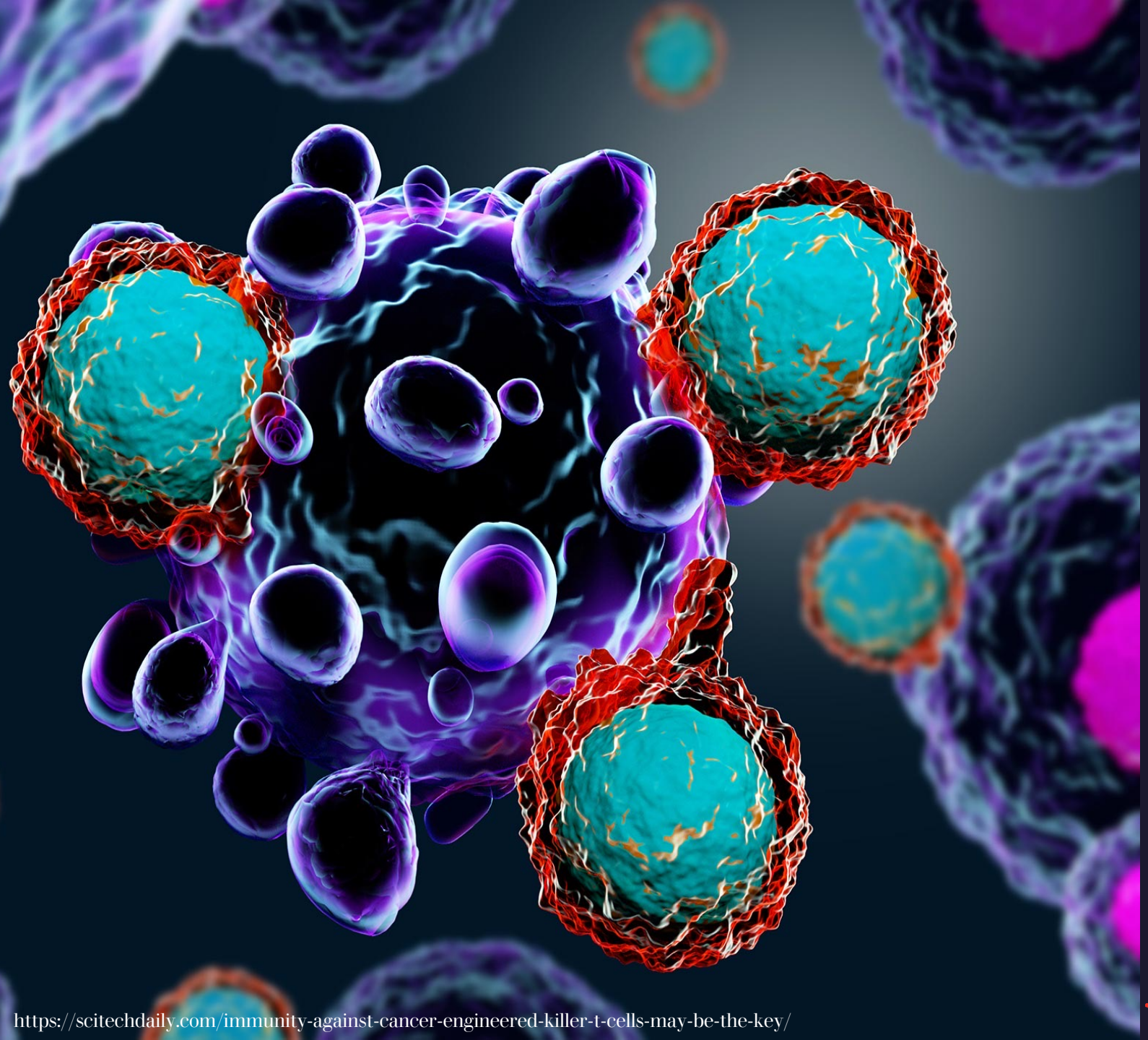

https://scitechdaily.com/immunity-against-cancer-engineered-killer-t-cells-may-be-the-key/

TUMOR IMMUNE MICROENVIRONMENT IN MELANOMA

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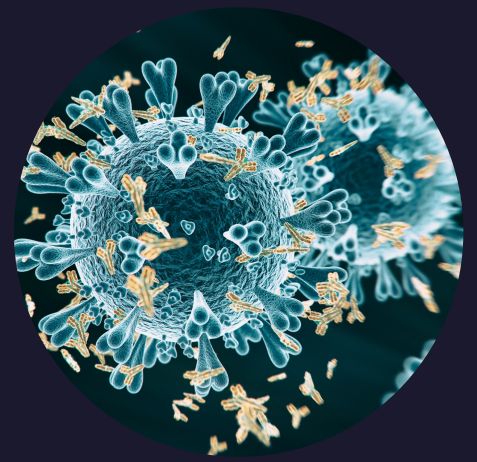
NEAR EAST UNIVERSITY
DESAM RESEARCH INSTITUTE

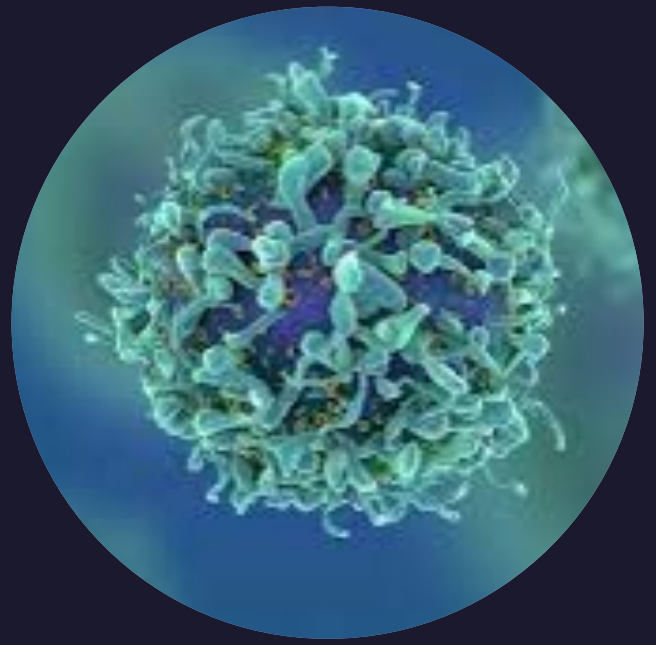
April 13, 2023

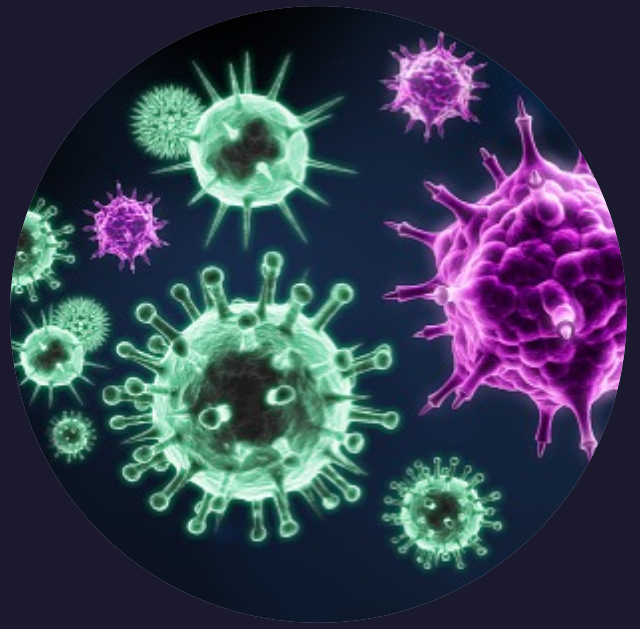
THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center®

Outline

- Introduction of myself
- Introduction of the pathway
- Overall Goal
- Key Questions
- Current Findings
- Future



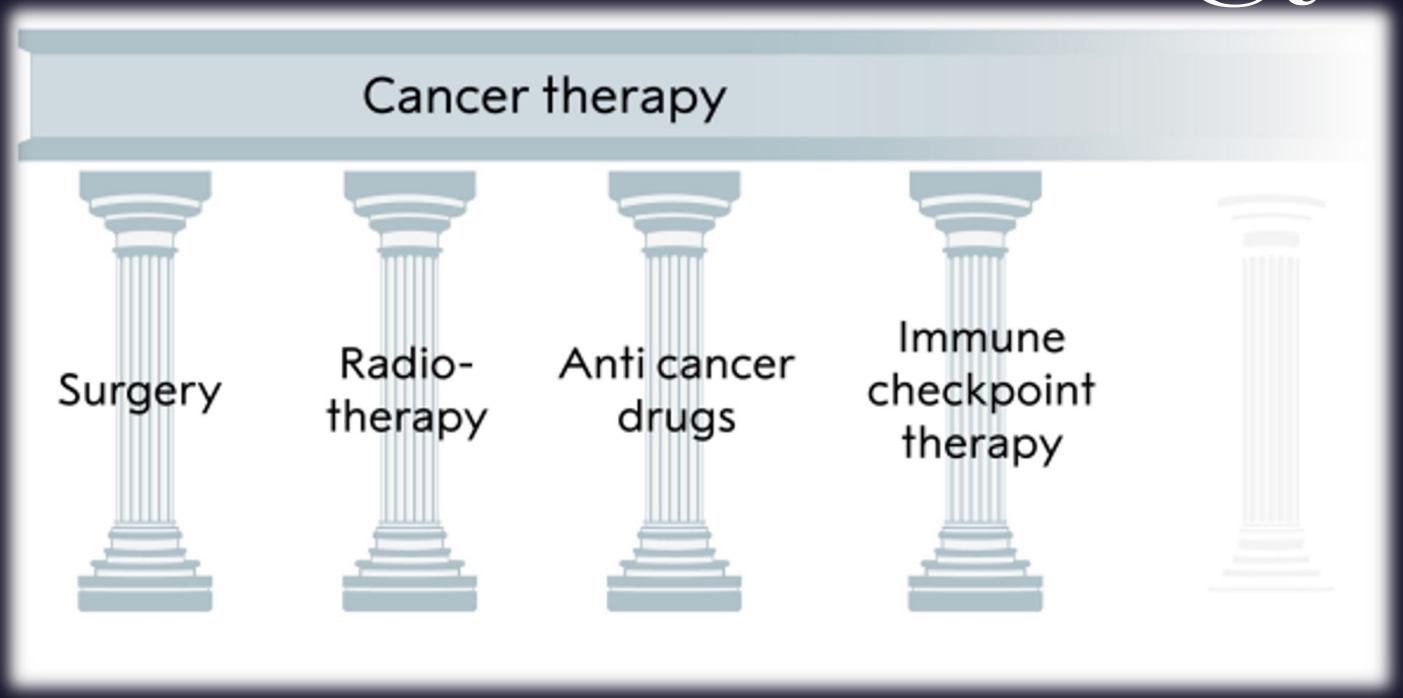




Introduction

- Immunotherapy has revolutionized cancer treatment and rejuvenated the field of tumor immunology. Several types of immunotherapy, including adoptive cell transfer (ACT) and immune checkpoint inhibitors (ICIs), have obtained durable clinical responses, but their efficacies vary, and only subsets of cancer patients can benefit from them.
- Immune infiltrates in the tumor microenvironment (TME) have been shown to play a key role in tumor development and will affect the clinical outcomes of cancer patients.
- Comprehensive profiling of tumor-infiltrating immune cells would shed light on the mechanisms of cancer–immune evasion, thus providing opportunities for the development of novel therapeutic strategies.

