U24: High-dimensional immune monitoring of NCI-supported immunotherapy trials

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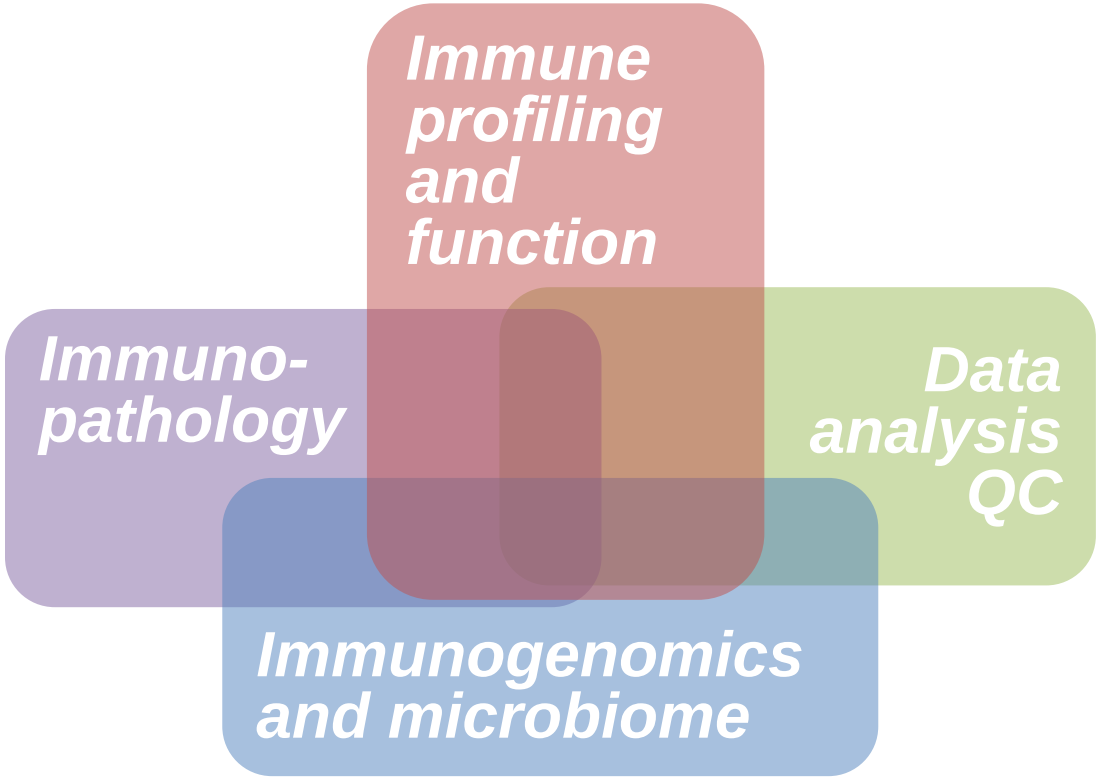
Precision Immunology Institute

The Tisch Cancer Institute

Objectives and scope

- Why do some patients respond and others don’t? To identify biomarkers with translational potential to optimize immunotherapeutic strategies
- To develop molecular signatures that define immune response categories to correlate with the clinical outcomes
- To define immunophenotype characteristics of response that will be valid for diverse immuno-oncology classes with different mechanisms of action

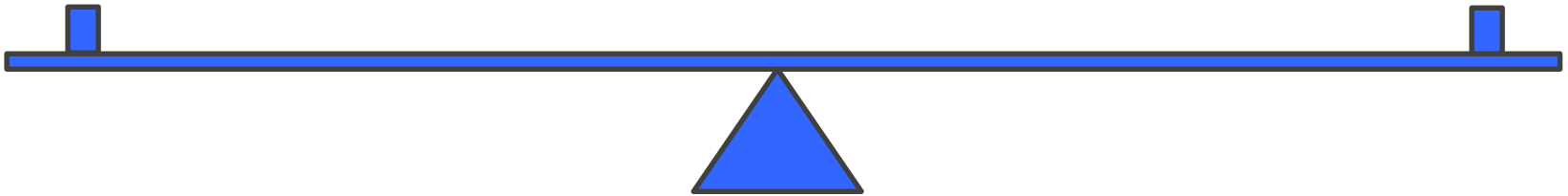


With analytically validated assays and procedures

The ideal immune monitoring program

Develop innovative assays to monitor disease-relevant immune signatures and discover new mechanisms, biomarkers and immune targets

Improve assay standardization and minimize experimental variability to maximize data quality and reproducibility

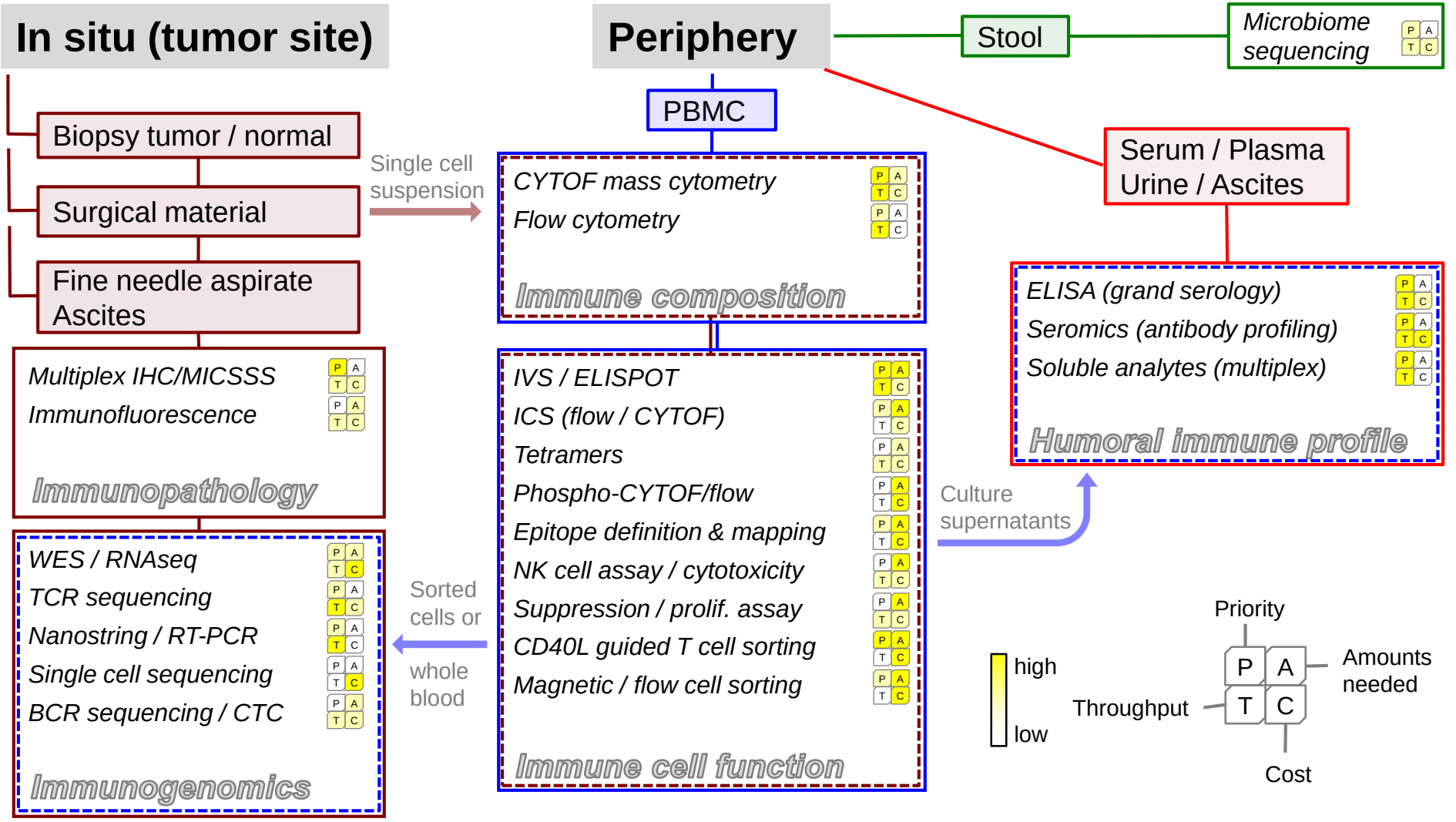


Balancing innovation and standardization.

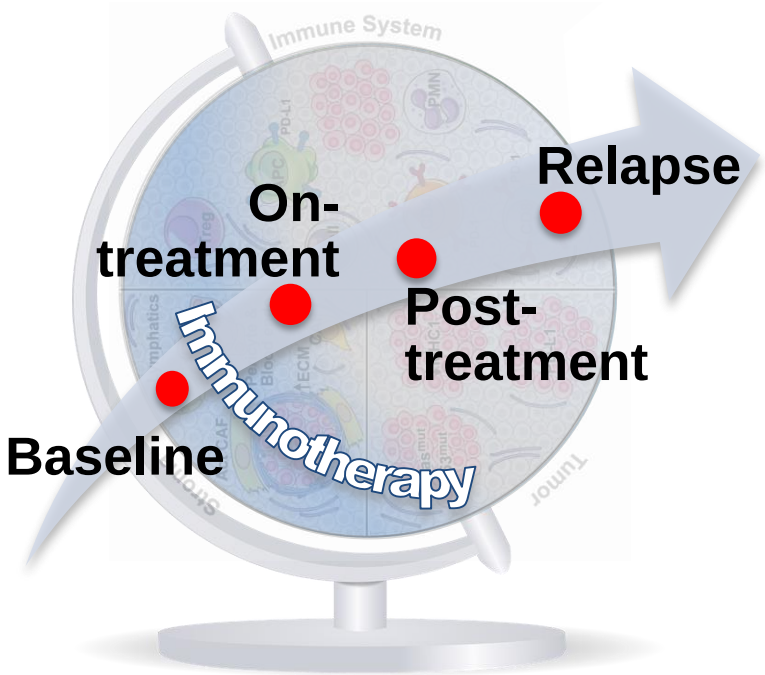
Assays/Platforms of CIMACs

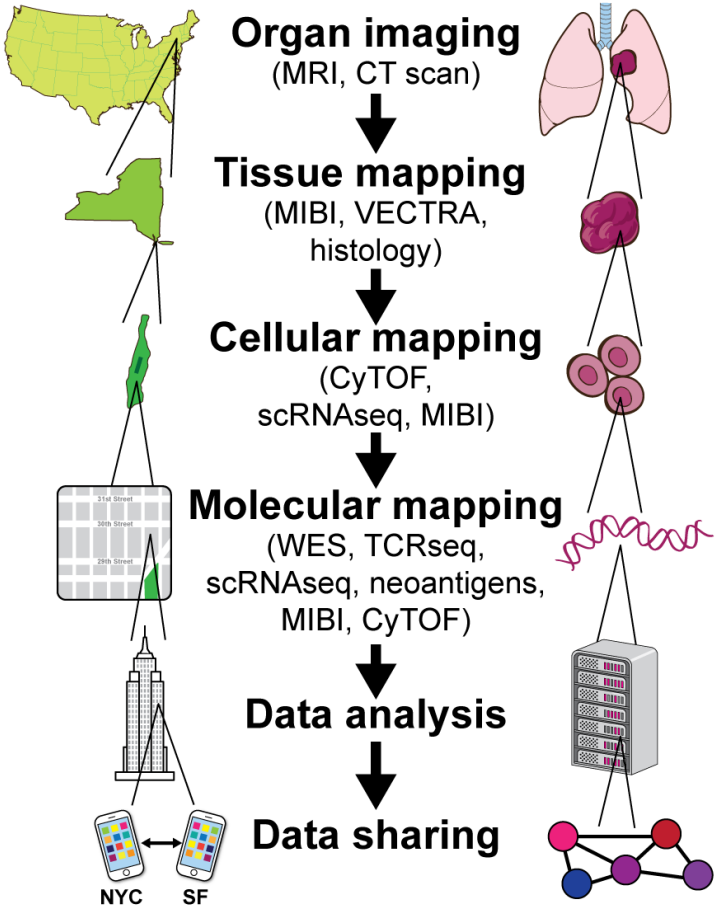
cfDNA [DF, MD]
CRISPR
CTC
CyTOF imaging
Cytokine analysis [MD, ST, MS]
ELISA, antibody profiling and seromics [MS]
ELISPOT, Intracellular Cytokines [MD, MS]
Epigenomics (RRBSeq, ATAC-seq) [ST]
Exosomes
FISH DNA
Gene expression – HTG-EgeSeq [MD]
Gene expression – Nanostring [MD]
High-dimensional flow cytometry [MD]
Image analysis for IHC [DF, MD, MS, ST]
Immunogenomics: HLA-seq [DF]
ISH DNA/RNA [MD]
KIR-KIR-L genotyping
Mass cytometry (CyTOF) [MS, ST]
Mass spectrometry epitope detection
Microbiome (16S, metashotgun) [MD, MS]
Multiplex IF [DF, MD]
Multiplex IHC [DF, MS]
Multiplexed Ion Beam Imaging (MIBI) [ST, MS]
Neoantigen prediction [DF, MS]
Peptide-MHC multimers
RNA-seq [DF, MD]
Serum markers – soluble analytes [MS]
Single cell transcriptome [DF, MS]
Standard flow cytometry [MD]
Standard IHC [MD]
TCR/BCR clonality [DF, MD]
Transcriptome/TCR/BCR analysis [DF]
WES/targeted [DF, MD]

Preferred and **Tier 1** assays noted for Dana Farber (DF), MD Anderson (MD), Mt. Sinai (MS), Stanford (ST)


