Correlative High-Dimensional Analyses in Cancer Immunotherapy: *From Infrastructure to Assays to Useful Predictive Biomarkers*

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Disclosure information: Sacha Gnjatic, PhD

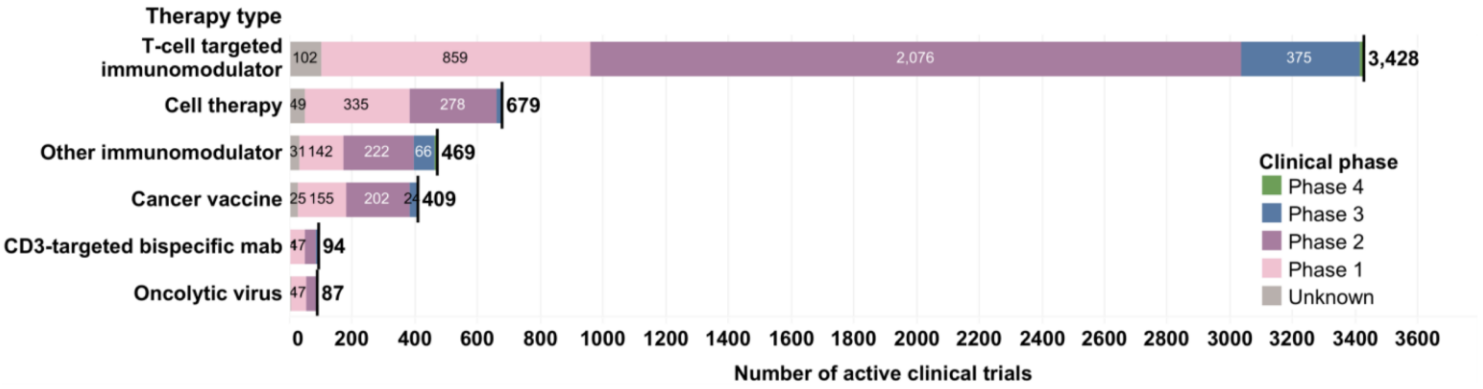
The following relationships exist:

Immune Design: *Research Support*
Janssen R&D: *Research Support*
Agenus: *Research Support*
Genentech: *Research Support*
Pfizer: *Research Support*
Regeneron: *Research Support*
Takeda: *Research Support*

Co-inventor on an issued patent for multiplex immunohistochemistry to characterize tumors and treatment responses. The technology is filed through Icahn School of Medicine at Mount Sinai (ISMMS) and non-exclusively licensed to Caprion. Mount Sinai has received payments associated with licensing this technology and both Mount Sinai and Dr. Gnjatic are entitled to future payments.

Third Rock Ventures/Neon Therapeutics: *Consulting Fees (e.g., advisory boards)*
B4CC: *Consulting Fees (e.g., advisory boards)*
OncoMed: *Consulting Fees (e.g., advisory boards)*
Merck: *Consulting Fees (e.g., advisory boards)*

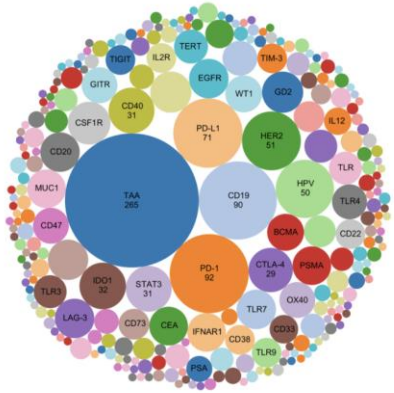
Immunotherapy's continued explosive growth



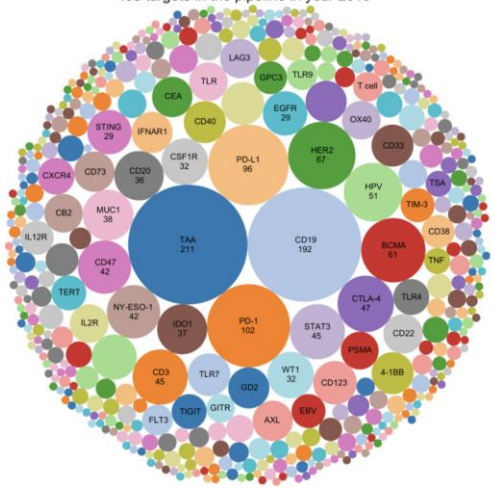
Over 5000 active immuno-oncology trials worldwide