Immune Oncology

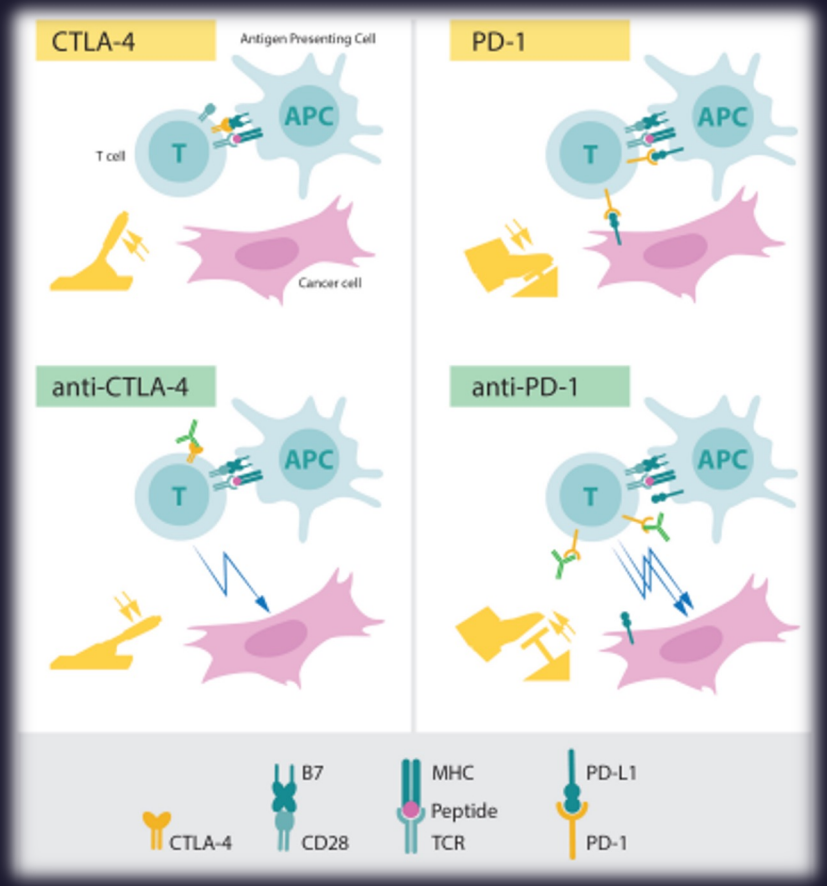


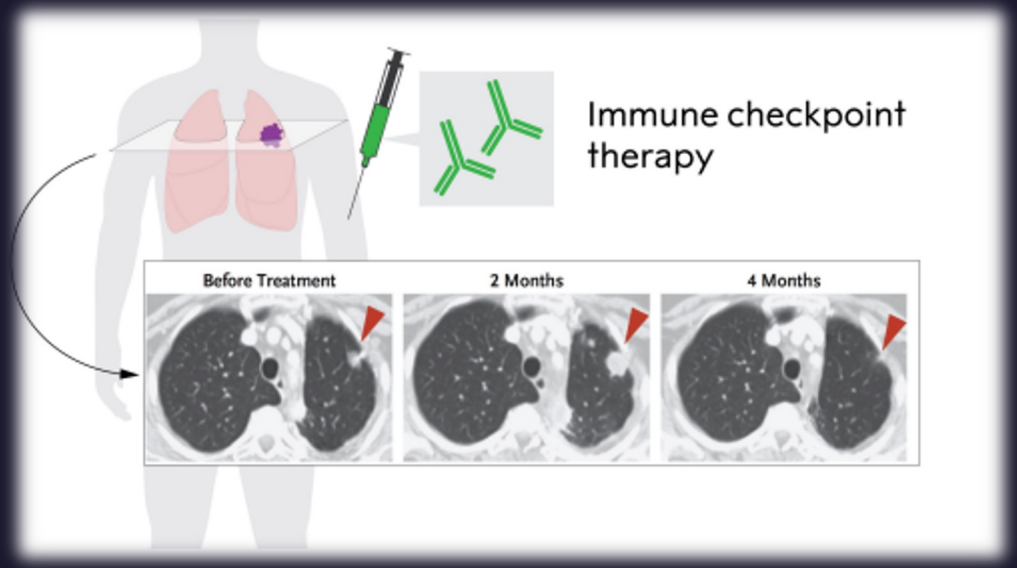
https://www.nobelprize.org/uploads/2018/10/advanced-medicineprize2018

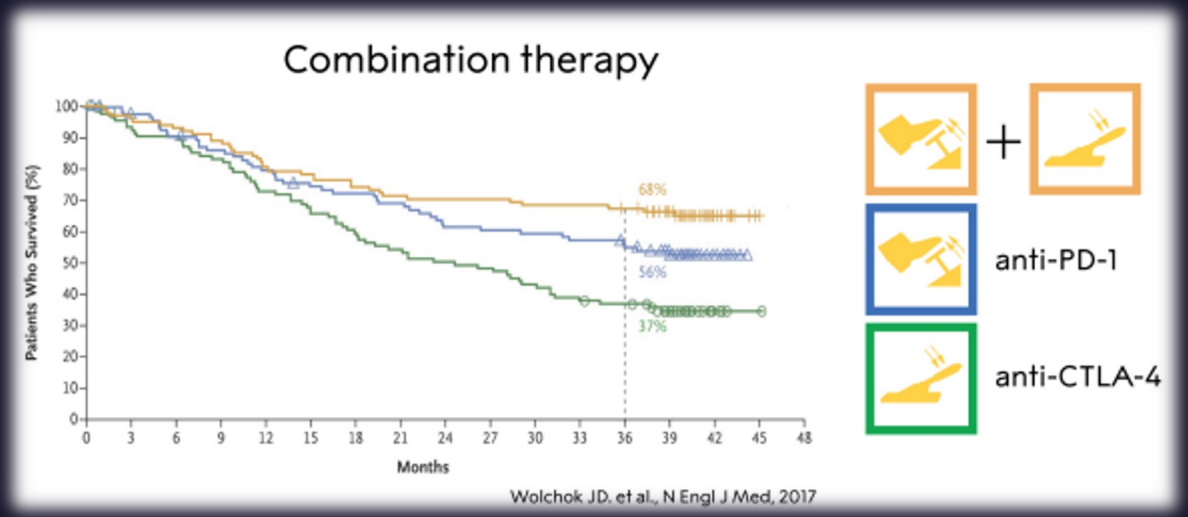
The Nobel Prize in Physiology or Medicine 2018 was awarded jointly to James P. Allison and Tasuku Honjo "for their discovery of cancer therapy by inhibition of negative immune regulation“

Drs James P. Allison, PhD (UT MDACC, USA) and Tasuku Honjo, MD (Kyoto University, Japan) were the first to identify an immune checkpoint pathway, the CTLA-4 receptor. Their discovery then led to the development of ipilimumab, an anti-CTLA-4 checkpoint immunotherapy, which was first approved by the FDA in 2011 for melanoma Currently, there are six FDA-approved checkpoint immunotherapies. Two of them, ipilimumab and nivolumab (an anti-PD-1 checkpoint immunotherapy) are approved in combination for the treatment of melanoma, while pembrolizumab (anti-PD-1) is approved as a first-line option for patients with advanced lung cancer and atezolizumab (anti-PD-L1) is approved as a first-line option for patients with advanced bladder cancer who are ineligible for chemotherapy.

Immune Oncology







https://www.nobelprize.org/uploads/2018/10/advanced-medicineprize2018

Overall Goal

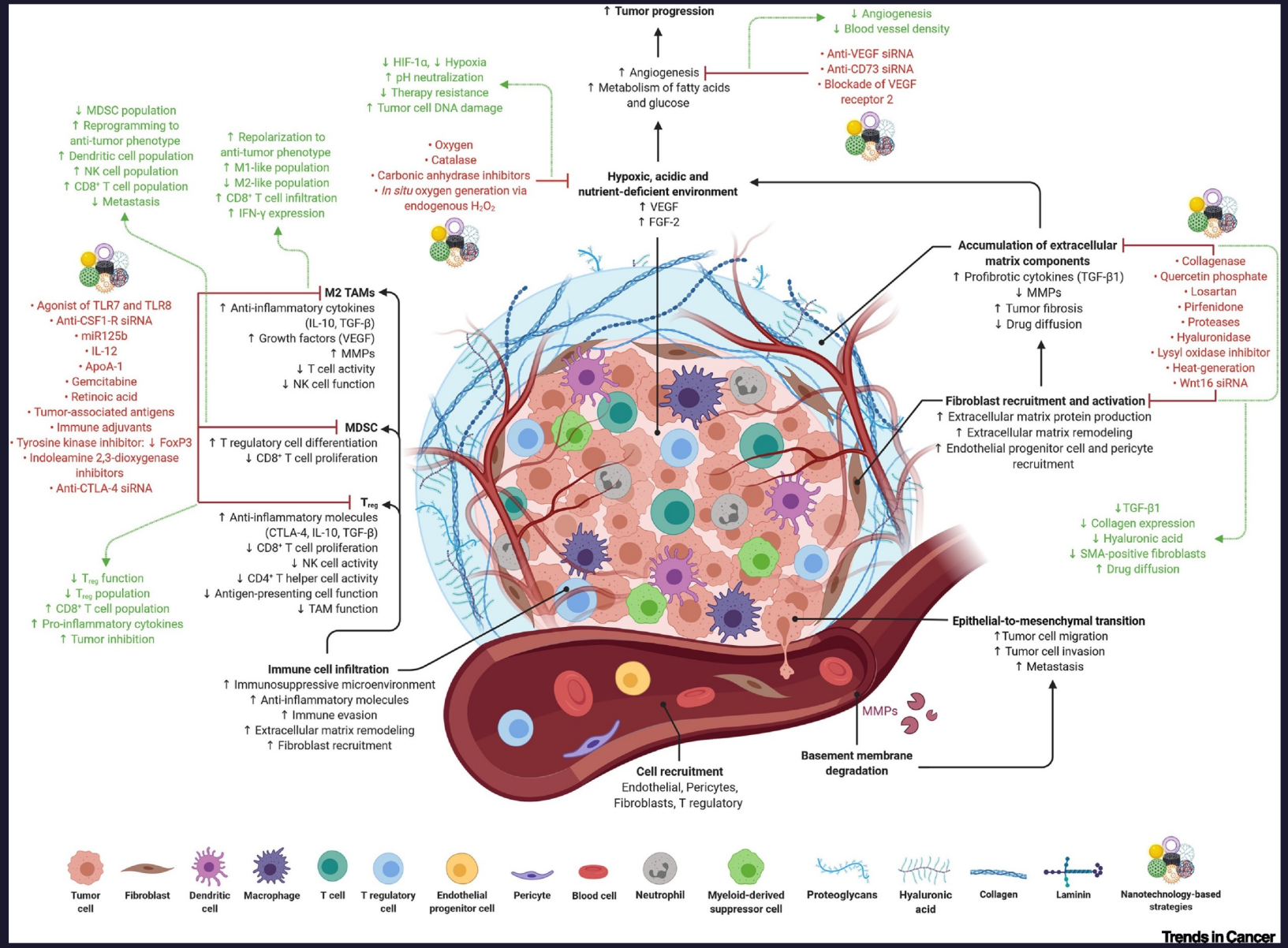
Specifically, we aim to clarify the predictive role and validate an initial set of immune-related markers. We target stably expressed innate inflammatory enzymes and their mediators (iNOS, COX-2/mPGES1, and CD74/CD44/MIF) along with their associated downstream post-translational modifications (PTM) as Nitrotyrosine (NT).