In situ (tumor site)
Periphery
Stool
Microbiome sequencing
PBMC
Biopsy tumor / normal
Surgical material
Fine needle aspirate Ascites
Single cell suspension
CYTOF mass cytometry
Flow cytometry
Immune composition
Serum / Plasma Urine / Ascites
ELISA (grand serology)
Seromics (antibody profiling)
Soluble analytes (multiplex)
Humoral immune profile
Multiplex IHC/MICSSS
Immunofluorescence
Immunopathology
IVS / ELISPOT
ICS (flow / CYTOF)
Tetramers
Phospho-CYTOF/flow
Epitope definition & mapping
NK cell assay / cytotoxicity
Suppression / prolif. assay
CD40L guided T cell sorting
Magnetic / flow cell sorting
Immune cell function
Culture supernatants
Sorted cells or whole blood
WES / RNAseq
TCR sequencing
Nanostring / RT-PCR
Single cell sequencing
BCR sequencing / CTC
Immunogenomics
high
low
Throughput
Priority
Amounts needed
Cost
P
A
T
C

Multiscale, dynamic atlas of immune changes

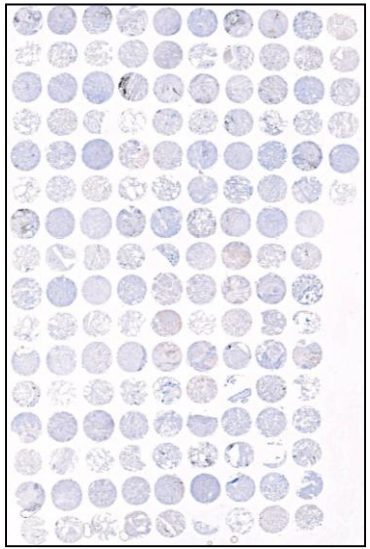




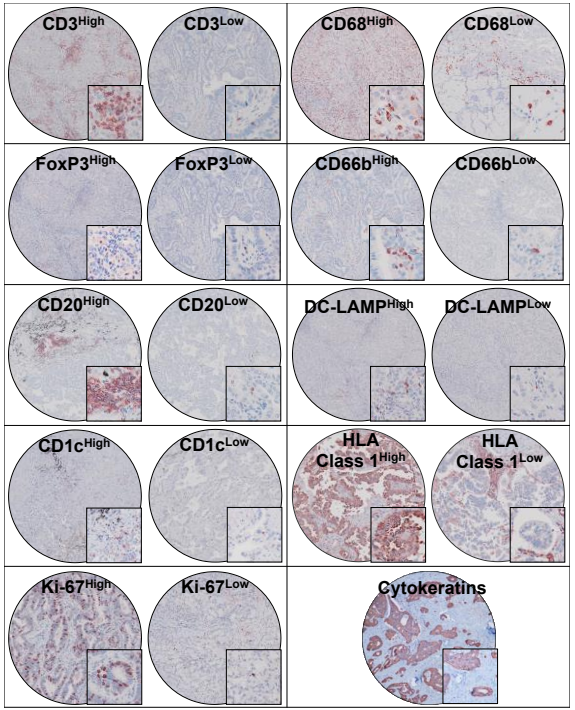
Multiplex IHC on tissue microarrays to identify prognostic biomarkers

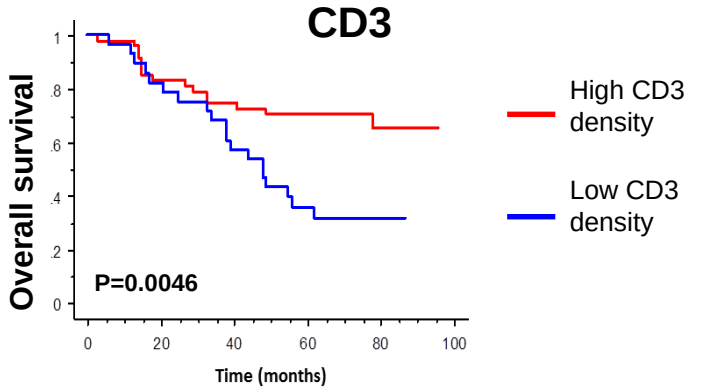
Heterogeneity of immune markers in non-small cell lung cancer (NSCLC)

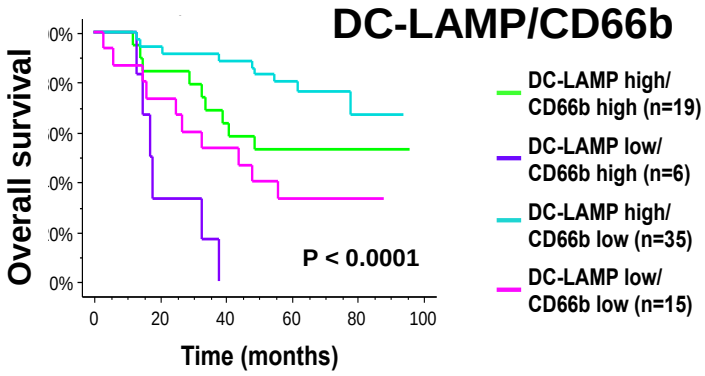
NSCLC tissue microarray



n=75 patients



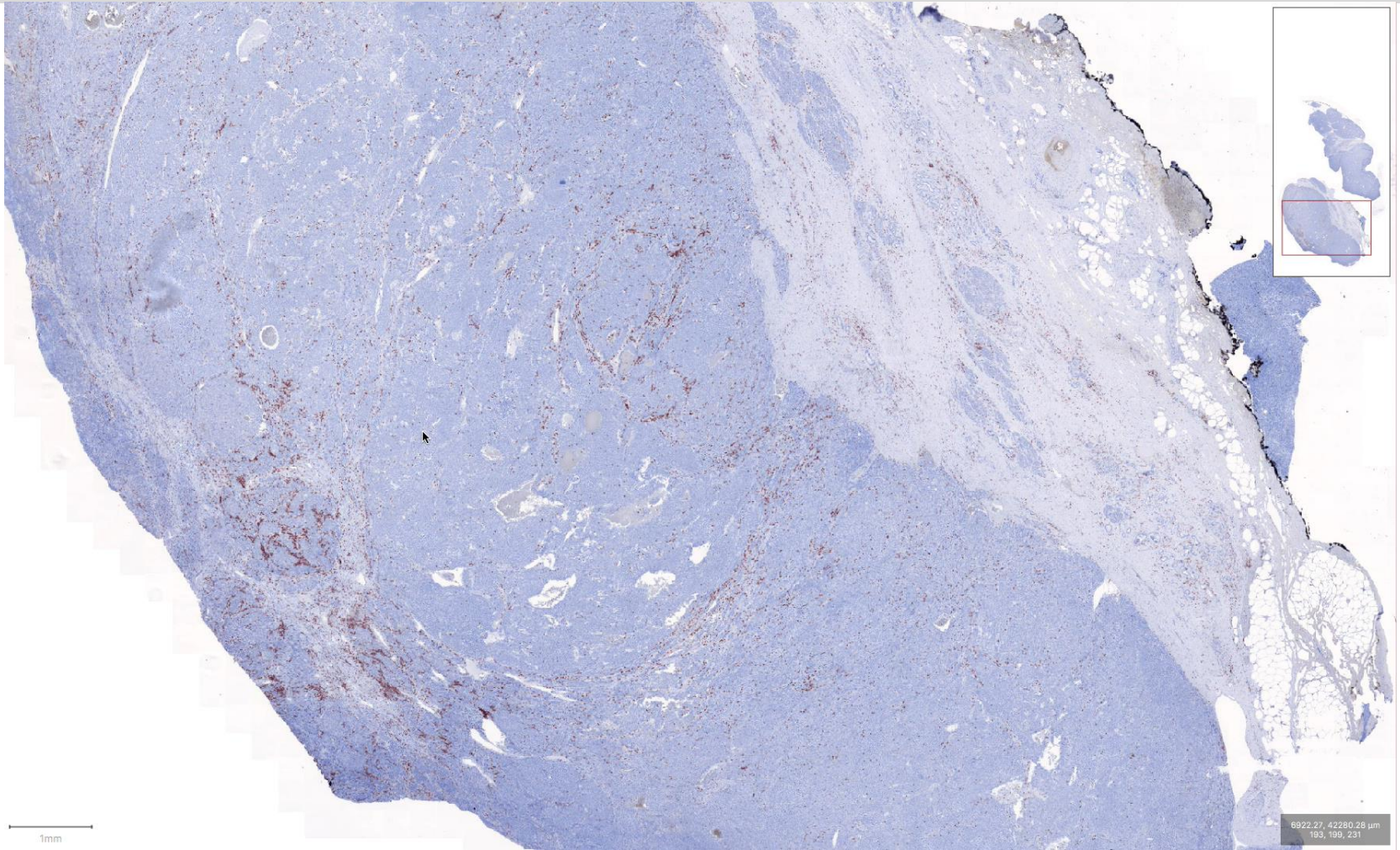




Remark R, Merghoub T, Grabe N, Litjens G, Damotte D, Wolchok JD, Merad M, Gnjatic S. *Science Immunology; 1:aaf6925 (2016).*

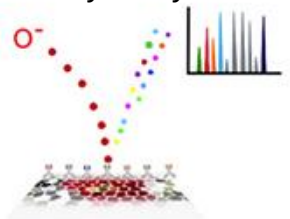
Review on NSCLC immune contexture in Am J Respir Crit Care Med. 2015;191:377-90

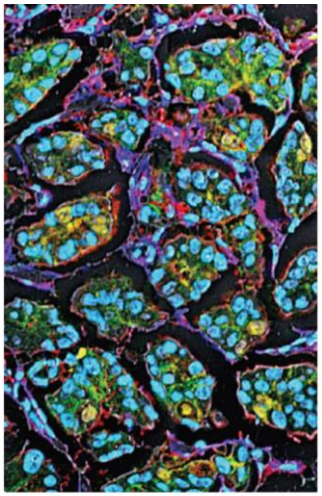
Pancreatic neuroendocrine tumor with high CD3 lymphocytic infiltration (TLS)



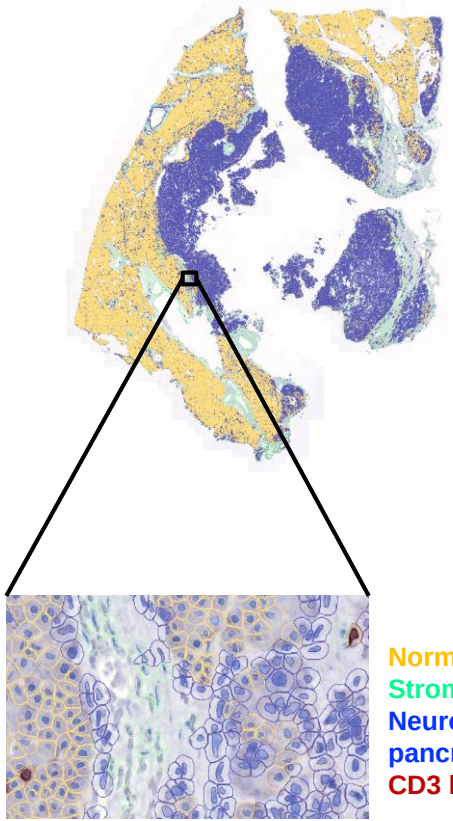
Next frontier in tissue imaging: higher multiplexing, 3D-4D analyses, and integrating neural network image learning and radiomics

More than 40 markers using metal-conjugated antibodies & an ion-beam or CYTOF for tissue mass cytometry analyses



