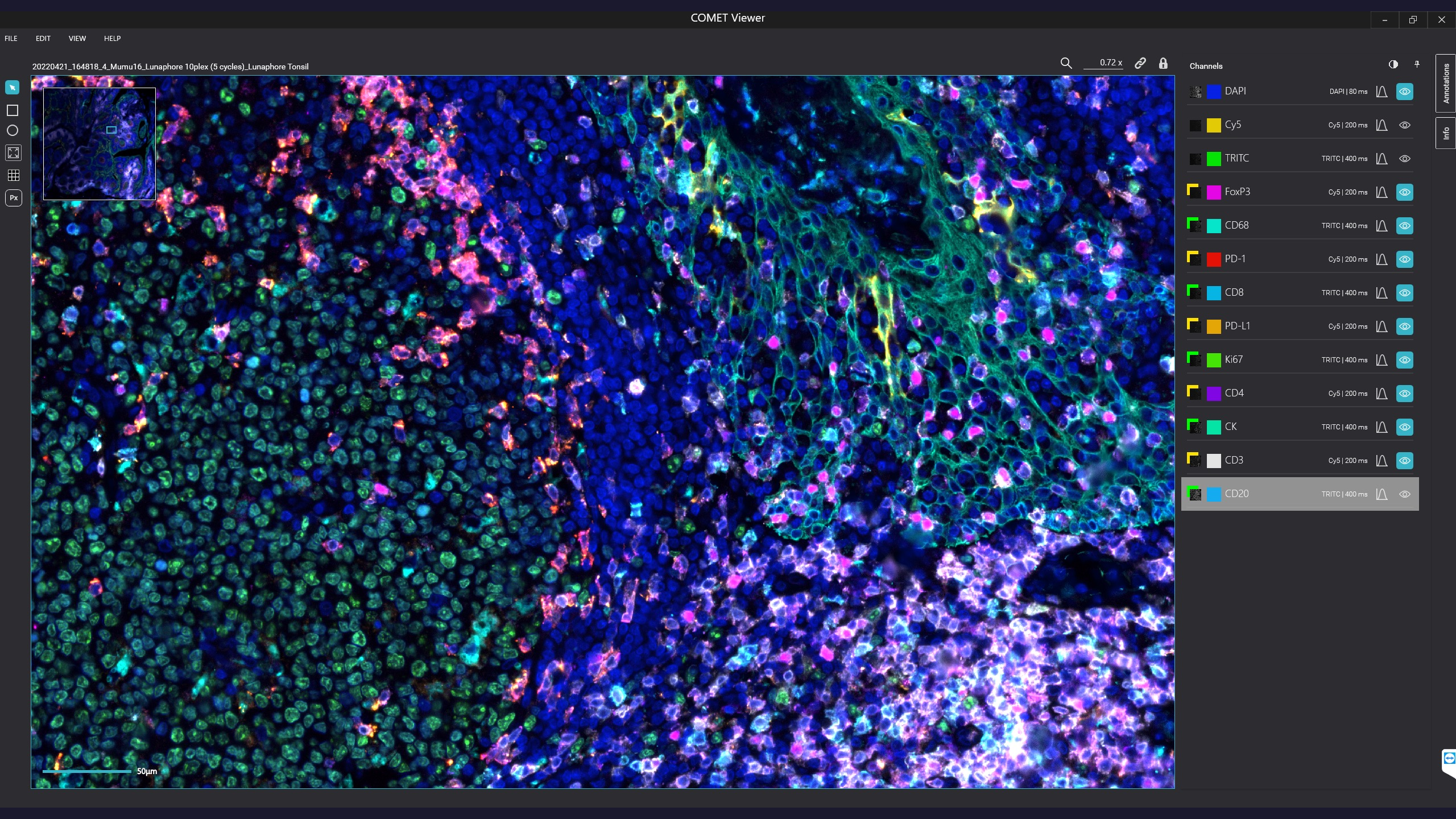
Stain & Elute



CD68 Stain in Green

CD68 Elution in Red




COMET Viewer
FILE
EDIT
VIEW
HELP
20220421_164818_4_Mumu16_Lunaphore 10plex (5 cycles)_Lunaphore Tonsil
0.72 x
Channels
DAPI
DAPI | 80 ms
Cy5
Cy5 | 200 ms
TRITC
TRITC | 400 ms
FoxP3
Cy5 | 200 ms
CD68
TRITC | 400 ms
PD-1
Cy5 | 200 ms
CD8
TRITC | 400 ms
PD-L1
Cy5 | 200 ms
Ki67
TRITC | 400 ms
CD4
Cy5 | 200 ms
CK
TRITC | 400 ms
CD3
Cy5 | 200 ms
CD20
TRITC | 400 ms
Annotations
Info
Px
50µm

Significant Findings

Our proposed markers of oxidative stress and immune-related enzymes and their mediators defines TIME architecture and predicts overall survival of advanced stage melanomas.