Each of these markers, individually as well as in “signatures” are being tested currently for predictive value.

Key Questions

- Why do some patients respond while others don't?
- Can we identify biomarkers that predict response?
- Can we identify markers for immune-related toxicities?
- Can we identify markers to enable patient selections to increase the number of responders?
    - Monotherapy?
    - Combination therapies –if so, what combo?
- Ultimately, can we identify other pathways that can be targeted!

THE TUMOR MICROENVIRONMENT



Schematic representation of the tumor microenvironment (TME), which comprises stromal and immune cells and extracellular matrix components, among others, involved in metabolic, cellular, and tissue remodeling.

CellPress - https://doi.org/10.1016/j.trecan.2021.05.001

THE TUMOR MICROENVIRONMENT – Melanoma

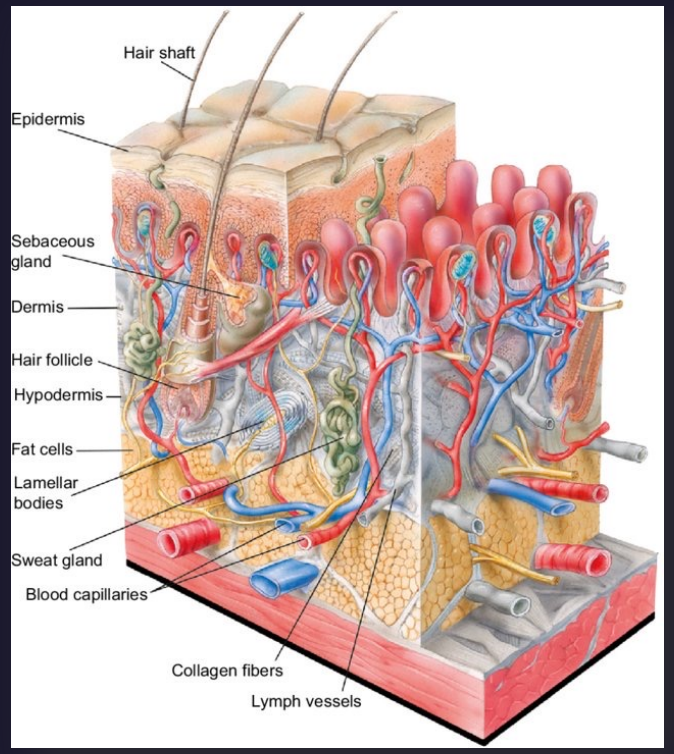
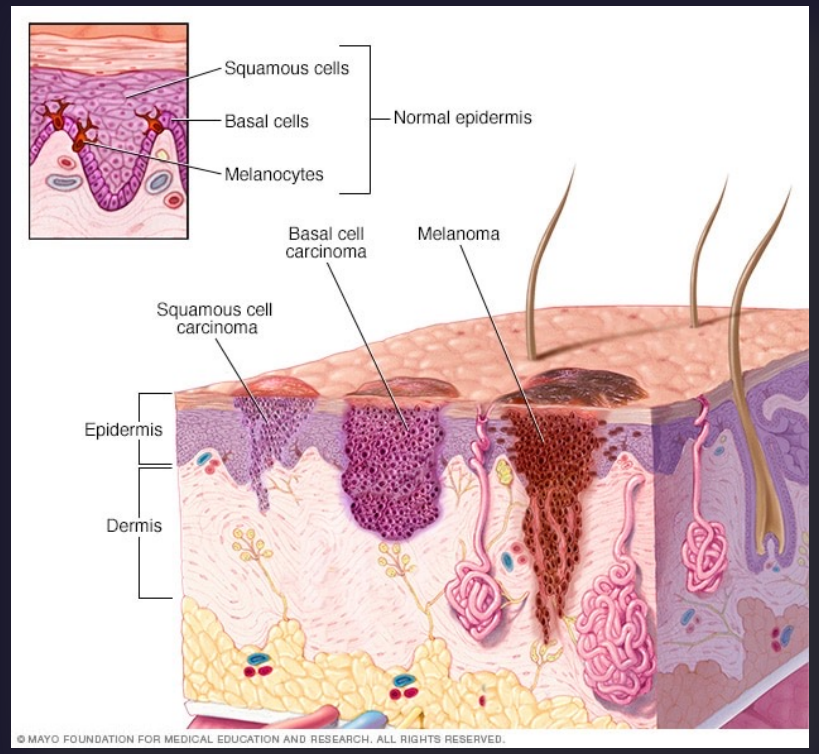
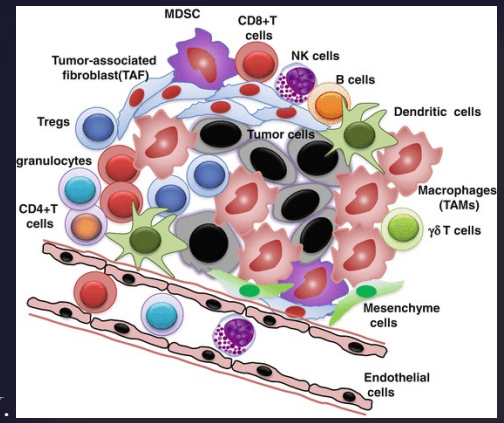


Illustration: © www.julius-ecke.de



https://www.mayoclinic.org/-/media/kcms/gbs/

Structure drives the normal function?



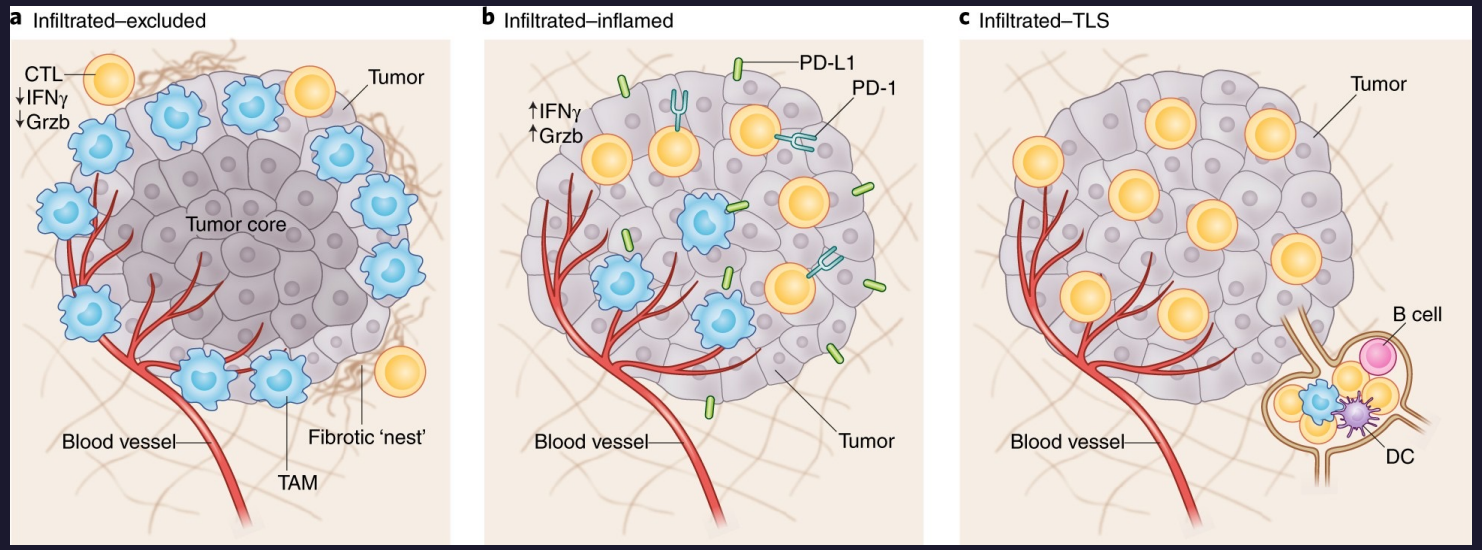
In: Torres-Cabala C., Curry J. (eds) Genetics of Melanoma. Cancer Genetics. Springer, New York, NY.

THE TUMOR IMMUNE MICROENVIRONMENT (TIME)

The past decade has seen a revolution in cancer treatments by moving away from drugs that target tumors broadly and toward the use of immunotherapies that modulate immune responses against tumors.

Retrospective analyses of patient populations treated with Immune Checkpoint Blockade (ICB) have revealed that there are classes of TIME that are associated with those tumors more inclined to ICB responsiveness.

Infiltrated–Excluded (I–E) TIMEs -- "cold" tumors.
TIMEs that are broadly populated with immune cells but are relatively void of CTLs in the tumor core. CTLs localized along the border of the tumor mass in the invasive margin or 'caught' in fibrotic nests. I–E TIMEs, compared with more inflamed TIMEs, contain CTLs with low expression of the activation markers *GZMB* (*GRZB*) and *IFNG* and poor infiltration of CTLs into the tumor core.
-melanoma



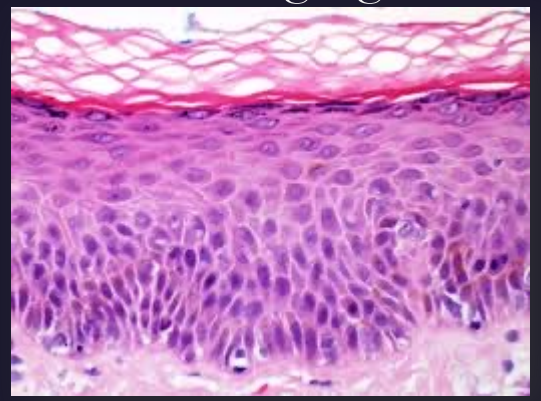
Infiltrated–inflamed (I–I) TIMEs -- "hot" tumors
High infiltration of CTLs expressing PD-1 and leukocytes and tumor cells expressing the immune-dampening PD-1 ligand PD-L1.

A subclass of I–I TIMEs, TLS-TIMEs, display histological evidence of tertiary lymphoid structures (TLSs), cellular composition is similar to that in lymph nodes.
TLSs are often correlated with a positive prognosis. Similarly to lymph nodes, TLSs can contain a substantial diversity of lymphocytes, including naive and activated conventional T cells, Treg cells, B cells and DCs. TLSs are generally present at the invasive tumor margin and in the stroma, and are thought to act as sites of lymphoid recruitment and immune activation.