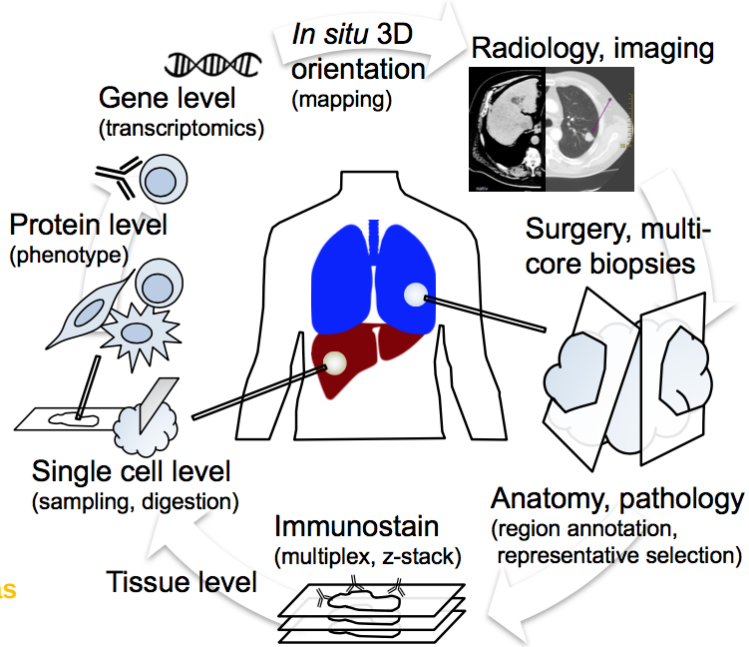


From Stanford.edu

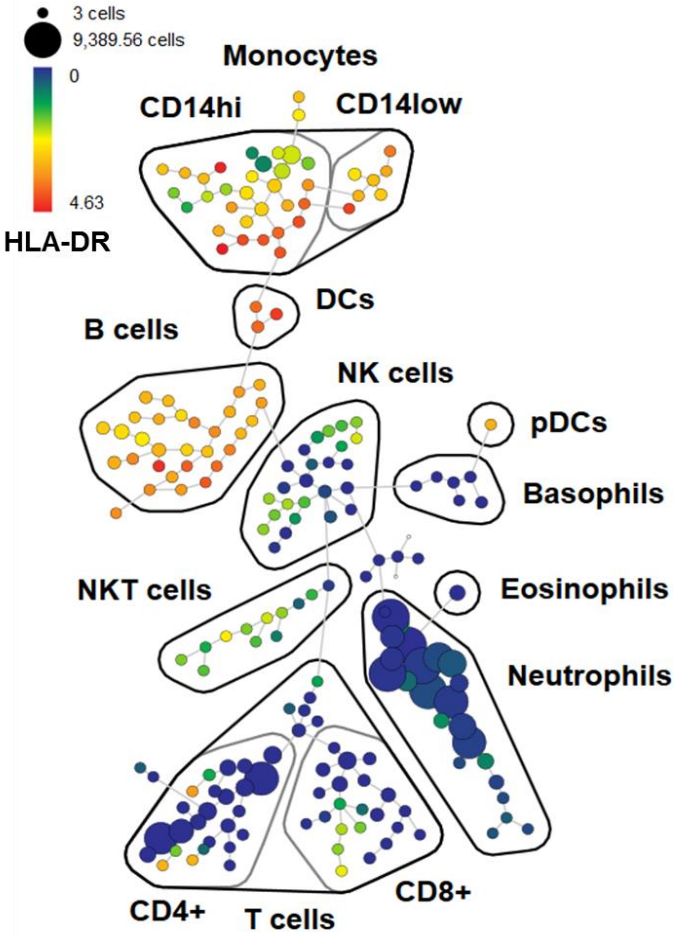


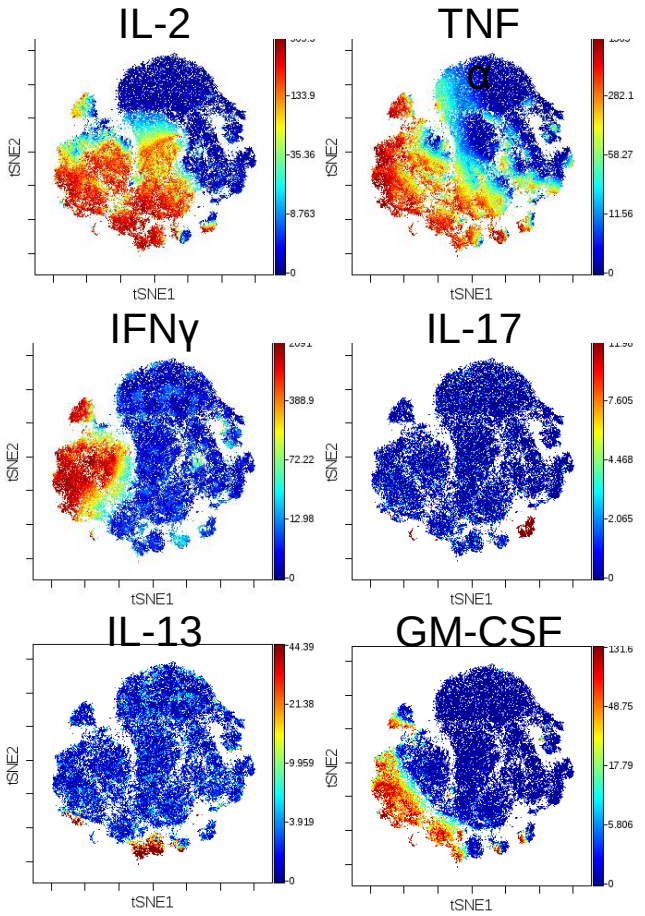
Normal pancreas
Stroma
Neuroendocrine pancreatic tumor
CD3 lymphocytes

Gnjatic et al. Unpublished. With QuPath software (http://biorxiv.org/content/early/2017/01/12/099796)



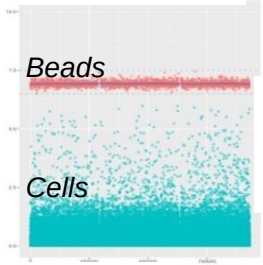
Functional applications of CyTOF mass cytometry



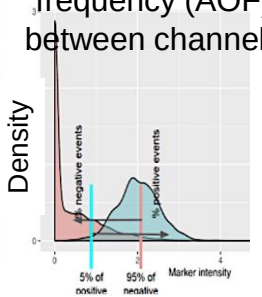


Optimization of CyTOF with beads and lyophilized panels

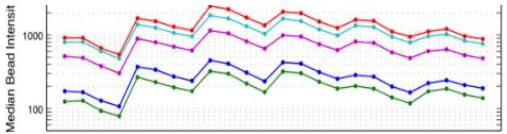
Beads for QC



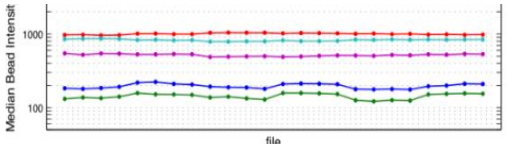
Average overlap frequency (AOF) between channels



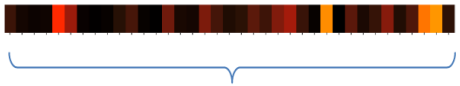
Bead intensity before normalization



Bead intensity after normalization

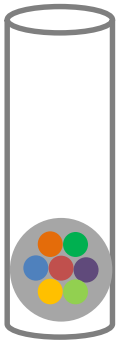


AOF for all antibody channels



Overall staining quality score

Core set of consensus markers to identify major immune cell subsets



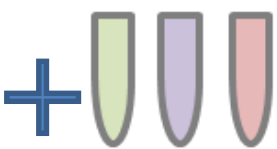
Core Panel	
CD45	CD19
CD3	CD27
CD4	CD56
CD8	CD16
CD45RA	CD14
CD38	CD123
HLADR	CD11c

Optimized panels to provide detailed characterization of specific subsets

T cell module	
CD161	ICOS
CD57	OX40
TIM3	CD39
CD103	CD73
CCR7	PD-1
CXCR3	CD25
CCR6	CD127
CCR4	CD69
CXCR5	CD28
41BB	TCRgd
2B4	CD44

Myeloid module	
CD33	CX3CR1
CD64	CD85j
CD1c	CD141
CD66b	CD11b
CD163	CD86
CD206	CD40
CD169	CD117
CD15	PDL1
CD141	PDL2
TLR2	TLR4
CCR7	SIRPa

NK cell module	
NKp30	CD244
NKp44	CD103
NKp46	CD69
NKG2A	CD96
NKG2C	CD94
NKG2D	Siglec7
KIR3DL1	DNAM1
KIR2DL3	CD132
CD57	CD25
CD161	TIM3
LILRB1	PD-1



Additional custom conventional liquid antibodies

Rahman et al. Unpublished

Automated analysis pipeline for CyTOF

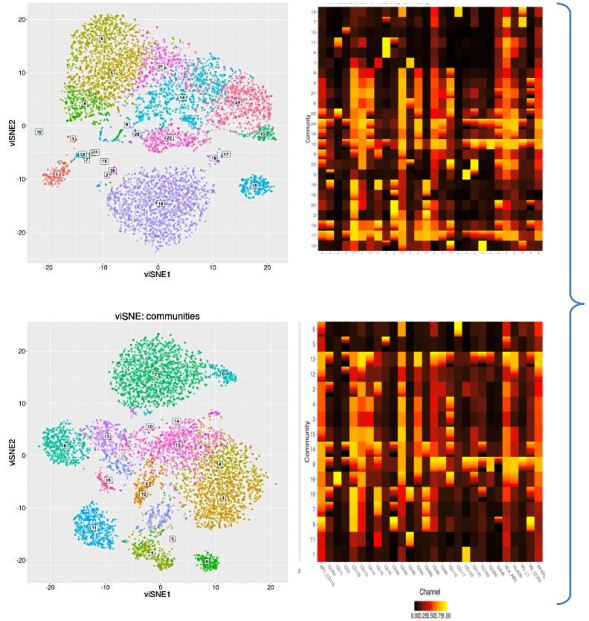
Unbiased identification and characterization of cell populations in individual CyTOF samples

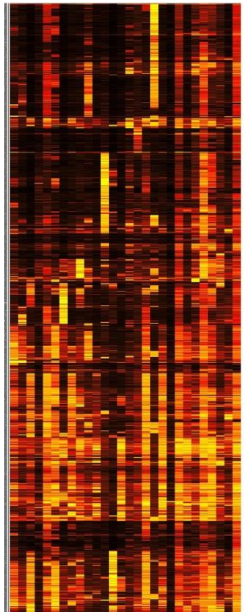
Automated meta-clustering of populations across multiple samples

Automated analytics to identify populations and protein expression patterns that differ between treatment groups

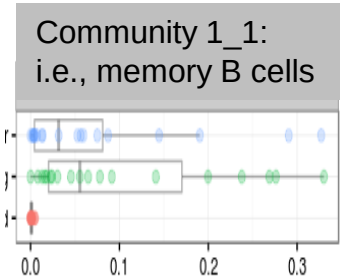
Sample 1

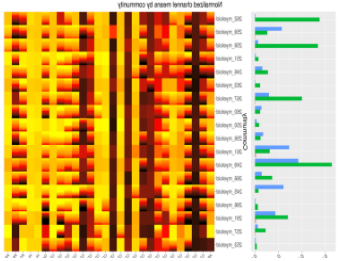
Sample 2



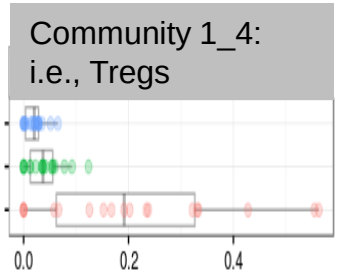


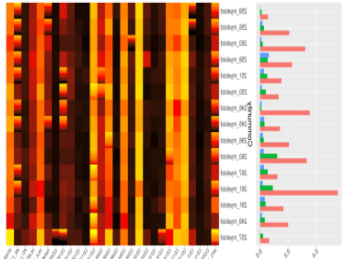
Community 1_1:
i.e., memory B cells





Community 1_4:
i.e., Tregs



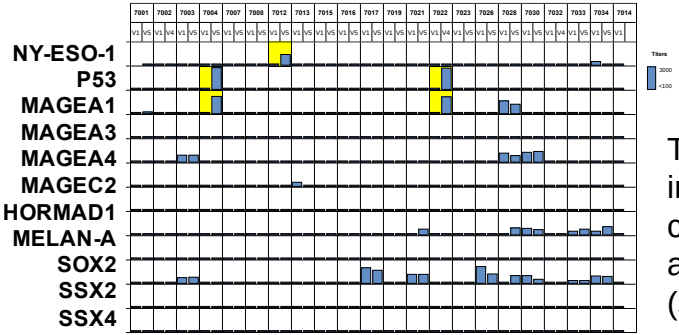


… multiple samples

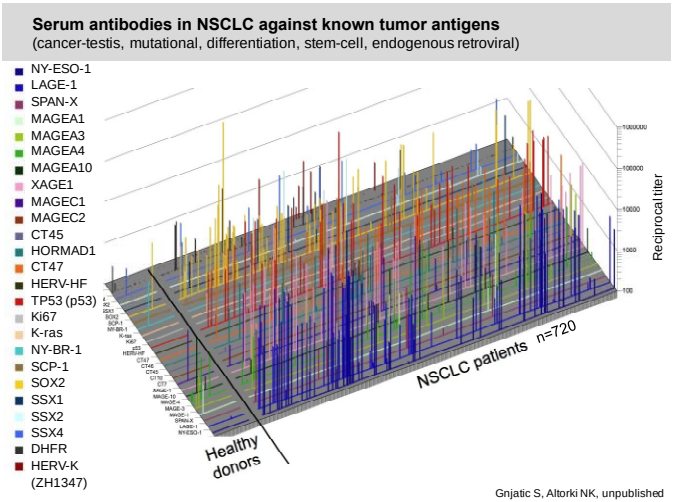
… multiple differing features across samples

Proposing two universal unifying assays

Grand Serology

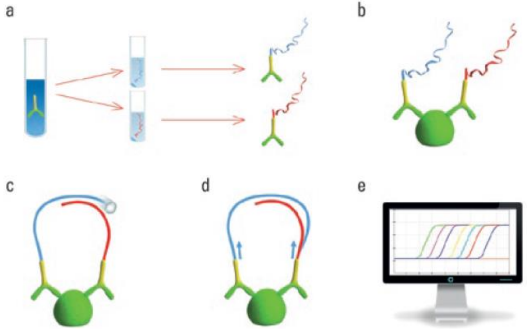


Titers to known tumor antigens in sera from head and neck cancer patients before and after chemoradiotherapy (seroconversion in yellow)



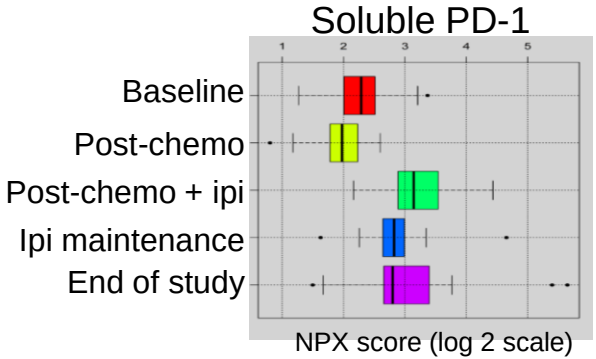
Gnjatic S, Altorki NK, unpublished

O-Link Soluble Proteome Analytes



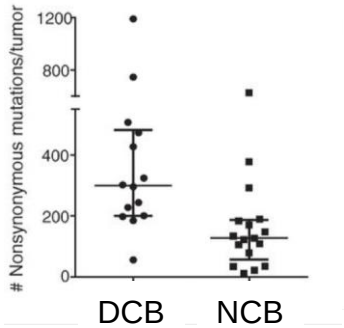
Nature Methods, Sep 2011

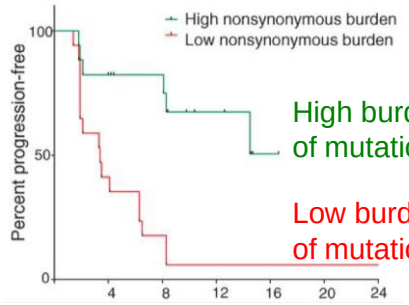
Analyze serum at various time points with 92 multiplex panel probing immuno-oncology analytes such as cytokines, chemokines, and soluble checkpoint molecules using O-Link's platform with oligo extensions. Mount Sinai CIMAC is the only approved site by O-Link in US.