- Most of the melanoma cells reside in iNOS/mPGESl NBH in both LTS and STS in Stage III and IV melanomas
- iNOS/mPGESl and iNOS/NT expressing cells reside significantly closer to each other than additional inflammatory profiles.

Inflammatory signatures (defined by iNOS/NT/mPGESl, and CD74/CD44/MIF expression characteristics) regulates immune profiles;

- There is a significantly higher average proportion of CTLs in LTS than in STS
- Anti-tumor immune cells (NK and CTLs) are more clustered together in LTS than STS.
- The average distance from a tumor community to the nearest CTL community is shorter in LTS than STS.
- The average distance from an average M2 TAM to the nearest B cells is longer in LTS than STS in Stage IV melanomas
- The proportion of M2 TAM cells that are also in the MIF CD44 NBH, out of all M2 TAM cells is higher in STS than in LTS

Future

More to Do – As always...

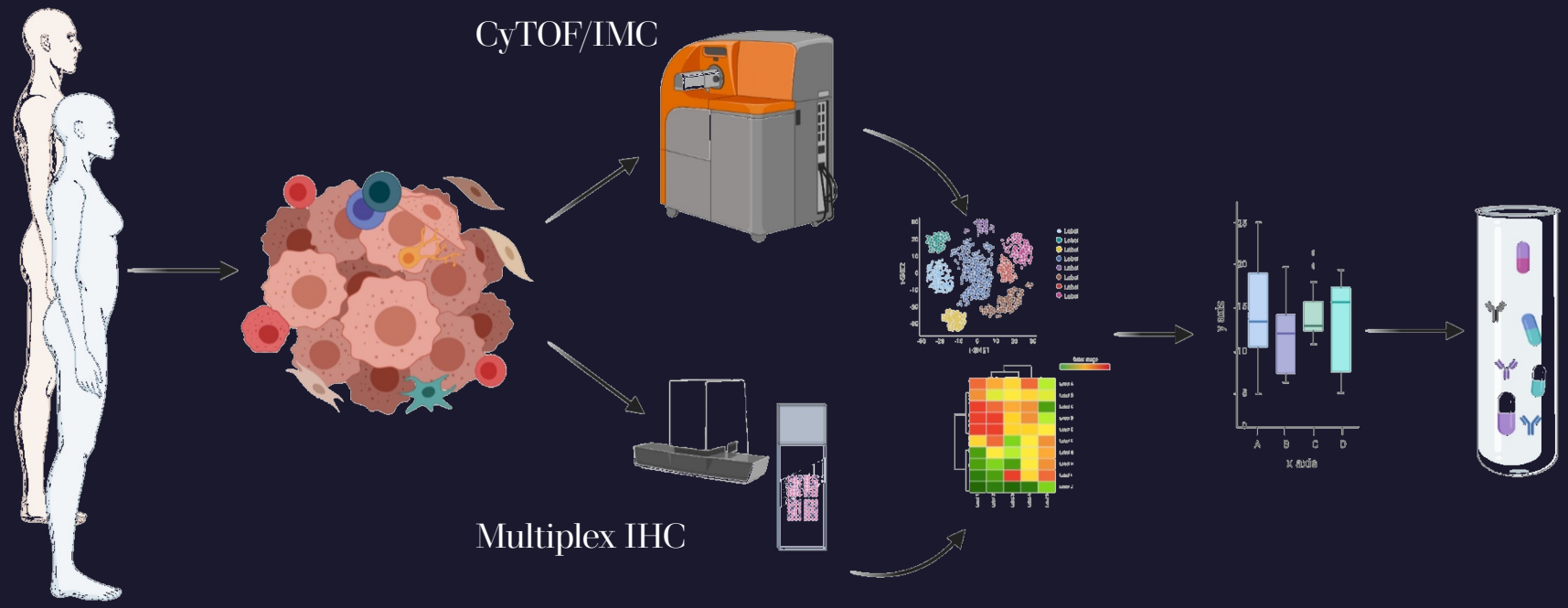
Correlate TIME signatures with multiscale radiomic properties that can be derived from routine CT scans to inform a mathematical model for the early prediction of the response to IO agents.