Nature Medicine | VOL 24 | MAY 2018 | 541–550 | www.nature.com/naturemedicine

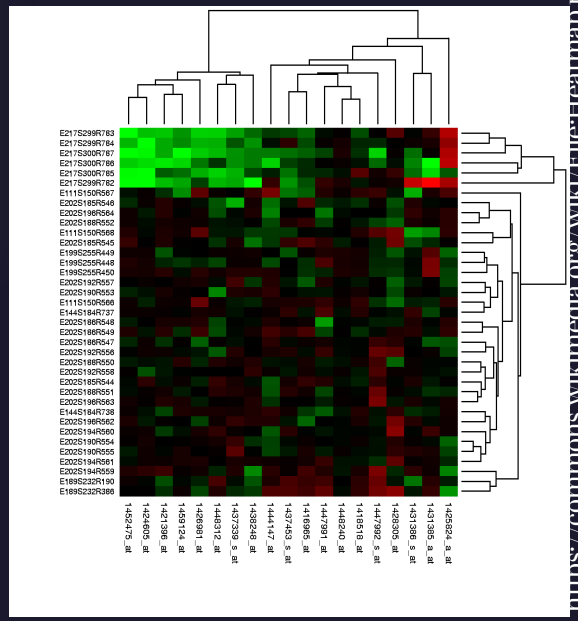
HOW DO WE STUDY TUMOR MICROENVIRONMENT

Imaging



Tissue Intact
Few Proteins
Lots of Tissue Use

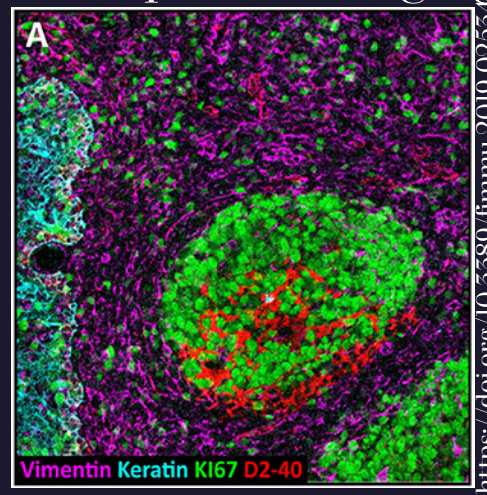
Omics



https://commons.wikimedia.org/wiki/File:Heatmap.png

Many Genes
Minimal Tissue Use
No Tissue Architecture

Multiplexed Imaging



https://doi.org/10.3389/fimmu.2019.02554

Tissue Intact
Many Proteins
Minimal Tissue Use

THE PANELS

Hyperion Metal

CD3
CD4
CD8a
CD11b
CD11c
CD19
SOX-10
CD20
CD31
CD44
CD45
CD56
CD68
CD134
HLA-A,B,C
HLA-DR
EOMES
CXCR2
CXCR-4
SOX-10
S100A9
Vimentin
FoxP3
VEGF
Arginase-1
PTEN
p-Tyrosine
T-bet
HIF-1a
PD-1
PD-L1
PD-L2
pERK
GzmB
Ki67

Vectra Opal

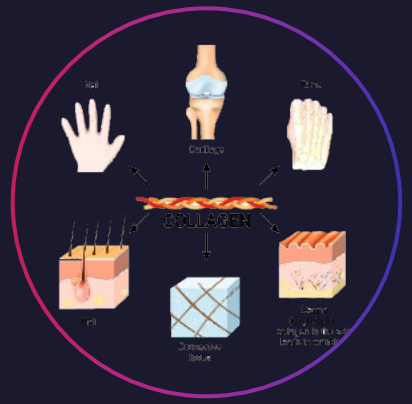
iNOS
NT
mPGES1
CD74
MIF
CD44
DAPI

Lunaphore Comet

SOX10
Ki67
iNOS
NT
mPGES1
CD74
CD44
MIF
Arginase-1
CD45
CD3
CD4
CD8
CD20
CD56
CD68
CD163
GrzB
HLA-DR

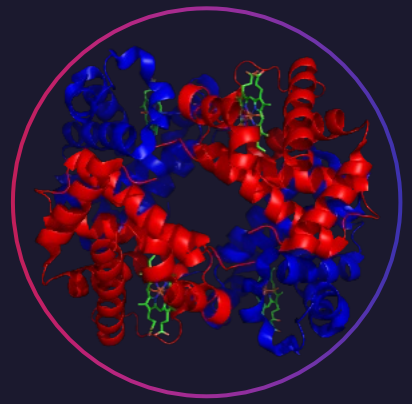
Phenotypic Protein

Characteristic molecules for cell lineages, e.g. cell surface markers.



Structural Protein

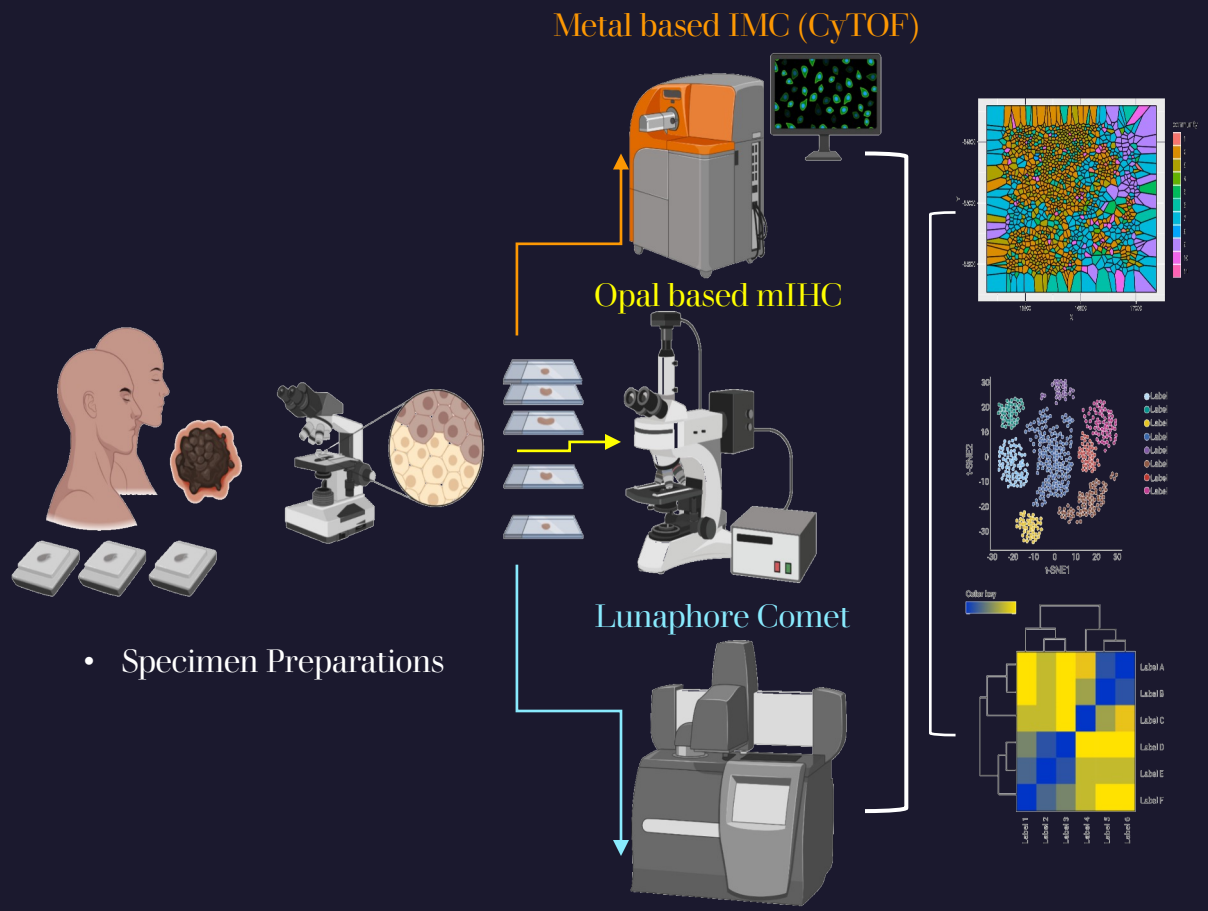
Fibrous proteins, e.g. the most familiar of the fibrous proteins are probably the keratins.



Functional Protein

More than building blocks, these generally form complex mixtures of active proteins that carry out a function, e.g. help support and maintain normal immune function.

COMPUTATIONAL ANALYSIS PIPELINE



- Immune composition variability could be measured
- Spatial enrichment analysis may reveal subtypes of immune-tumor organization
- Immune composition and tissue architecture could reveal more information on the same marker's functional outcome
  - ➢CD74+ cells could be in both tumor and immune cells area but MIF+ cells proximity may change the outcome.
- Tissue organization (mixed versus compartmentalized) may correlate with immune response to the given treatment.
  - ➢Compartmentalized phenotype correlates with better overall survival.

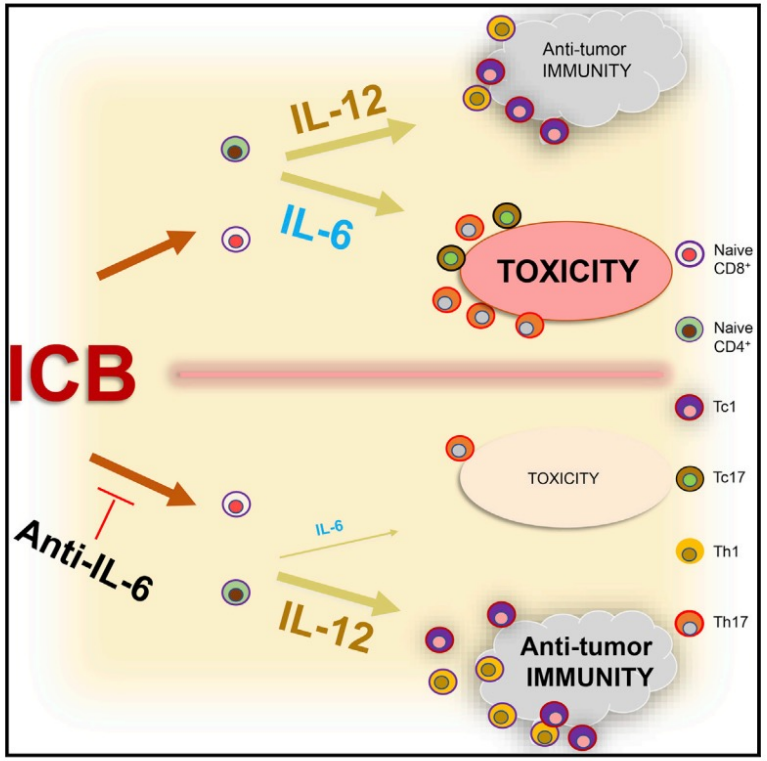
Current Findings

Cancer Cell

Article

Interleukin-6 blockade abrogates immunotherapy toxicity and promotes tumor immunity

Graphical abstract



Authors

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In brief

Hailemichael et al. find that expression of interleukin-6, a Th17-cell differentiation cytokine, and neutrophil and chemotactic markers increase in inflamed tissue of patients and mice receiving immuno-therapy. Blockade of IL-6 reduces Th17 and increases Th1 and CD8$^+$ T effector cell density in tumor, mitigates ICB-induced autoimmunity, and potentiates antitumor immunity.