Assessing tumor mutation burden (neoepitopes) and microbiome

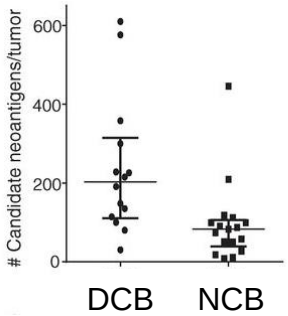
Mutational burden identified by whole exome sequencing correlates with response to PD-1 blockade in NSCLC

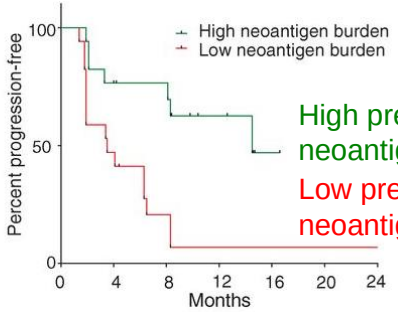




High burden of mutations

Low burden of mutations





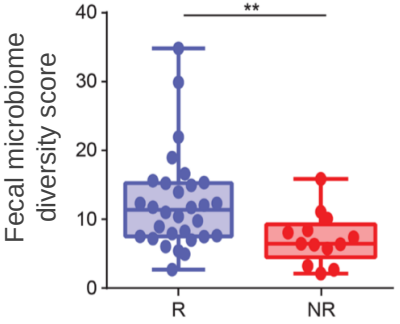
High predicted neoantigens

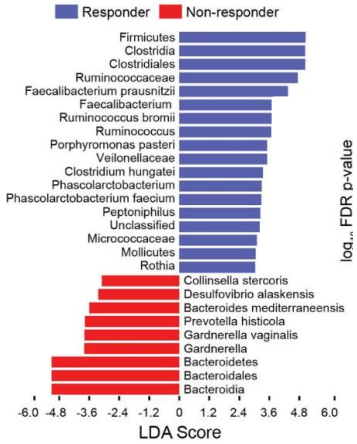
Low predicted neoantigens

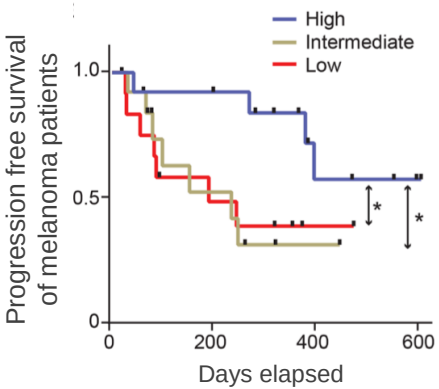
DCB = durable clinical benefit
NDB = no durable clinical benefit

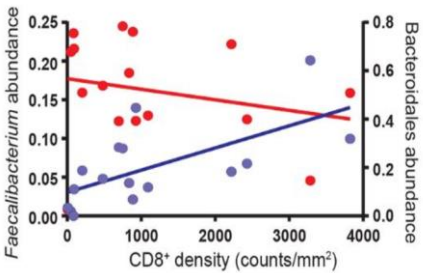
Rizvi et al. Science. 2015. 348-124

Cancer patients treated with PD-1 blockade, sequenced for gut bacteria by 16S or shotgun metagenomics





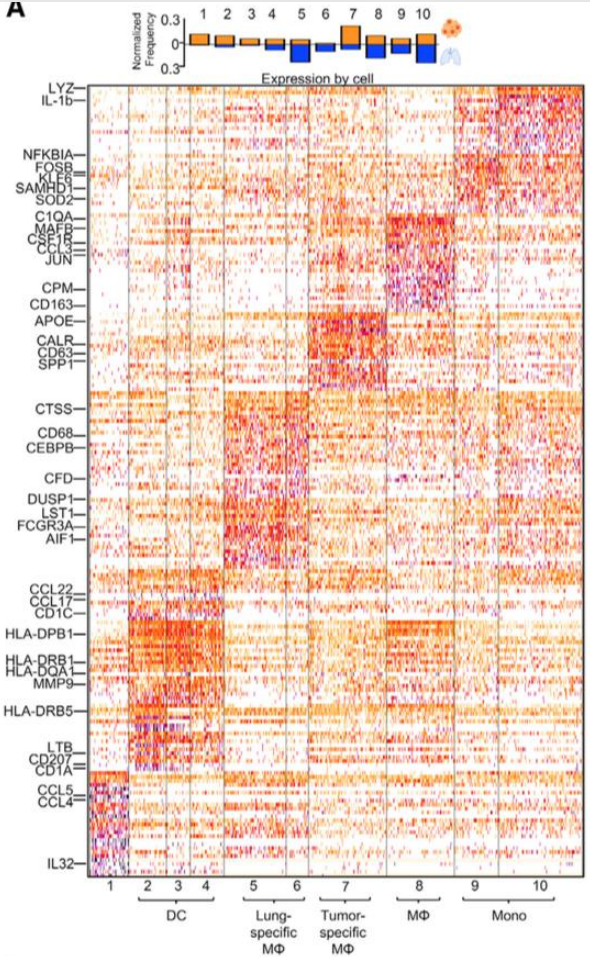


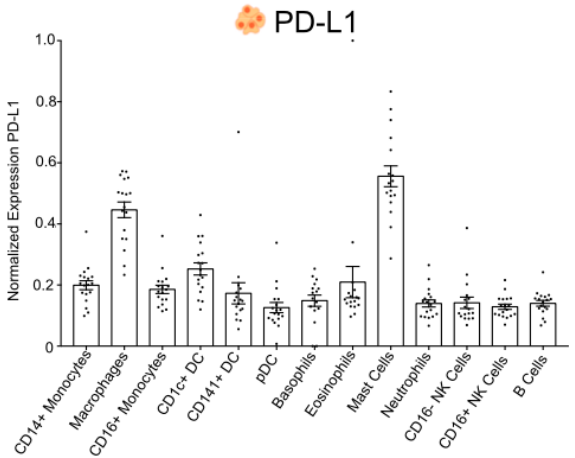


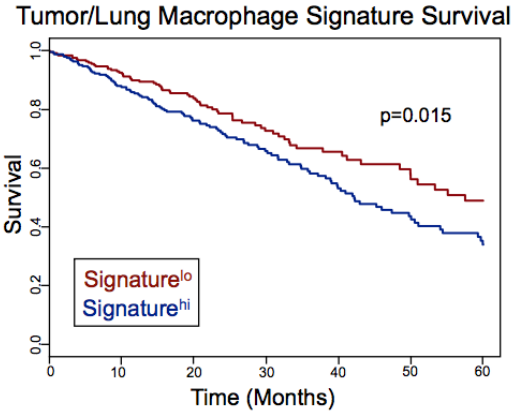
Gopalakrishnan, Spencer, Nezi et al. Science. 2017

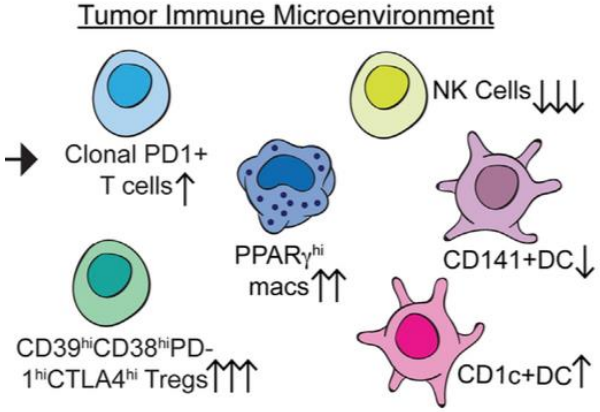
Routy al. Science. 2017

Single cell analyses reveal impaired immune profiles at the tumor site vs. adjacent non-involved tissue in early NSCLC



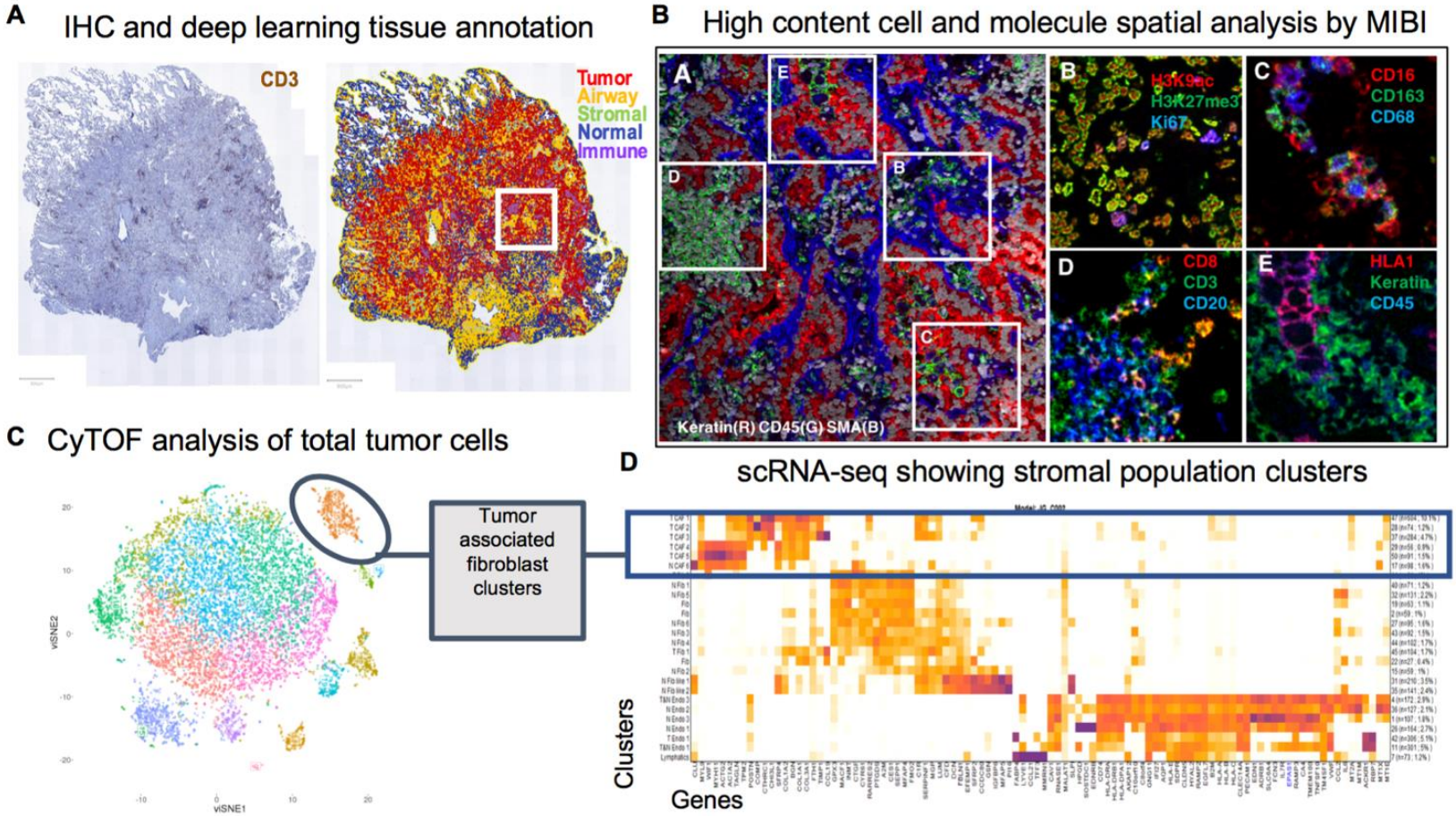






Lavin, Rahman, Gnjatic, Merad, Cell 2017;169:750-765

Integrating multiplex IHC, MIBI, CYTOF, and Single Cell RNA-seq analyses



Gnjatic, Angelo, Rahman, Kenigsberg, Merad et al. Unpublished.