91% increase in the number of active agents in development since 2017, a 78% increase in active targets, and a 60% increase in companies and other organizations with an immuno-oncology pipeline

263 targets in the pipeline in year 2017



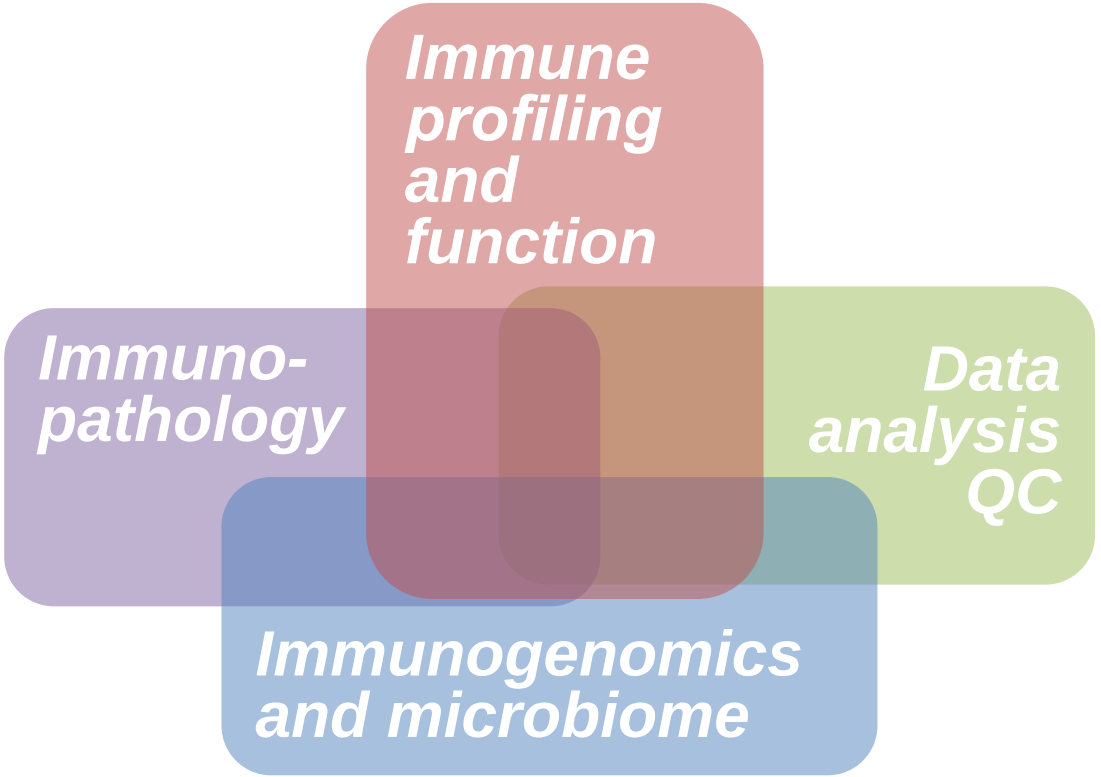
468 targets in the pipeline in year 2019



Yu et al (Cancer Research Institute). Nat Rev Drug Discovery 2019

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

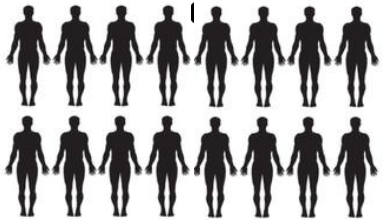


➢ Need for analytically validated assays and procedures, and harmonized interpretation of data

Identifying immunotherapy biomarkers

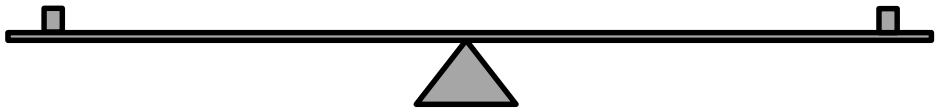
- Cutting-edge technology platforms
- Technical expertise in assay development and instrument platforms
- Scientific expertise in human immunology
- Computation expertise in data analysis and visualization