Cancer Cell

CellPress

Article

Interleukin-6 blockade abrogates immunotherapy toxicity and promotes tumor immunity

Yared Hailemichael,[1,18] Daniel H. Johnson,[1,14,18] Noha Abdel-Wahab,[1,2,15,18] Wai Chin Foo,[3] Salah-Eddine Bentebibel,[1] May Daher,[4] Cara Haymaker,[5] Khalida Wani,[5] Chantal Saberian,[1] Dai Ogata,[1] Sang T. Kim,[2] Roza Nurieva,[6,17] Alexander J. Lazar,[3,5,16] Hamzah Abu-Sbeih,[7] Faisal Fa'ak,[1] Antony Mathew,[7] Yinghong Wang,[7] Adewunmi Falohun,[2] Van Trinh,[8] Chrystia Zobniw,[8] Christine Spillson,[1] Jared K. Burks,[9] Muhammad Awiwi,[10] Khaled Elsayes,[10] Luisa Solis Soto,[5] Brenda D. Melendez,[1] Michael A. Davies,[1] Jennifer Wargo,[11,16] Jonathan Curry,[3] Cassian Yee,[1,6] Gregory Lizee,[1,6] Shalini Singh,[6] Padmanee Sharma,[6,12,13] James P. Allison,[6,13] Patrick Hwu,[1] Suhendan Ekmekcioglu,[1] and Adi Diab[1,19,*]

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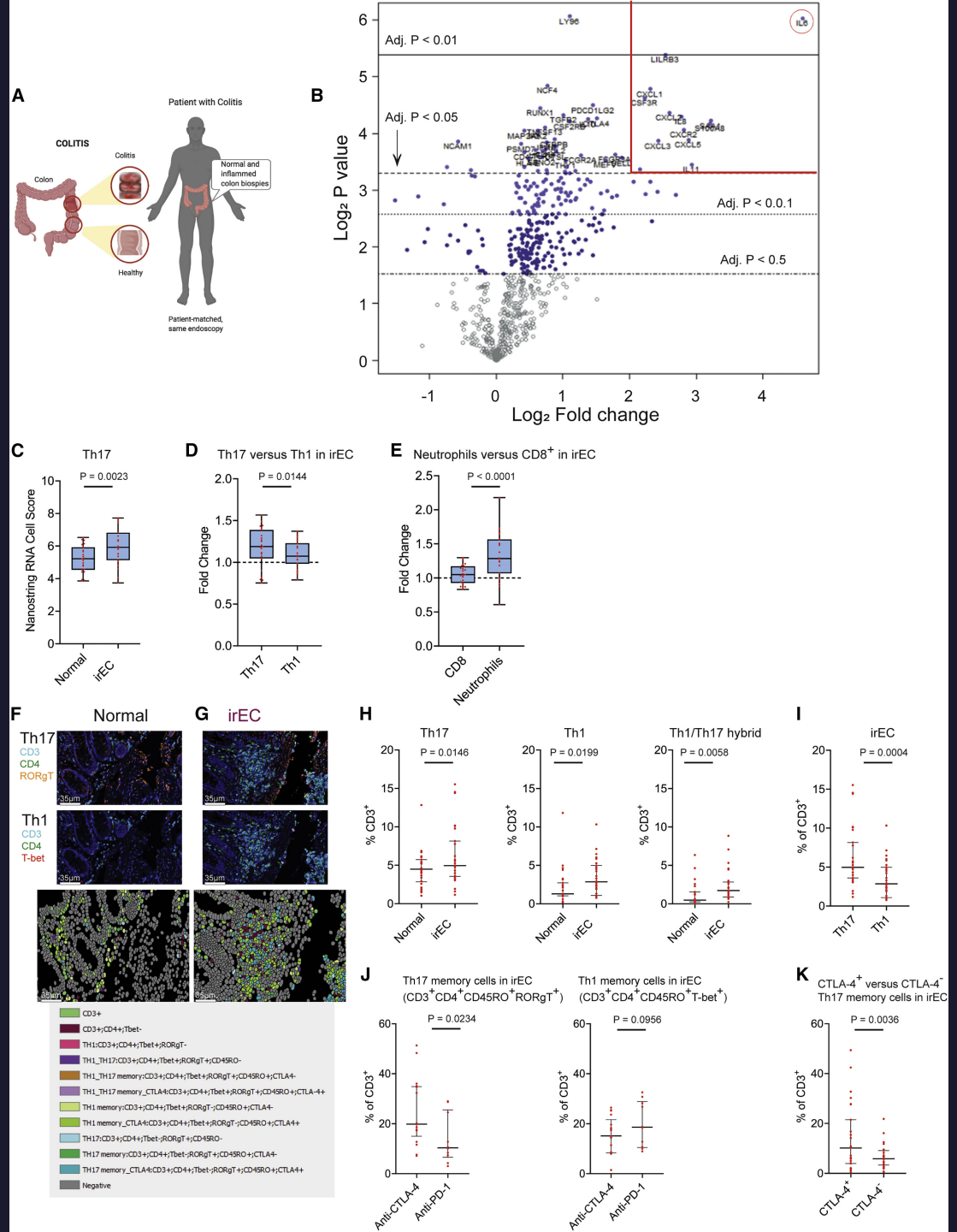
https://doi.org/10.1016/j.ccell.2022.04.004

SUMMARY

Immune checkpoint blockade (ICB) therapy frequently induces immune-related adverse events. To elucidate the underlying immunobiology, we performed a deep immune analysis of intestinal, colitis, and tumor tissue from ICB-treated patients with parallel studies in preclinical models. Expression of interleukin-6 (IL-6), neutrophil, and chemotactic markers was higher in colitis than in normal intestinal tissue; T helper 17 (Th17) cells were more prevalent in immune-related enterocolitis (irEC) than T helper 1 (Th1). Anti-cytotoxic T-lymphocyte-associated antigen 4 (anti-CTLA-4) induced stronger Th17 memory in colitis than anti-program death 1 (anti-PD-1). In murine models, IL-6 blockade associated with improved tumor control and a higher density of $CD4^+/CD8^+$ effector T cells, with reduced Th17, macrophages, and myeloid cells. In an experimental autoimmune encephalomyelitis (EAE) model with tumors, combined IL-6 blockade and ICB enhanced tumor rejection while simultaneously mitigating EAE symptoms versus ICB alone. IL-6 blockade with ICB could de-couple autoimmunity from antitumor immunity.

Highlights

- Immunotherapy increases expression of Th17 and Tc17 cell differentiation cytokine IL-6
- Th17 cells are more prevalent in enterocolitis than Th1
- IL-6 blockade reduces Th17, increases Th1 and Tc1 cell density in ICB-treated tumors
- Blockade of IL-6 decouples ICB antitumor immunity and toxicity



IL-6-mediated inflammation was observed in immune checkpoint blockade induced immune-related enterocolitis (irEC) samples from patients with cancer

(A) Schematic diagram for sample collection for gene expression profiling and multiplex IHC analyses.
(B) Volcano plot of irEC compared with normal intestinal tissue. Significantly upregulated genes with log2 fold change >2 are shown inside the red lines. IL-6 log2 fold change (red circle).
(C–E) Box plots visualize estimate of abundance of immune cell subset populations using expression of characteristic genes.
(C) Th17 cells within irEC compared with normal colon tissue.
(D) Th17 cells compared with Th1 cells in irEC.
(E) Neutrophils compared with $CD8^+$ cells in irEC.

(F and G) Example of multiplex IHC with cell type annotation and visualizations.
(F) Normal intestinal tissue
(G) irEC tissue samples.

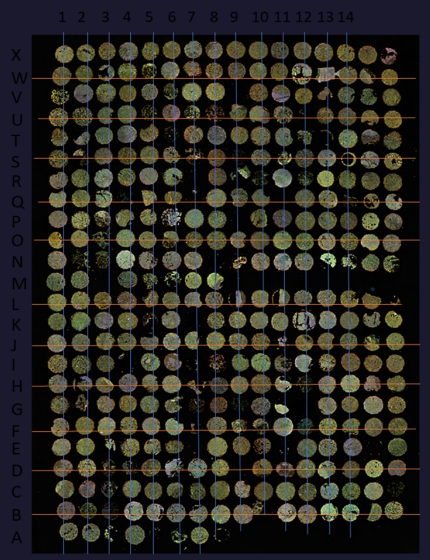
(H) Percentage of total T cells from multiplex IHC in normal intestinal tissue compared with irEC tissue samples.
(I) Percentage of Th17 cells compared with Th1 cells in irEC.
(J) Percentage of Th17 or Th1 memory cells in irEC induced by anti-CTLA-4 compared with anti-PD-1 monotherapy.
(K) CTLA-4 expression among Th17 memory cells in irEC. Data are presented as median and IQR (n = 27, unpaired t test).

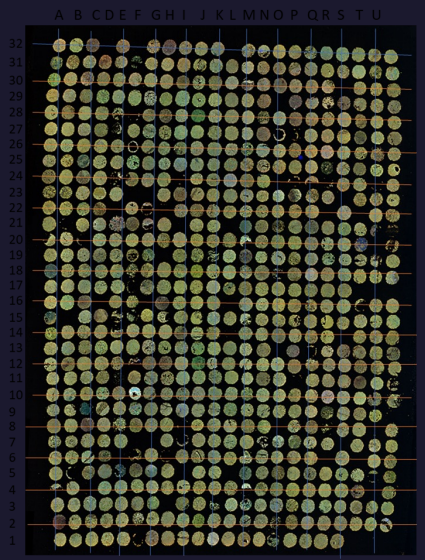
Current Cohorts to Establish Signatures

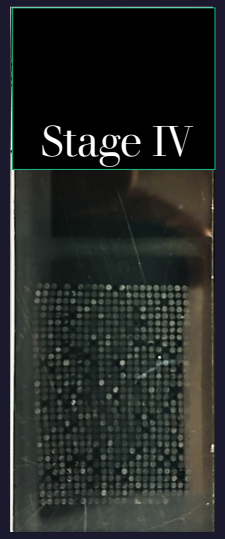
Slide	Stage III		Stage IV	
Antibody Conjugation	Fluorescent	Metal	Fluorescent	Metal
# of Core (ROI)	384	301	704	640
# of Plex	7	36	7	37
Type of file	.qptiff	.mcd & .txt	.qptiff	.mcd & .txt

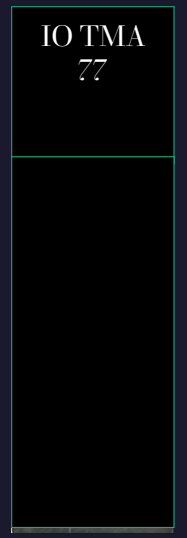
IO TMA	
Fluorescent	Metal
77	77
7	36
.qptiff	.mcd & .txt

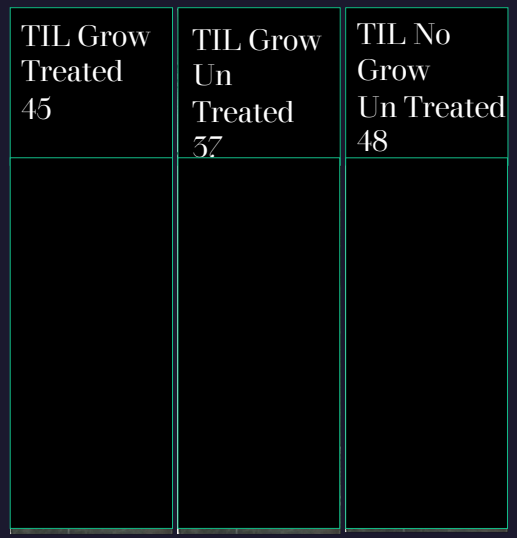
TIL Samples	
Fluorescent	Metal
130	45
7	36
.qptiff	.mcd & .txt