Acknowledgments

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Romain Remark
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El-Ad Amir
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Patricia Kovatch
Jose Clemente
Jeff Hammerbacher
Nina Bhardwaj
Seunghee Kim-S
Christian Becker
Sarah Nataraj
Ilaria Laface
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Ben Greenbaum
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Erika Ritter
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Mario Sznol
Ruth Halaban

Roswell Park Cancer Institute, Buffalo, NY

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Junko Matsuzaki
Takemasa Tsuji

Ludwig Institute for Cancer Research, New York, NY

Lloyd J. Old

Support from:

Cancer Research Institute
Ludwig Institute for Cancer Research
NCI P01 CA190174
NCI U24 CA224319 (CIMAC)
ARRA RC2 NCI

Aims of immune monitoring

To find better ways to predict patients who may benefit from immunotherapies, and to design new approaches for those who don’t

Just measuring tumor growth and survival in immunotherapy clinical trials leaves too many questions unanswered

Multidisciplinary approach to find molecular, genetic, microbial, or cellular signatures that are useful to select patients for the most appropriate treatment

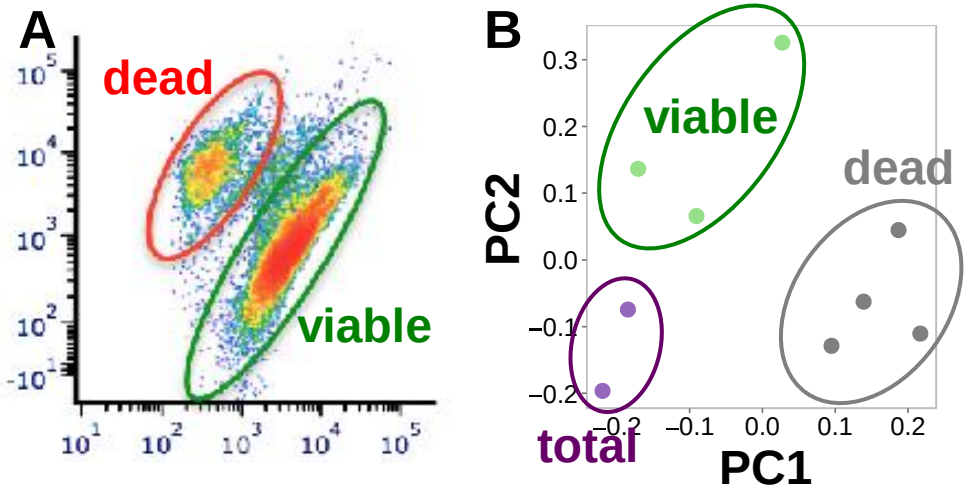
Explore markers at the tumor site and in the periphery

Learn from immune monitoring of untreated and treated tumors, and their antigenic profile for mechanisms and biomarker discovery

Need: High-dimensional immune monitoring and analysis tools

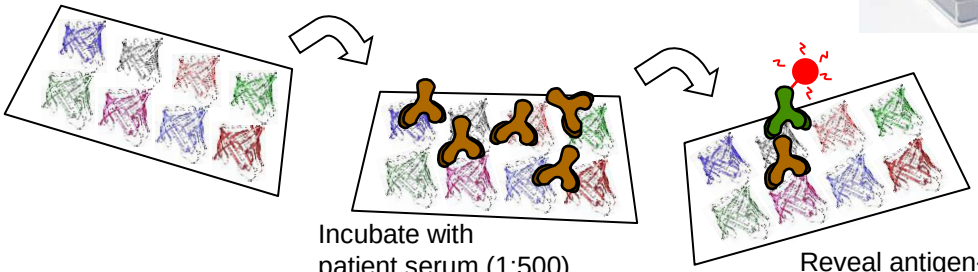
Microbiome shotgun metagenomics (Clemente, Faith)

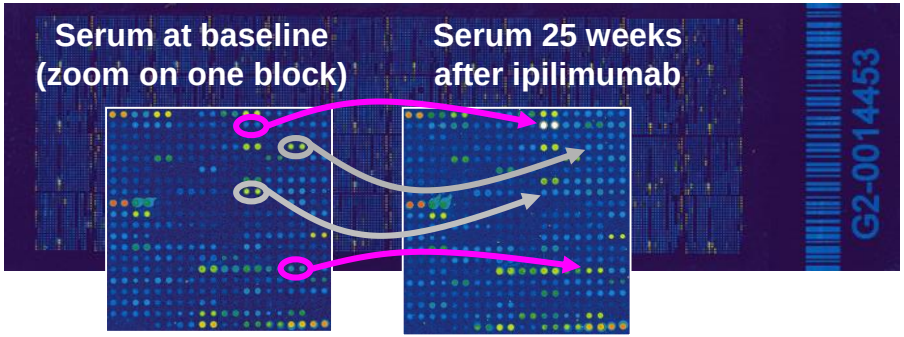
Importance of distinguishing live from dead bacteria for QC aspects



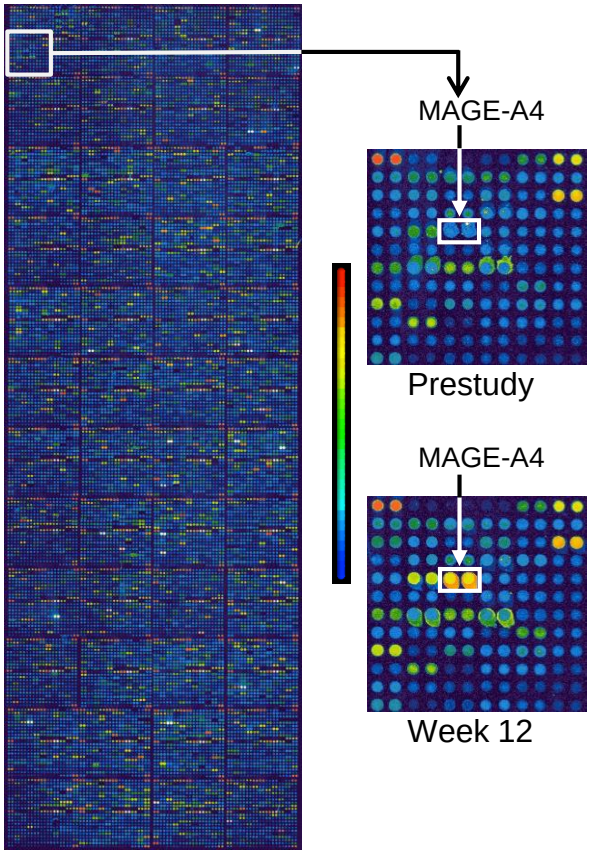
Seromics detects antigen-specific changes in autoantibody profiles during treatment (H. Wada, Osaka JP; H. Shiku, Mie JP; unpublished)

Protoarrays™ contain >9000 proteins mostly full-length baculovirus-produced GST-fusion proteins randomly selected, both known and predicted sequences



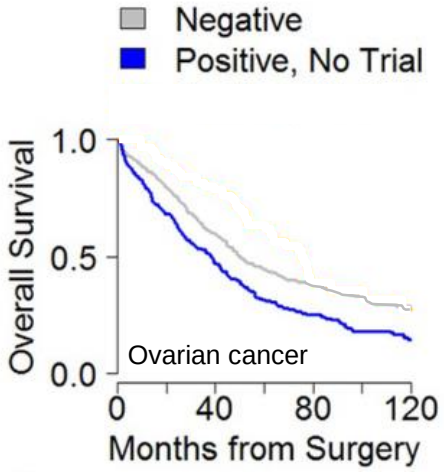


for biomarkers of treatment:

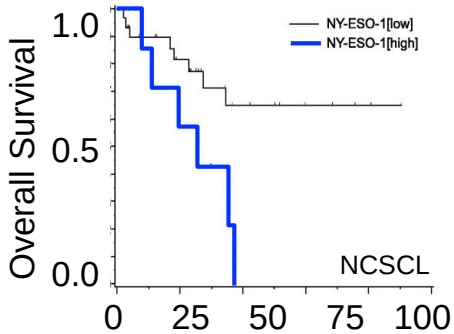


MAGEA4 protein vaccination of esophageal cancer patient

NY-ESO-1 expression is a poor prognostic factor but it may be a good predictive marker for immunotherapy



Based on IHC or RT-PCR expression of ovarian cancer patients and participation or not in immunotherapy clinical trials.



Based on RT-PCR expression of non-small cell lung adenocarcinoma cancer patients with stage II disease (p=0.02).

Szender et al. Gyn Oncol. 2017;145:420-425.
Güre AO et al. Clin Cancer Res. 2005;11:8055-8062.

Metastatic melanoma patients with baseline NY-ESO-1 serum antibodies before CTLA-4 (ipilimumab) treatment

Status at wk 24	# patients (%)	NY-ESO-1 seronegative # (%)	NY-ESO-1 seropositive # (%)
CR	4 (2.9%)	3	1
PR	14 (10.0%)	10	4
SD	30 (21.4%)	23	7
Clinical Benefit	**48** (34.3%)	**36** (30.5%)	**12** (54.6%)
No Clinical Benefit	**92** (65.7%)	**82** (69.5%)	**10** (45.4%)
Total	140 (100%)	118	22

According to immune-related response criteria:

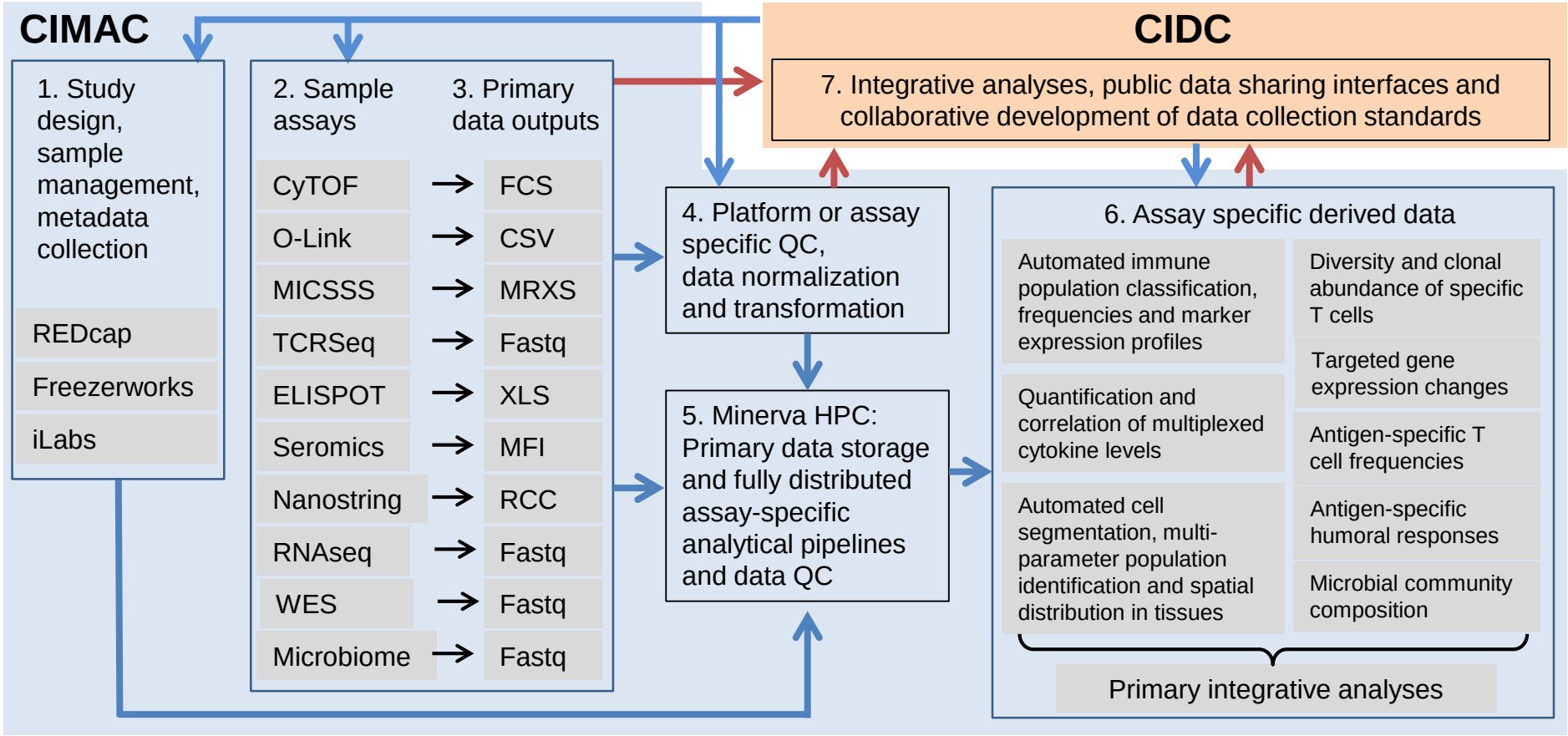
Clinical Benefit:
CR: Complete Response
PR: Partial Response
SD: Stable Disease

No Clinical Benefit:
POD: Progression of Disease (includes MR)
DOD: Dead of Disease

Fisher's exact test (two-tailed):
P value 0.0481
RR = 1 .8 (1.1-2.9)

Yuan, Gnjatic, et al. PNAS, 2011;108:16723

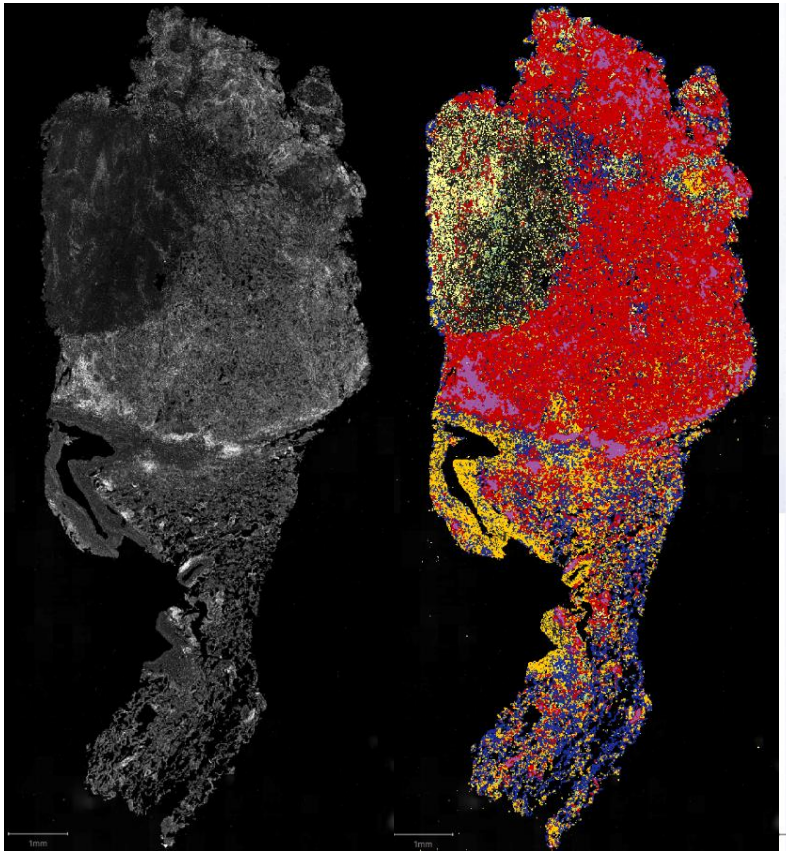
Sample and data handling, outputs (Kovatch, Kim-Schulze)