Patient

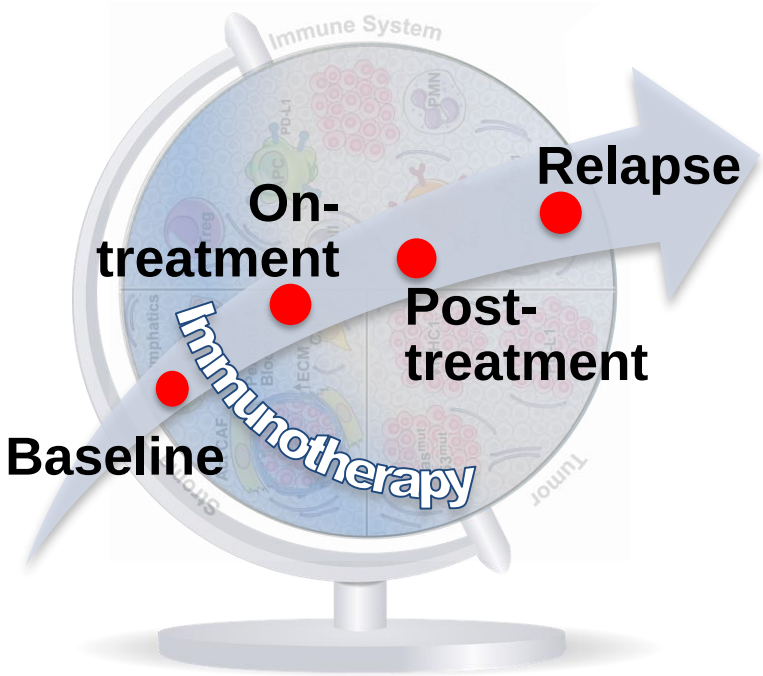


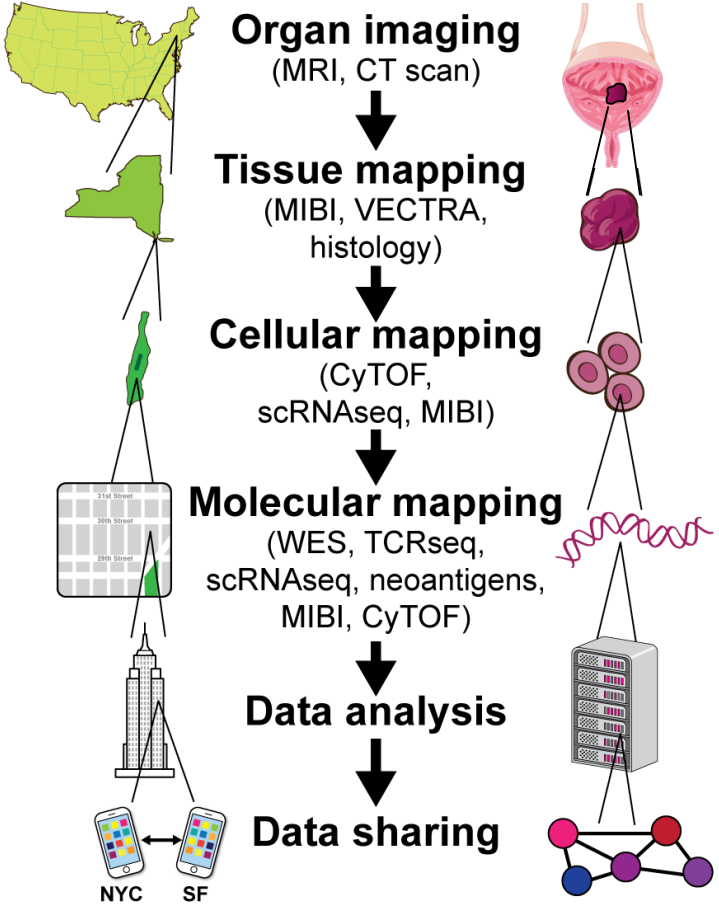
Develop innovation with high-dimensional assays to monitor disease-relevant immune signatures and discover new mechanisms, biomarkers and immune targets

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



Multiscale, dynamic atlas of immune changes





Challenges of immune monitoring

Except adoptive transfer, most immunotherapies directly target the immune system rather than the tumor itself: how to find a good biomarker when targeting a network?