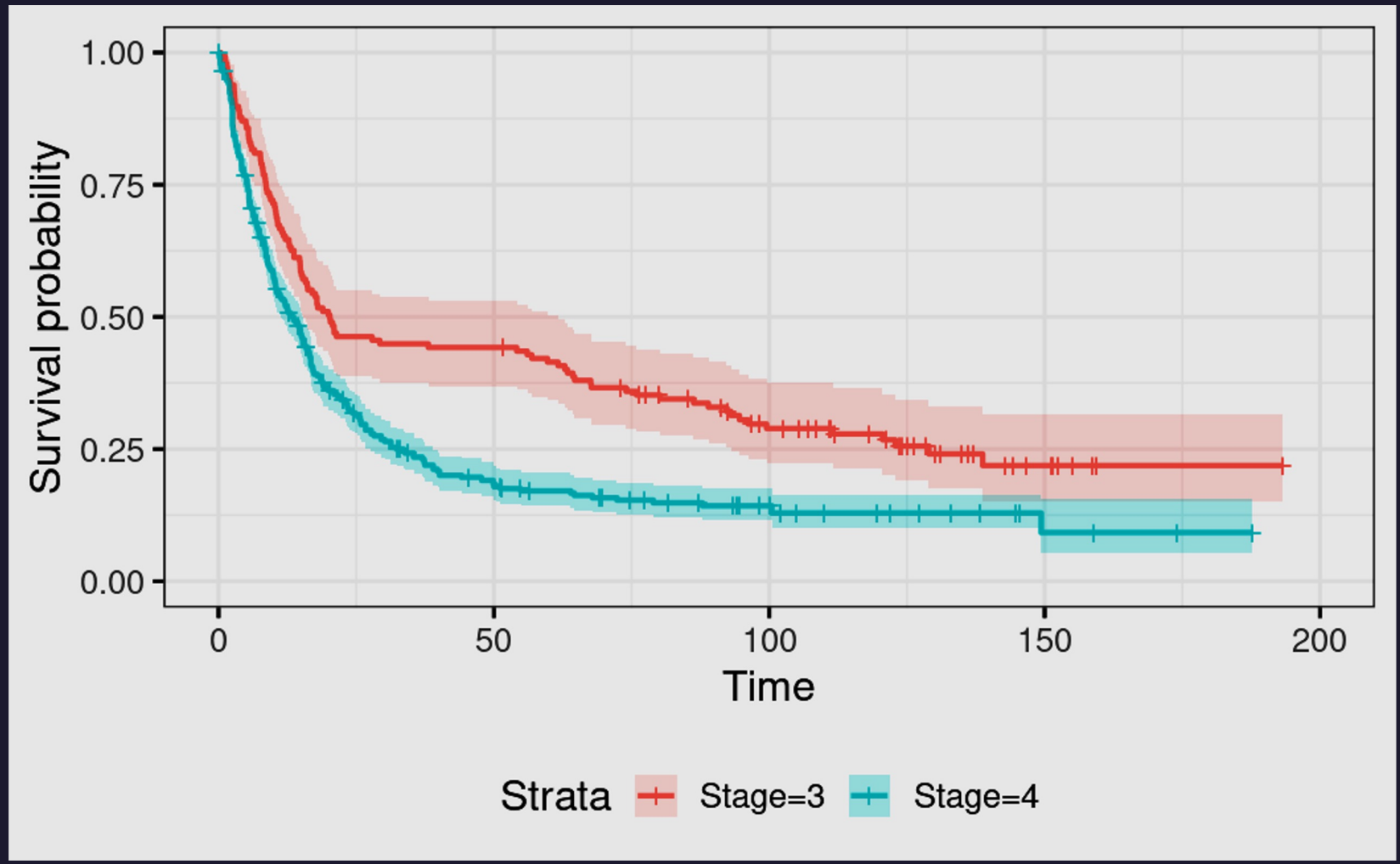




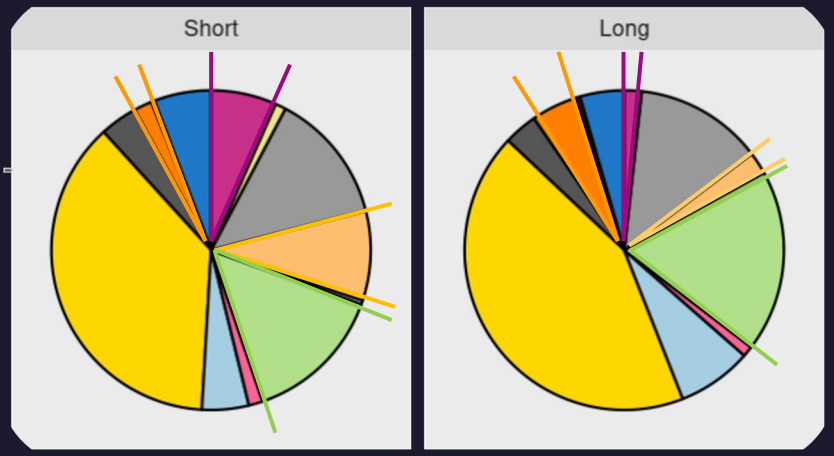




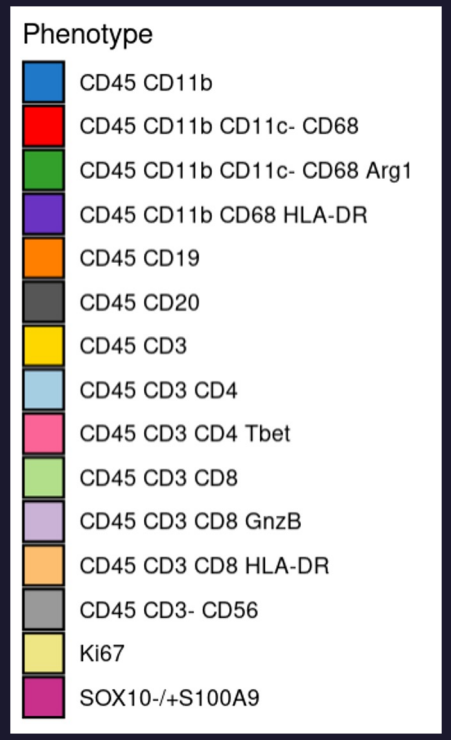
KM Survival Plots - Stage III and IV Melanoma TMA