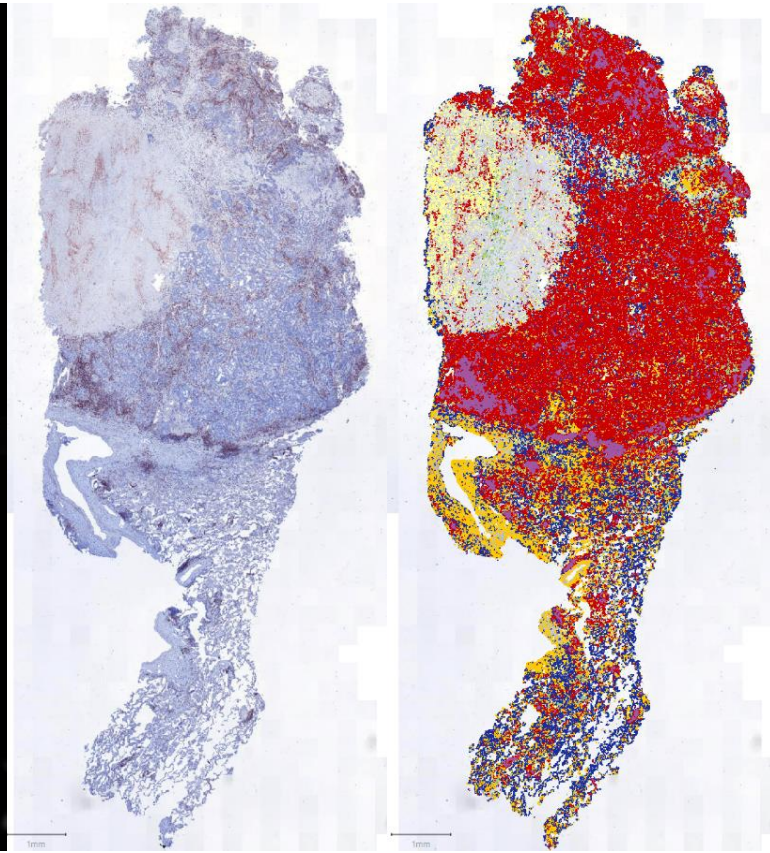


Annotating tumor areas with the help of artificial intelligence and deep learning tools

- Tumor (NSCLC)
- Normal lung epithelium
- Lymphocytes
- CD3 T cells

- Necrosis
- Airway
- Stroma





Gnjatic et al. Unpublished. With QuPath software (http://biorxiv.org/content/early/2017/01/12/099796).

Take home message

High-dimensional immune monitoring assays are poised to explain mechanisms of novel drugs or treatment and provide complex signatures to predict outcome

It is unlikely that a single predictive biomarker will be found for immuno-oncology

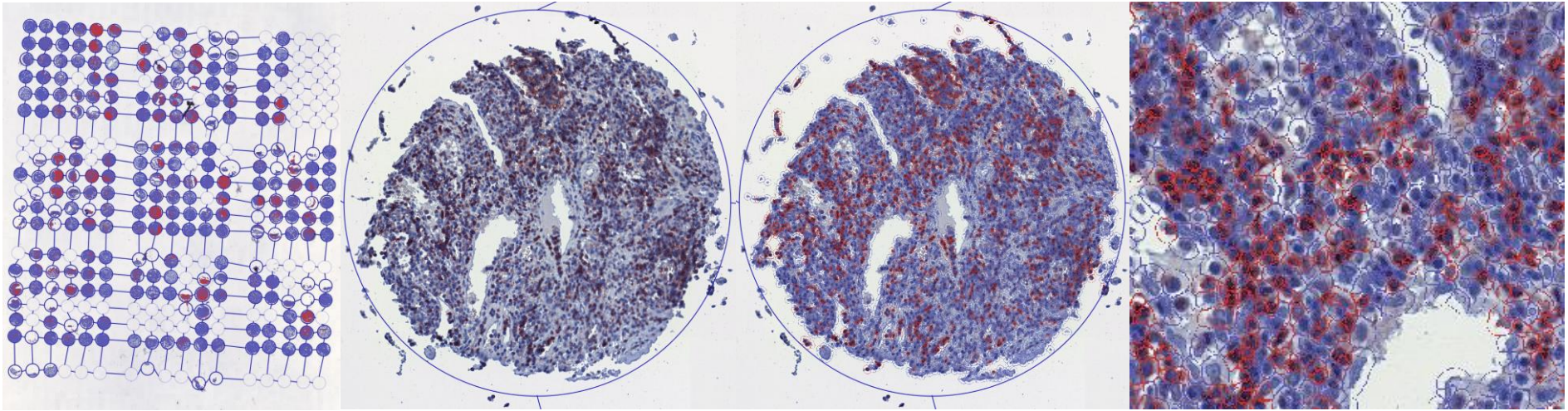
Single cell data analyses and data mining are the next frontiers for discoveries in immunotherapy

Immune monitoring supports immune atlas efforts, to define baseline characteristics and mechanisms of response or resistance to various immuno-oncology drugs

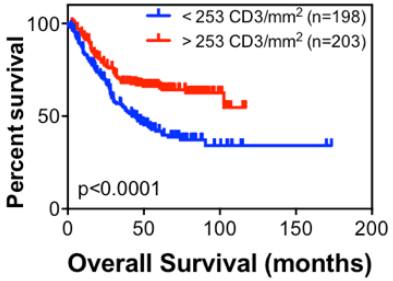
Era of personalized combined biomarkers

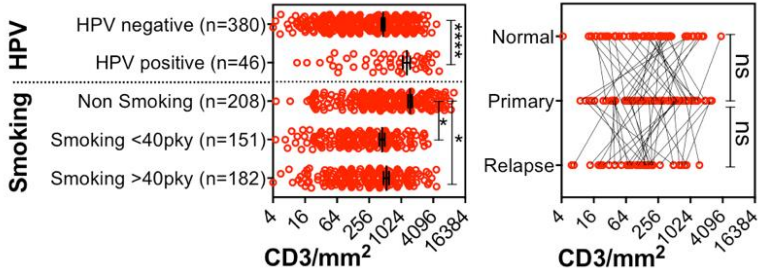
Automated quantification of tissue microarrays with grid detection and single cell segmentation

Head and neck squamous cell cancer tissue microarrays stained for CD3



Automated detection and quantification of markers in tissue microarrays, using grid finding (left), nuclei segmentation (blue outlines), and determination of chromogen-positive cells (red outline), shown here for CD3 in HNSCC before (middle) and after (right) counting – zoomed area (far right)

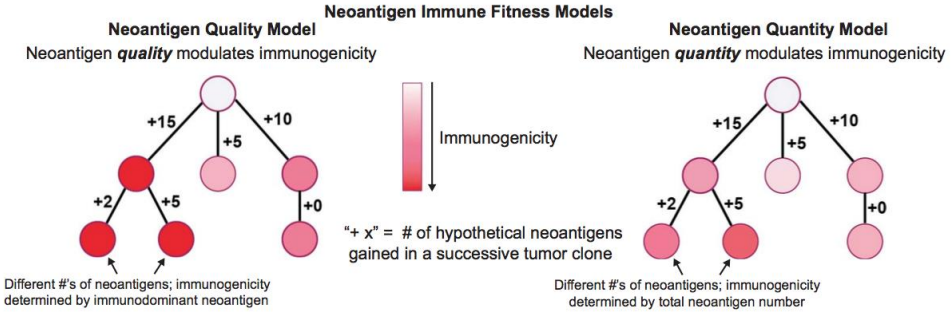


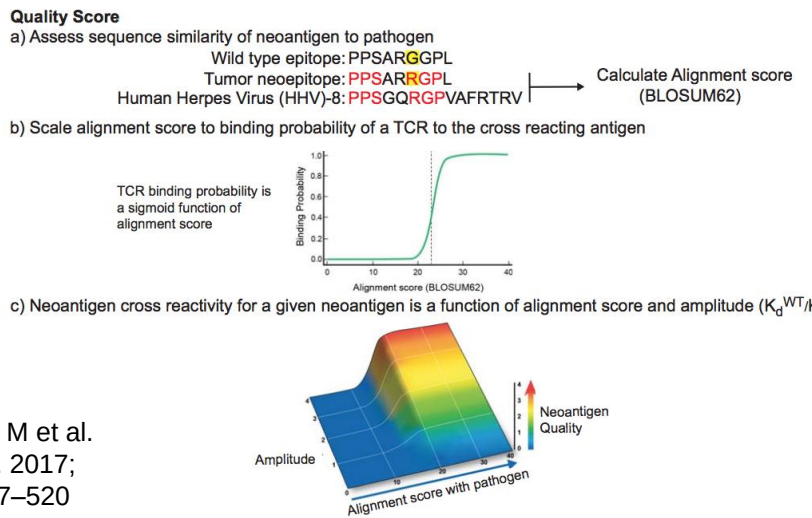


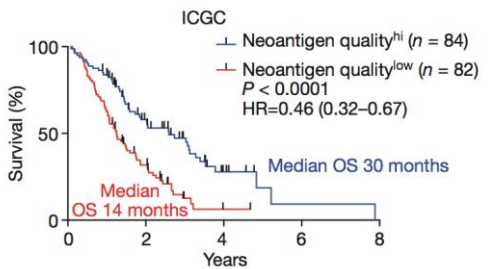
Gnjatic, Laban, Perner, Klumper, Ribbat et al. Unpublished.

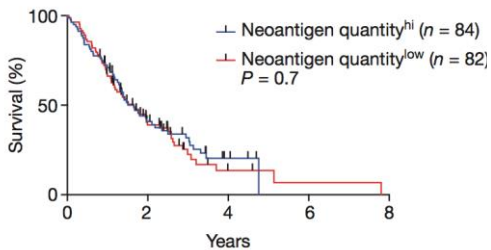
In silico modeling improves prognostic value of neoantigens by assessing their quality

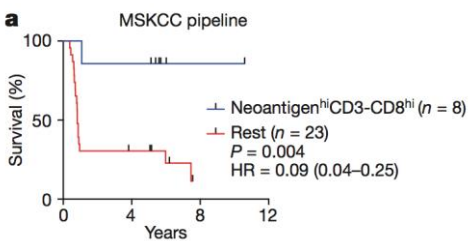
Pancreatic cancer patients, including subset with long-term survival

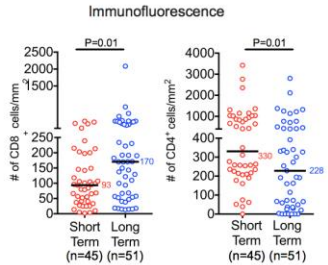


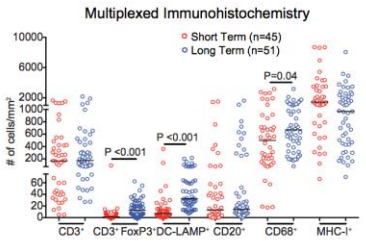


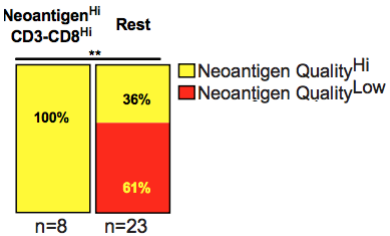










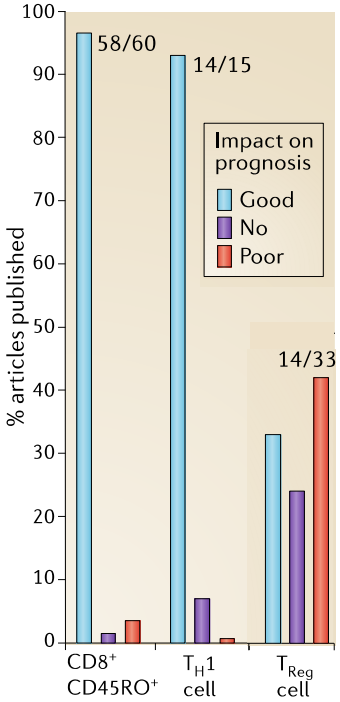


Łuksza M et al. Nature. 2017; 551:517–520

Balachandran V et al. Nature. 2017;551:512–516

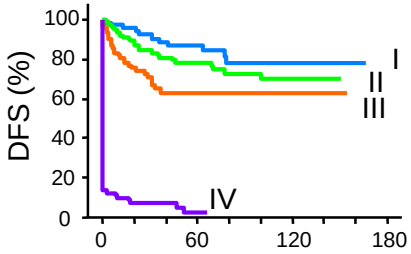
T cell tumor infiltration as a prognostic marker in various tumors and a predictive biomarker of PD-1 response in melanoma

Meta-analysis of 124 articles (20 cancer types)

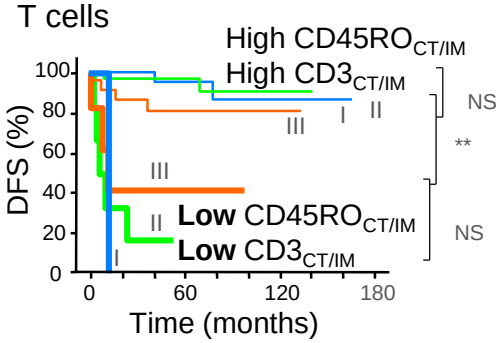


Immunoscore
(colorectal cancer)

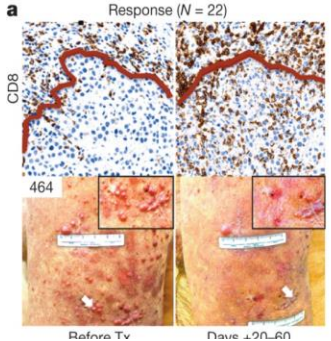
TNM classification



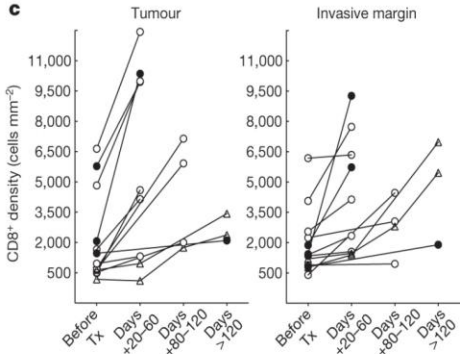
T cells



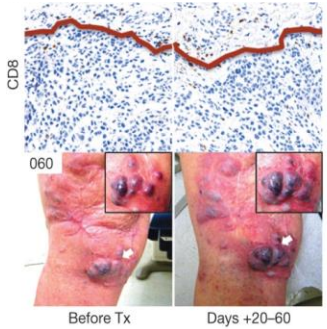
Responders (n=22)



Tumor / Invasive margin



Progressors (n=24)



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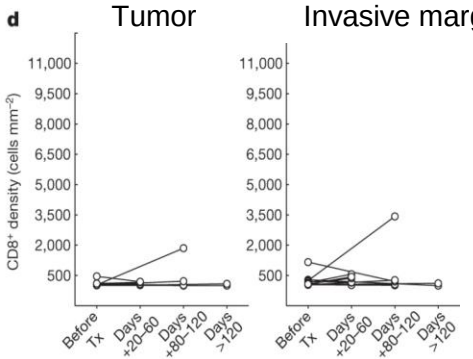


The immune contexture of the tumor influences prognosis

Fridman et al., *Nature Rev. Cancer*, 2012

Galon et al., *Science*, 2006;
Pagès et al., *J Clin Oncol*, 2009

CD8 T cell infiltration before and during pembrolizumab in advanced melanoma.