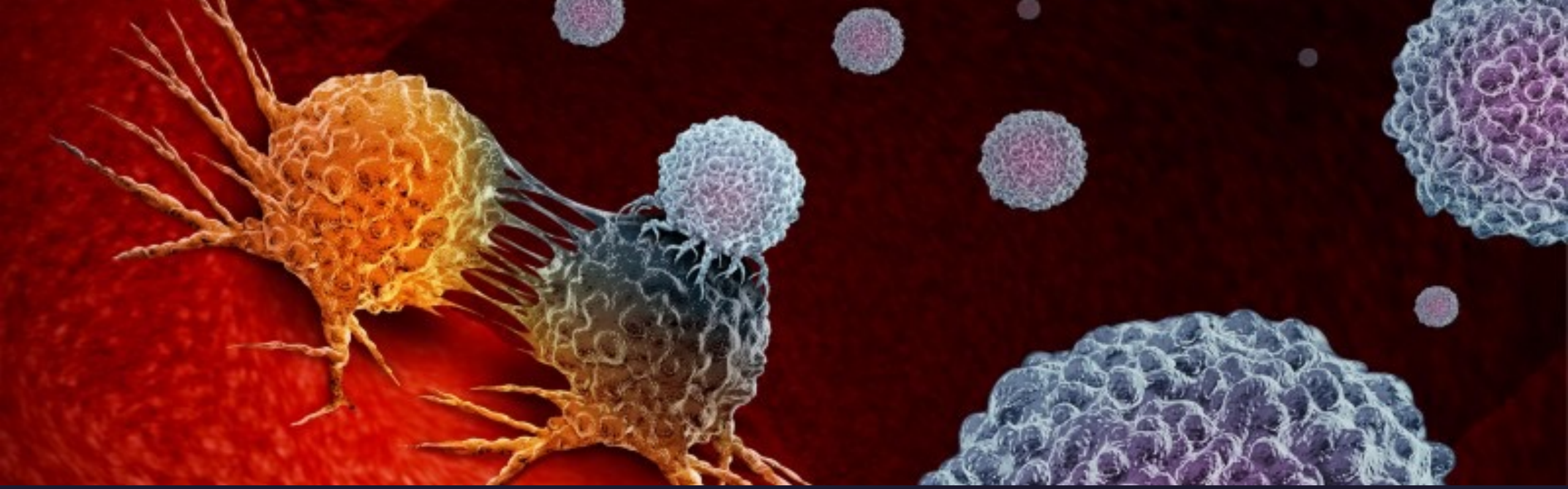
Extend and validate the mathematical model to predict melanoma response to immuno-oncology (IO) agents using the following data types;

- Imaging data: standard of care imaging, including pre-immunotherapy (T0) and post-immunotherapy (T1, T2, etc.) CT scans to quantify lesion volume and volume change over time. Our typical approach in the clinic is to obtain CT scans once every 3 months.

- Multiplex IHC data: We will correlate the refined set of markers in the mIF and CyTOF immune signatures, including iNOS, CXCR4, and CXCR7 for tumor cell proliferation rates (model parameter α;, IFNgR1, CD3+, CD4+, CD8+ and macrophage markers CD11b+F4/80+CD11c−Ly6G− as quantitative measures of tumor immune infiltration (model parameter Λ); and CD44 and dendritic cell CD11c+Ly6G−F4/80−, and MDSC markers CD11b+Gr-1Ly6G+ as quantitative indicators of immune cell kill efficacy (model parameter μ).

Validate each marker individually, and then in sets signatures, for prognostic and then by testing of predicting response to immunotherapy using (ongoing as well as retrospectively collected) human melanoma (and others) biopsy samples from patients with known immunotherapy outcomes.



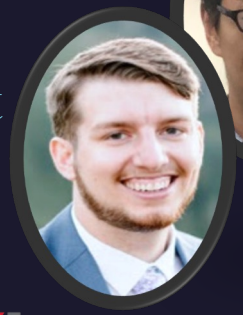


Summary

Our platform serves as comprehensive “molecular diagnosis” tool to support precision medicine approach by providing access to high-density proteomics and radiomics information.

Team

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Thank You

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