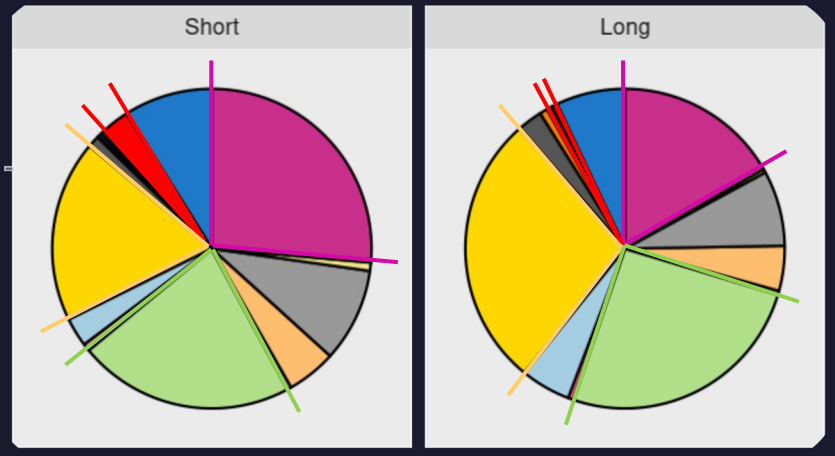


Phenotype Frequencies Across Survival Groups



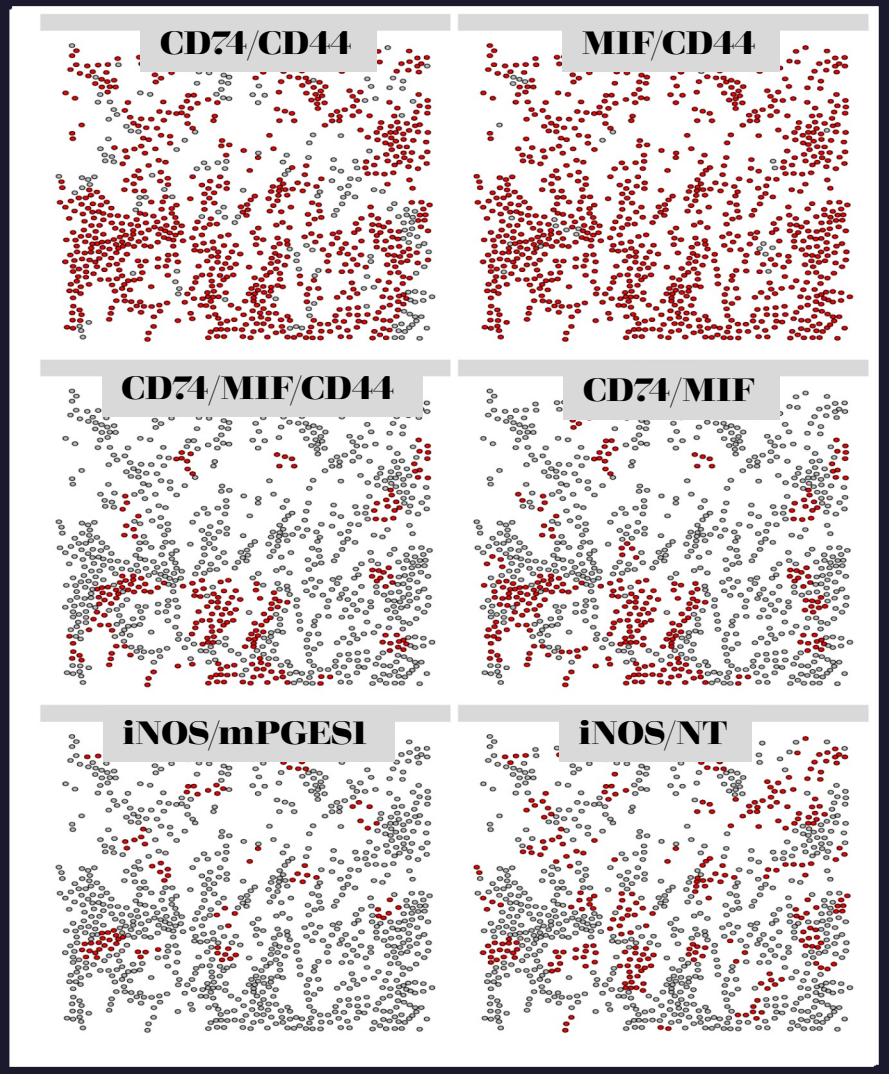
Stage III



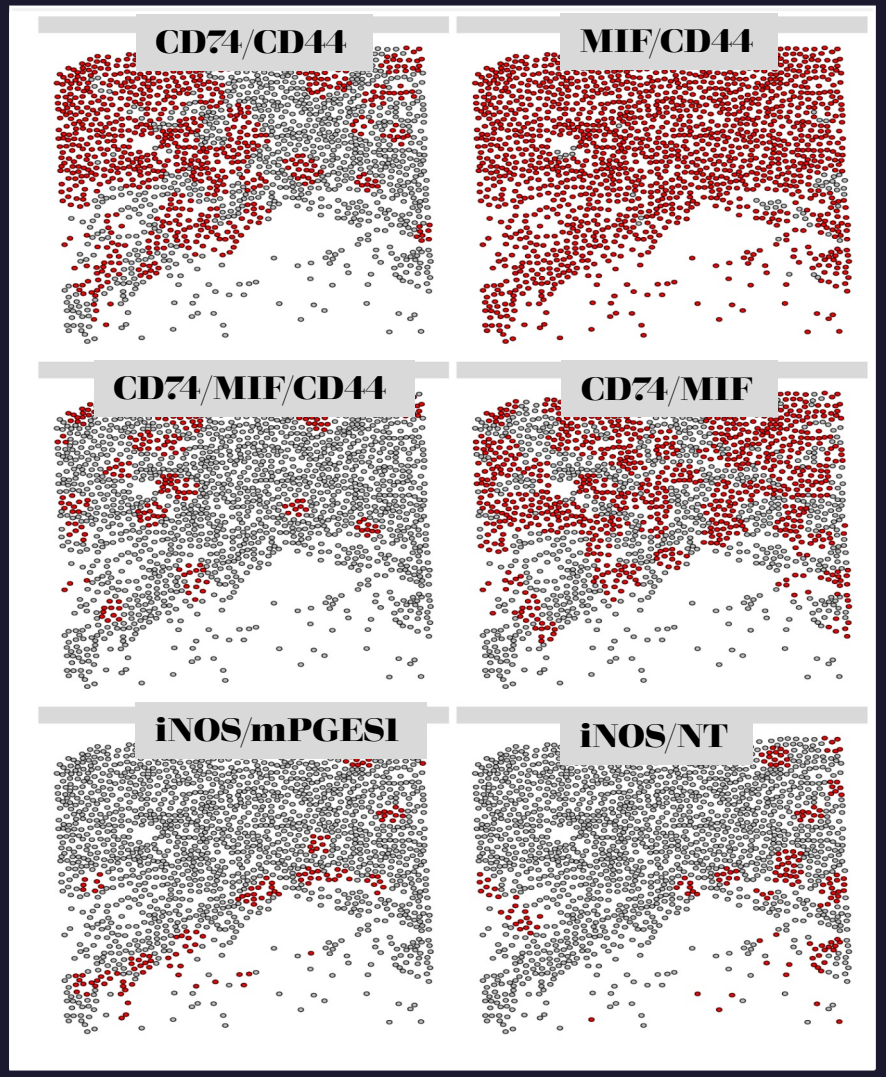


Stage IV

Spatial Analyses on Neighborhoods

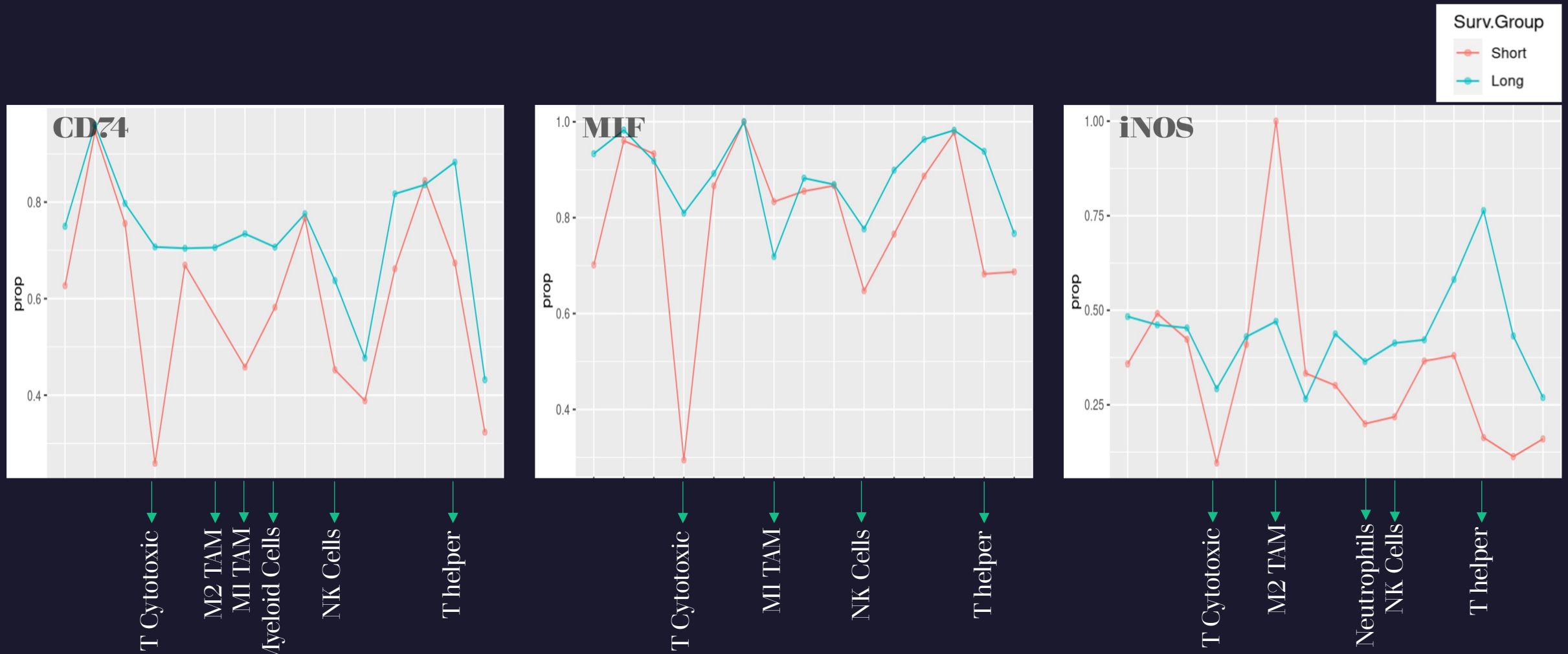


Stage III

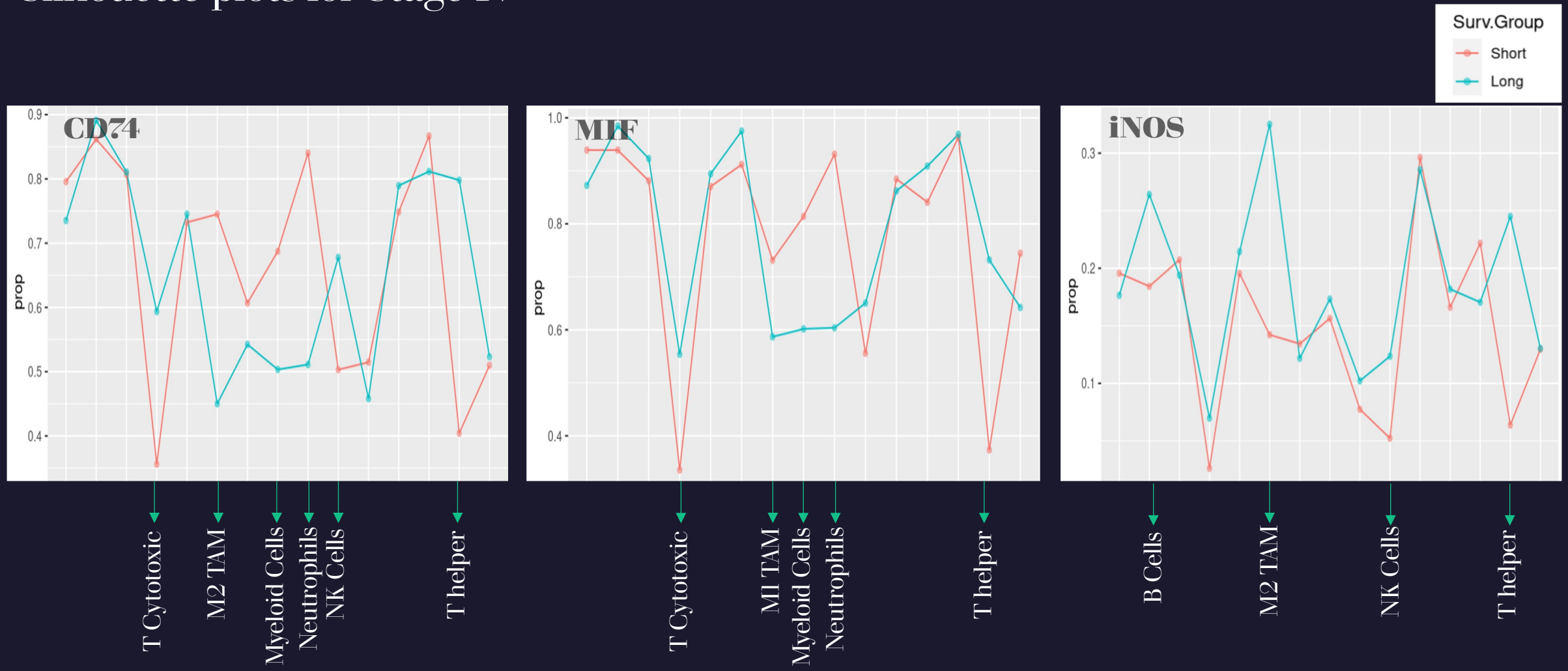


Stage IV

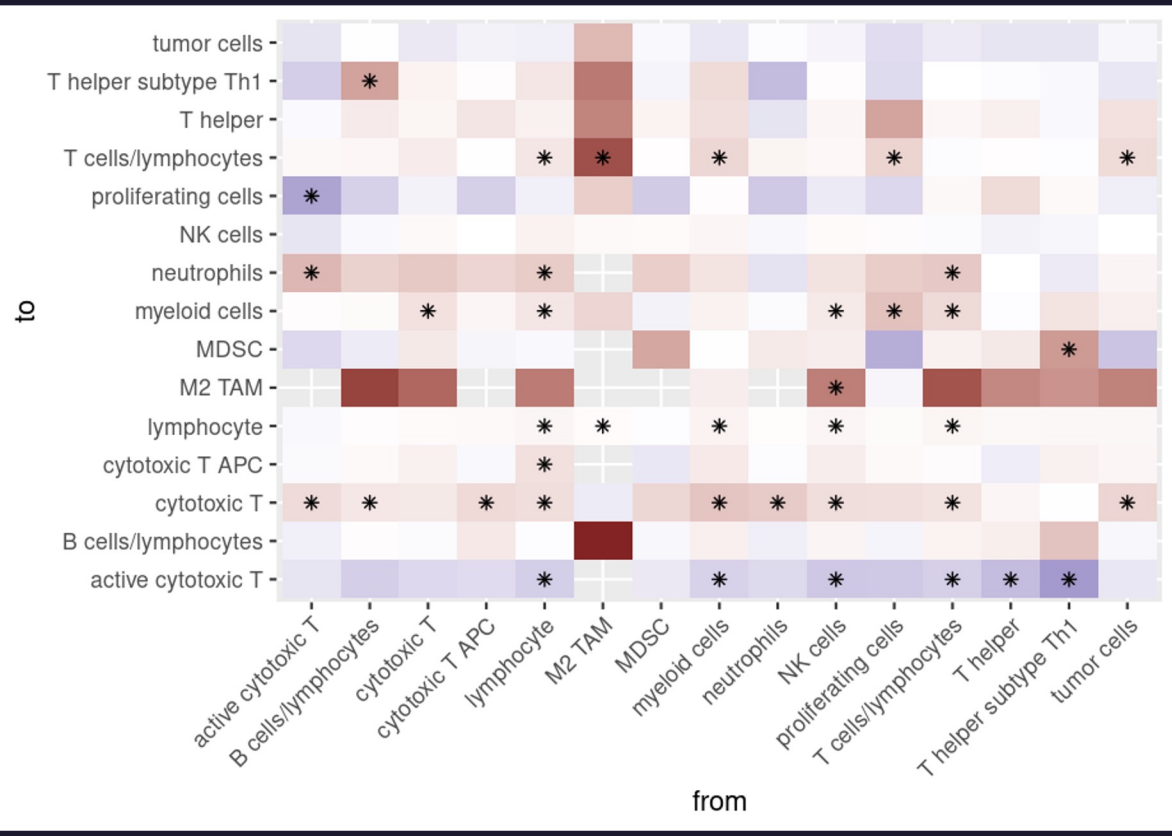
Cell Type Proportions in Neighborhoods
Silhouette plots for Stage III