PC Tumeh *et al. Nature* 515, 568-71 (2014)

Example of budget prioritization plan for assays

CITN-09

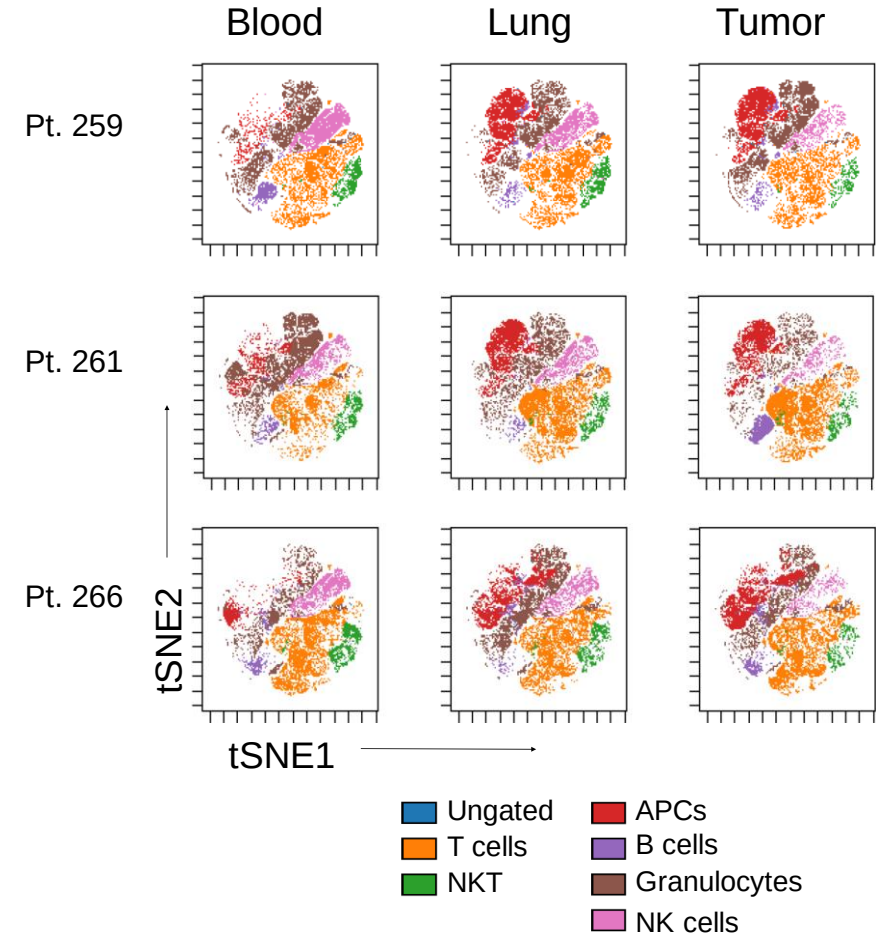
Pembrolizumab in Merkel Cell Carcinoma.

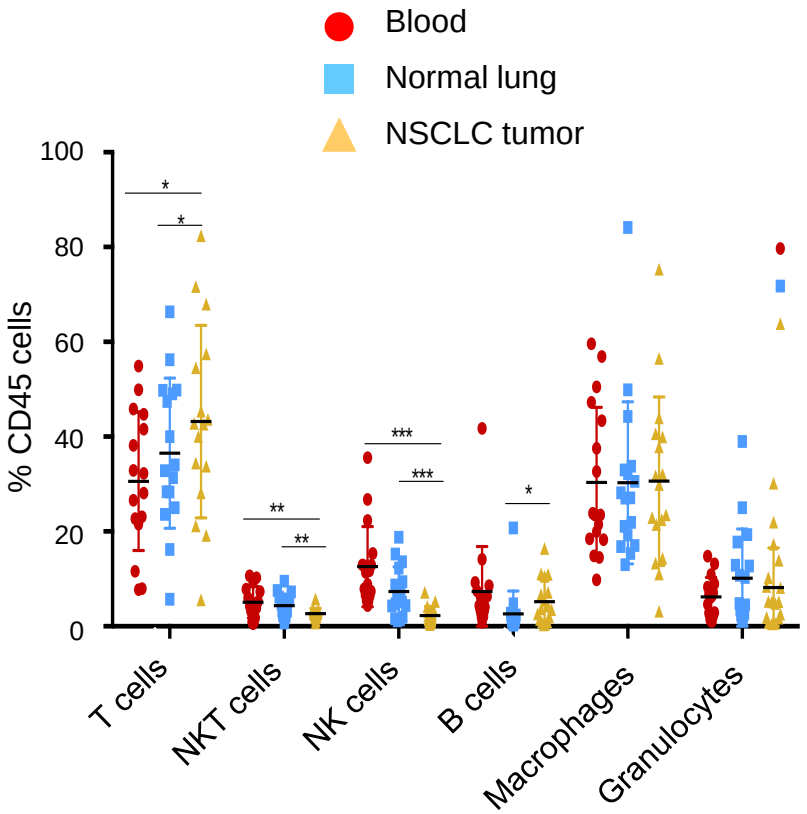
Assay and cost rundown per patient.

		Priority
Sample reception, barcoding, storage, management, realiquoting post-assay	$	
Serum antibodies to MCPyV		
ELISA (3 time points)	$	#6
Serum antibody profiling for tumor specificity		
Grand Serology (3 time points)	$$	#2
Seromics (subset only, 50%)	$$$	#12
Soluble protein analytes, including Flt3L		
O-Link (3 time points)	$	#7
Phenotyping of biopsies and of peripheral blood		
CyTOF of tissue (2 time points)	$$	#4
CyTOF of blood (3 time points)	$$	#5
Tissue multiplex IHC from biopsies		
MICSSS (2 time points)	$$	#1

		Priority
Tumor gene expression from biopsies		
Nanostrings (2 time points)	$	#3
Tumor mutational profile and neoepitope prediction		
WES / RNAseq (1 time point)	$$	#8
Peptides for neoantigen	$$	#9
Neoantigen identification of T cell, characterization (priced at 50% of cost if planned only in subset)		
IVS + ELISPOT (2 time points)	$$	#10
CD154 sort / tetramer (subset)	$$	#14
T cell diversity from biopsies or peripheral blood		
TCRSeq (2 time points)	$$$	#11
Microbiome analyses		
16S sequencing (2 time points)	$	#13
Data analysis pricing included in assays		
Data management, storage, sharing	$	

Immune composition of early non-small cell lung carcinoma (NSCLC) by mass cytometry (Lavin, Rahman, Gnjatic, Merad, Cell 2017;169:750-765)



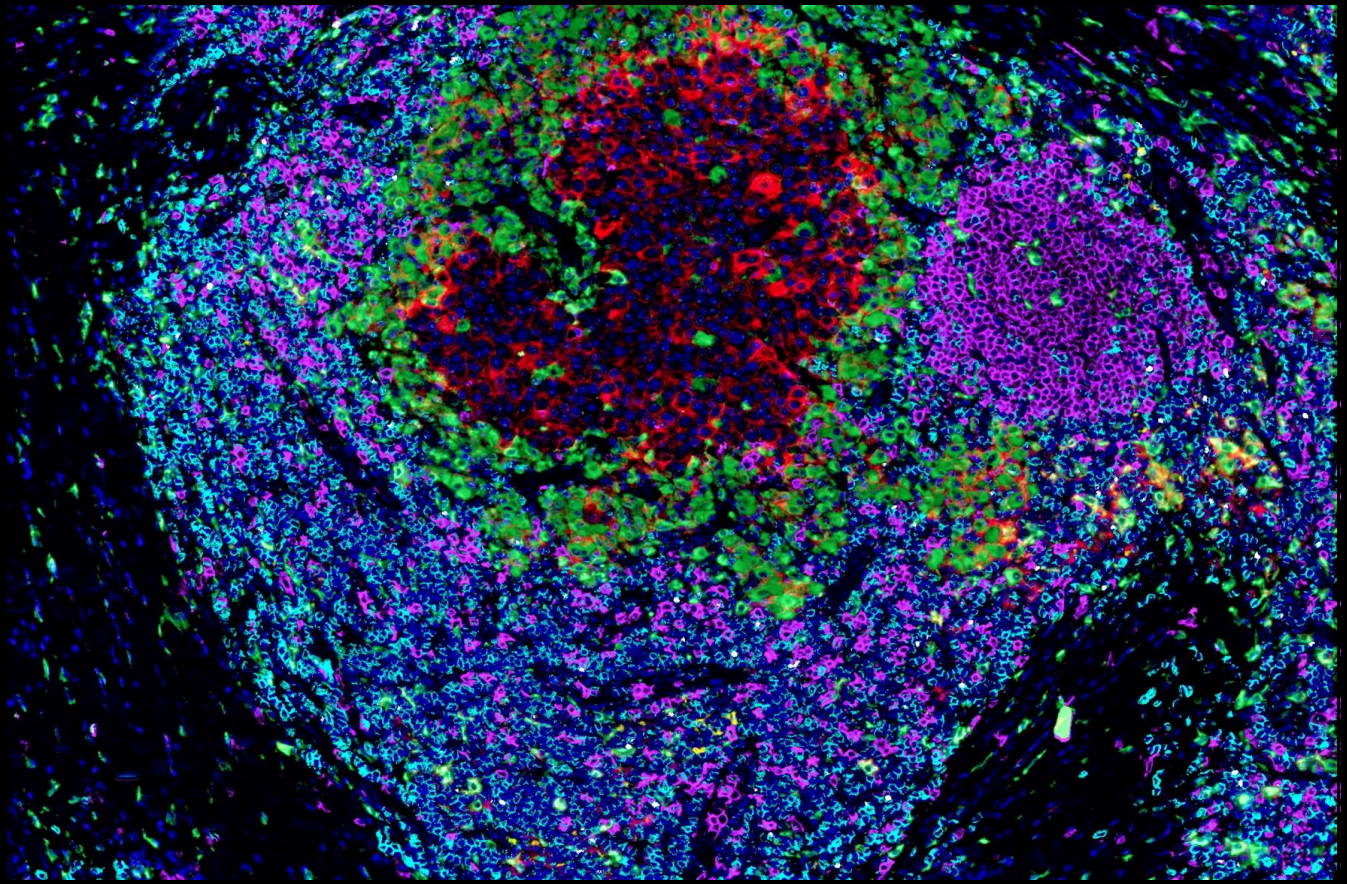


Areas of focus

- Immune microenvironment by multiplex immunohistochemistry
- Phenotyping by CYTOF mass cytometry
- Immunosupportive role of microbiome composition
- Defining antigen specificity and quality (neoantigens, seromics)
- Modeling, integration of data, and automated analyses pipelines

Applying multiplex IHC to query effect of checkpoint blockade

Hematoxylin/PD-L1/CD68/DC-LAMP/CD20/CD3/FoxP3



Melanoma lesion biopsied after CTLA-4 treatment (1 FFPE slide) **x100**

Remark R, Merghoub T, Grabe N, Litjens G, Damotte D, Wolchok JD, Merad M, Gnjatic S. *Science Immunology; 1:aaf6925 (2016).*

Output in 2016

- 49 active clinical trials and studies

Mount Sinai examples

- Cancer (tumor immune responses, immune monitoring of antitumor therapies)
- Cardiovascular disease (gene therapy trial, monocyte in cardiac disease)
- Food allergy (mechanisms of disease)
- Genetics (disease pathogenesis)
- IBD (mechanism of disease)
- Infection disease (infection induced immune response)
- Ophthalmology (biomarkers of dry eye disease)
- Pediatrics (vaccine-induced immune reactions)
- Psychiatry (immune targets of depression)

Pharma / Biotech examples

- Immune Design (cancer vaccine)
- Imclone (new target of disease)
- Dendreon (immunomodulation drug)
- Genentech (immunomodulation drug)
- Advaxis (cancer vaccine)
- Ludwig Institute Cancer (cancer vaccine)
- Janssen Inc. (IBD study)

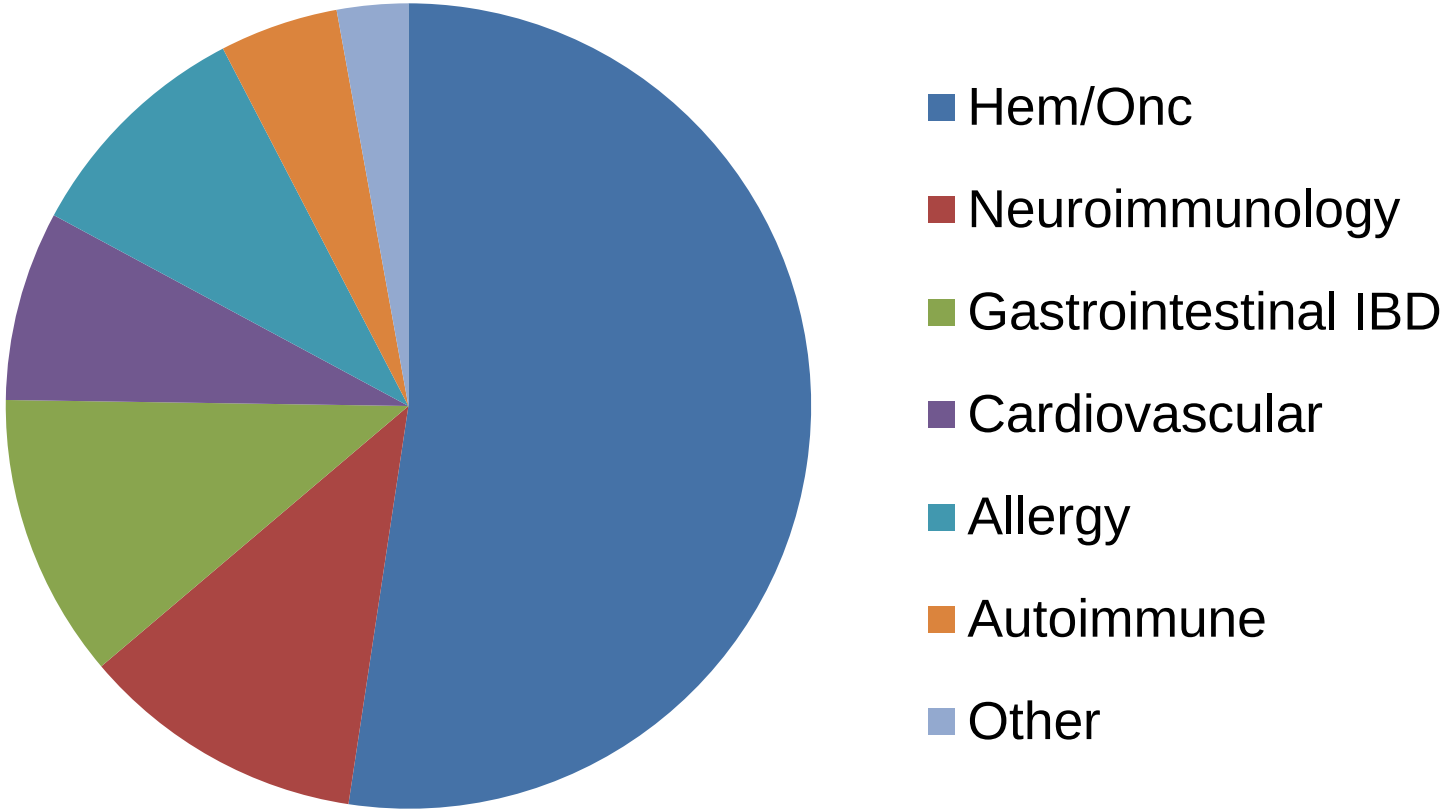
- Average of 85 patient collection visits **per month** generating 4531 monthly bio-specimen aliquots tracked by barcode for storage and analysis (blood, tissues, stool, urine, skin swabs, impression cytology)

Work output and capacity in 2017

Types of immune assays and sample processing	# of samples
Mass Cytometry, CyTOF2	2,550
Flow Cytometry, immunophenotyping/ICS	1,350 (3,500 panels)
Antigen specific (ELISPOT)	432
Antigen specific (Seromics, ELISA)	288 + 520
Multiplex assay cytokine/chemokine	3635
Multiplex IHC	450
Whole blood processing into PBMC, serum, plasma	2,216
Immune cell enrichment and sorting	358
DNA/RNA isolation assays	850
Microbiome processing and library synthesis	355

CYTOF usage in 2017	Internal	External Academic	External Industry	Total
Experiments #	573	50	225	848
Samples #	1,585	140	825	2550

Studies handled by HIMC by area of research



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Cancer Immune Monitoring and Analysis Centers and Cancer Immunologic Data Commons

The CIMAC-CIDC Network

CDP: Magdalena Thurin

CTEP: Helen Chen, Minkyung Song, Elad Sharon, Howard Streicher, Bill Merritt

BRP: Yingdong Zhao

CBIIT: David Patton

TRP: Andrew Hruszkewycz, Jeff Abrams

Administrative Support: Rebecca Enos (contractor)

NIH NATIONAL CANCER INSTITUTE

National Cancer Institute

Cancer Immune Monitoring and Analysis Centers (CIMACs)
Cancer Immunologic Data Commons (CIDC)

A Cancer Moonshot Initiative, funded under the cooperative grants (U24)

- **The CIMAC-CIDC network will provide a standing infrastructure of bioassays and data commons for correlative studies in NCI-funded trials involving immunotherapy**
  - 4 CIMACs for scientific expertise and a wide range of highly specialized services using state-of-the-art equipment
  - One CIDC for centralized bioinformatics resources for data collection and integration across trials and clinical databases

- **Scope of work**
  - Support correlative studies in early (phase 1 and phase 2) immunotherapy trials in the CTEP Trial Networks and Grant-supported trials
  - 500 patients / multiple timepoints / year for comprehensive profiling

- **Utilization of the CIMAC-CIDC resource is voluntary, but desired studies in collaboration with CIMAC require approval by CTEP.**

Awardees

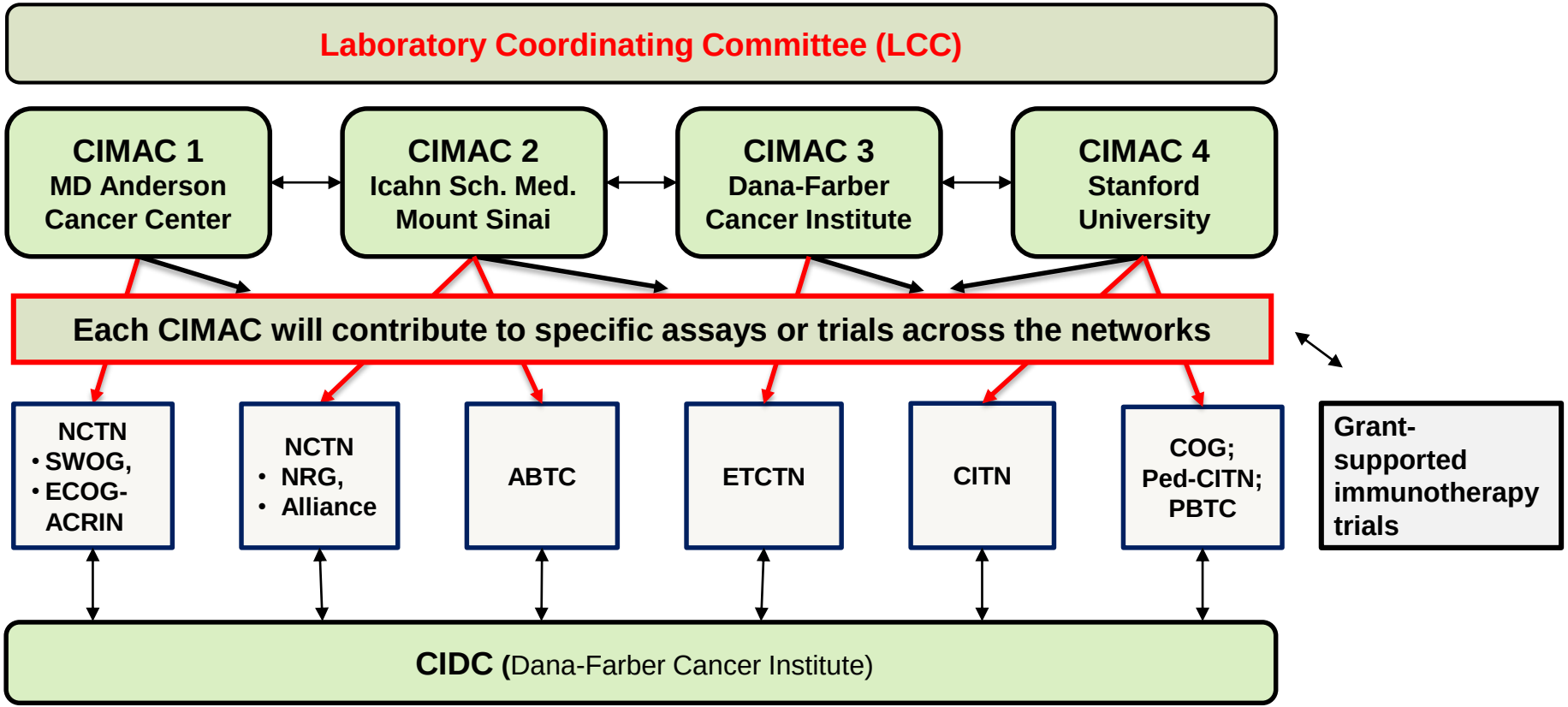
CIMACs

- **The University of Texas MD Anderson Cancer Center**
  PIs: Ignacio Wistuba, Elizabeth Mittendorf / Gheath Al-Atrash, and Chantale Bernatchez
- **Icahn School of Medicine at Mount Sinai**
  PI: Sacha Gnjatic
- **Dana-Farber Cancer Institute**
  PIs: Catherine Wu and F. Stephen Hodi
- **Stanford University**
  PIs: Holden Maecker and Sean Bendall

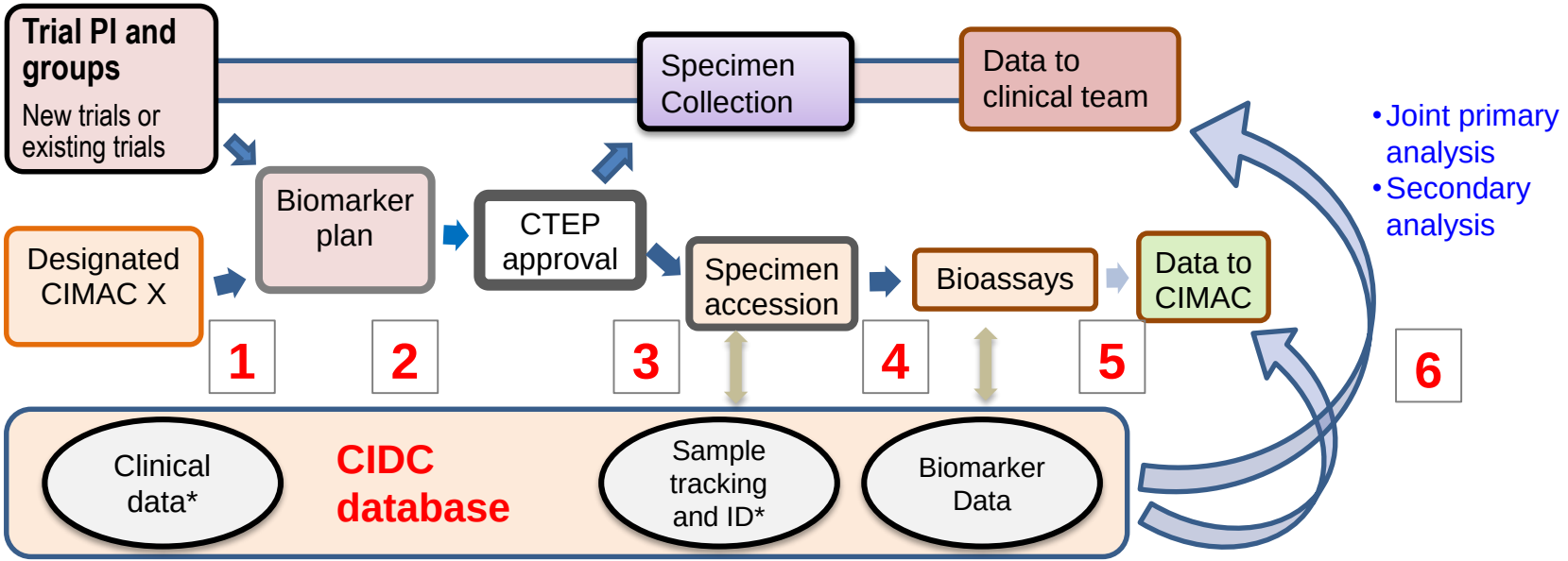
CIDC

- **Dana-Farber Cancer Institute**
  PIs: Xiaole Shirley Liu and Ethan Cerami

CIMACs-CIDC Network Structure



Proposed workflow for CIMACs/CIDC with the clinical networks



1. **Selection of trials**
2. **Proposal of Biomarker plans** - will be jointly proposed by CIMAC and Clinical investigators. Requires CTEP (PRC) approval.
3. **Specimen accession** – universal tracking system (across biobanks) under discussion. .
4. **Database at CIDC** – clinical annotation; bioassay results; and sample tracking system
5. **Data formats and informatics**
6. **Data analysis and publication** – Primary analysis: With trial investigators;