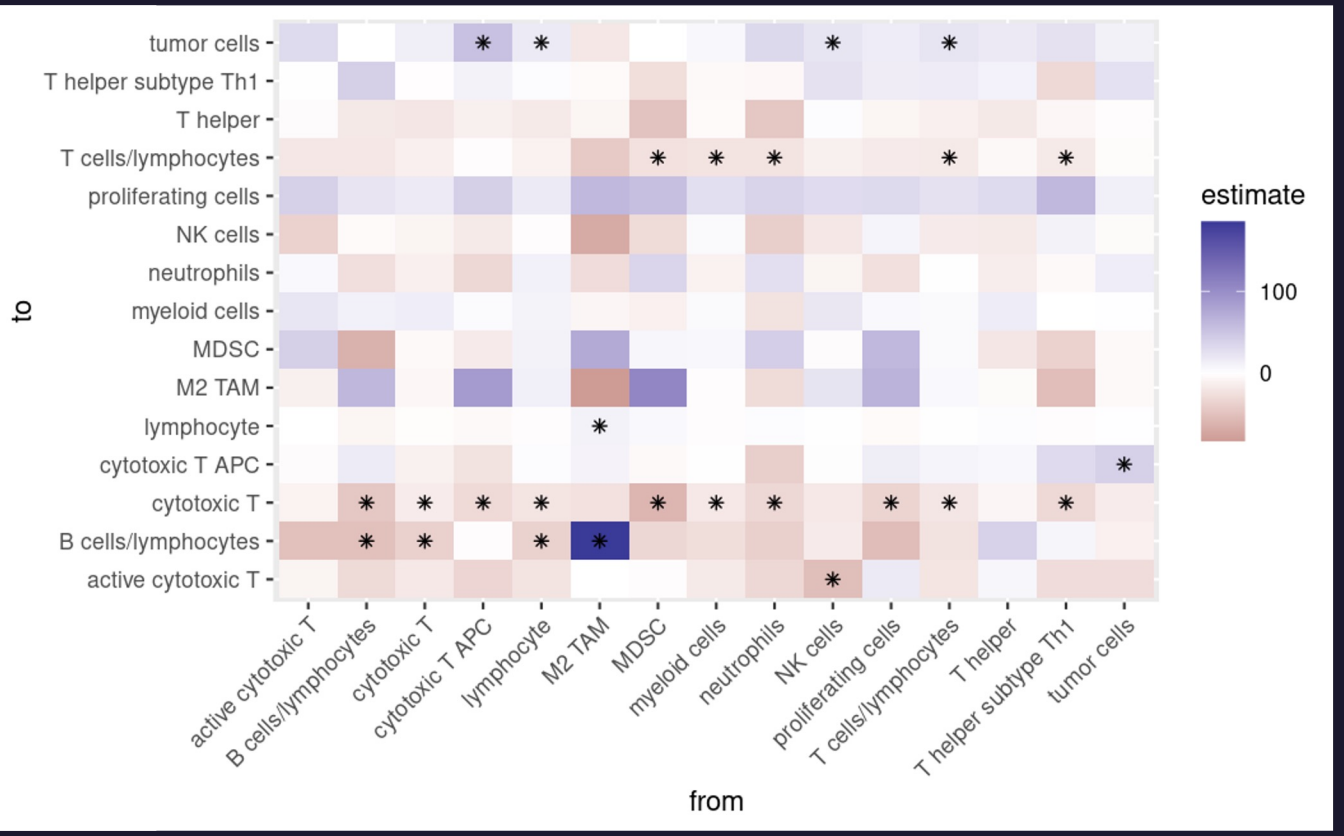


Cell Type Proportions in Neighborhoods
Silhouette plots for Stage IV



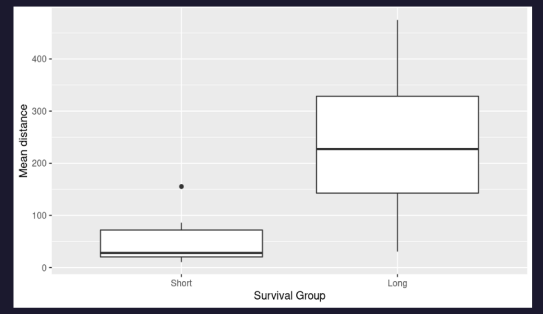
Nearest Neighbor Distances Between Cell Types





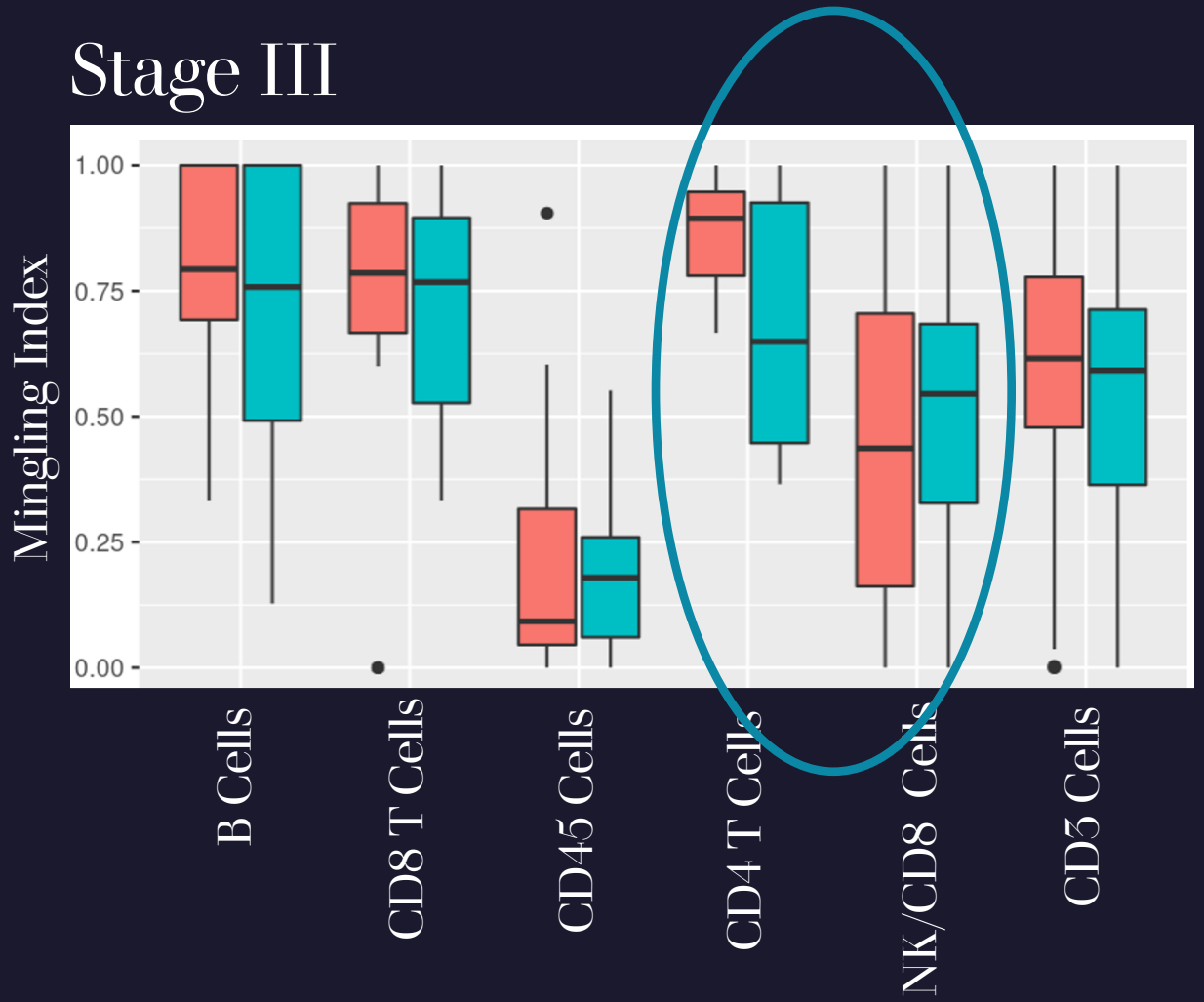
Stage III

Stage IV

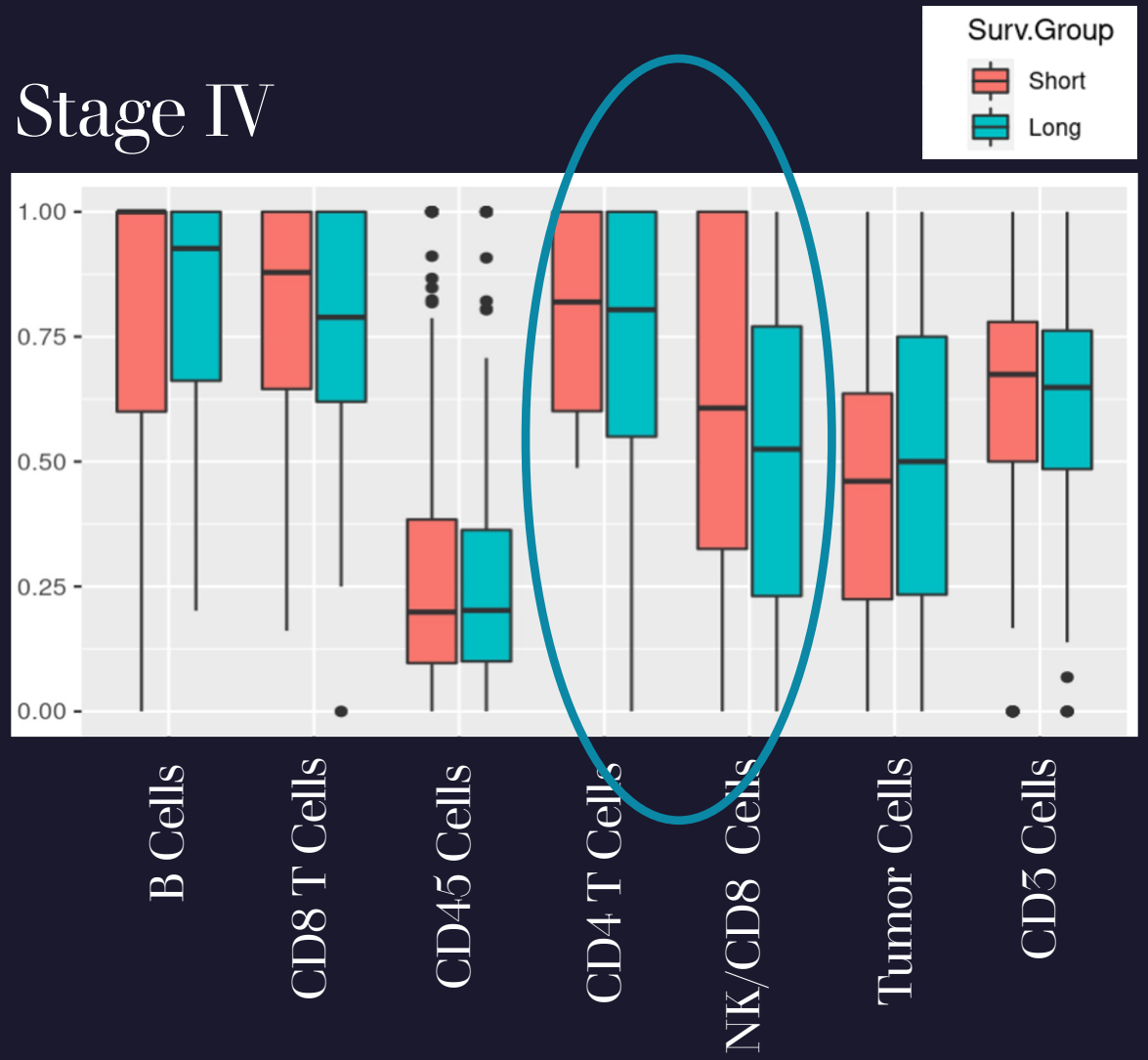


Mingling of Communities

Stage III



Stage IV



Structured Immune Composition and Organization in Melanoma

Tissue Segmentation → Pseudo Coloring → Assessment of Proximity/ Immune Cell Signature → Spatial Architecture → Survival Information