Assembling a multidisciplinary team for molecular, genetic, microbial, and cellular signatures

Ensuring reproducibility and robustness while fostering innovation

Obtaining adequate biospecimens to explore markers at the tumor site and in the periphery

Interpreting the complex data generated using computational tools

Correlating disparate data sets with clinical outcome

Some lead biomarkers in immune checkpoint blockade

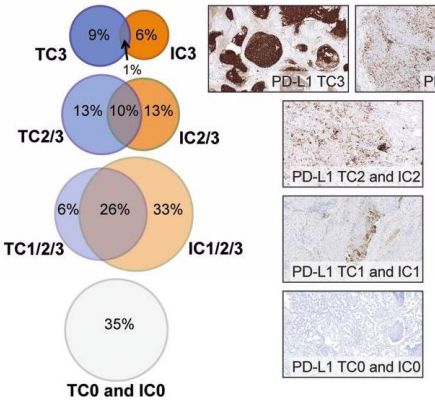
- Tumor immune infiltration
- Tumor mutation burden
- Stratifying biomarkers (inclusion/exclusion): driver alterations (EGFR/ALK in NSCLC)
- MSI-H/dMMR (FDA approved general indications)
- PD-L1 tissue expression (FDA companion/complementary test)

Promising other candidates

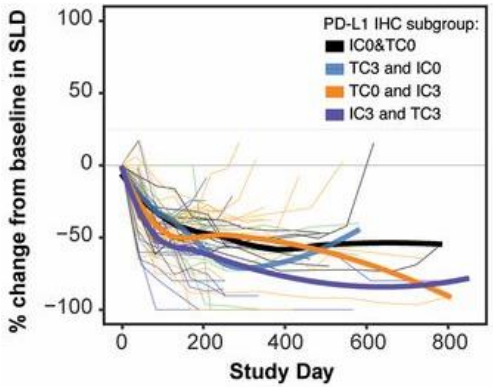
- Gut microbiota composition and metabolites
- Peripheral blood markers
  - Soluble: cytokine, protein analytes, antibody
  - Cellular: activation/suppression markers, cell populations (pre-exhausted T cells), tumor antigen specificity, T cell repertoire
- Stromal markers and tissue-resident immune cells
- ctDNA/cfDNA/CTC

PD-L1 expression matters…

Independent predictive value of PD-L1 on immune cells and tumor cells (n=4549 NSCLC treated with atezolizumab, using SP263 IHC assay)



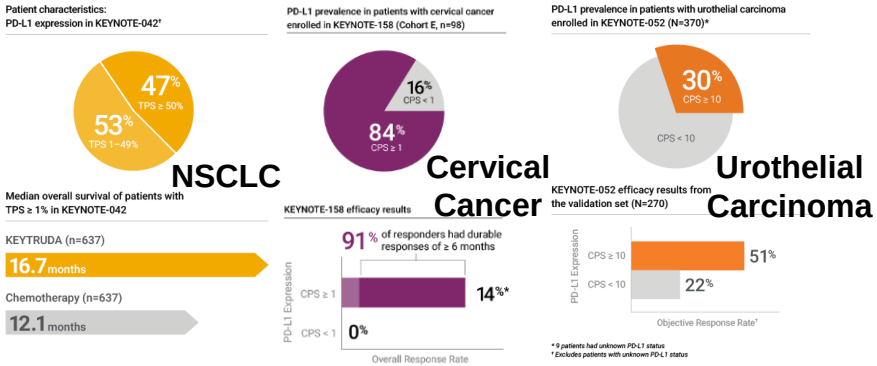
PD-L1 expression subgroup	n	Responders, n	Objective response rate (95% CI), %
TC3 and IC0	20	8	40% (19-64)
IC3 and TC0	108	24	22% (15-31)
TC3 and IC3	47	20	43% (28-58)
TC0 and IC0	73	6	8% (3-17)



TC: tumor cell PD-L1 score
IC: immune cell PD-L1 score

Kowanetz *et al.* Proc Natl Acad Sci U S A. 2018;115:E10119–E10126.

Pembrolizumab's FDA companion test 22C3 IHC



From Agilent.com

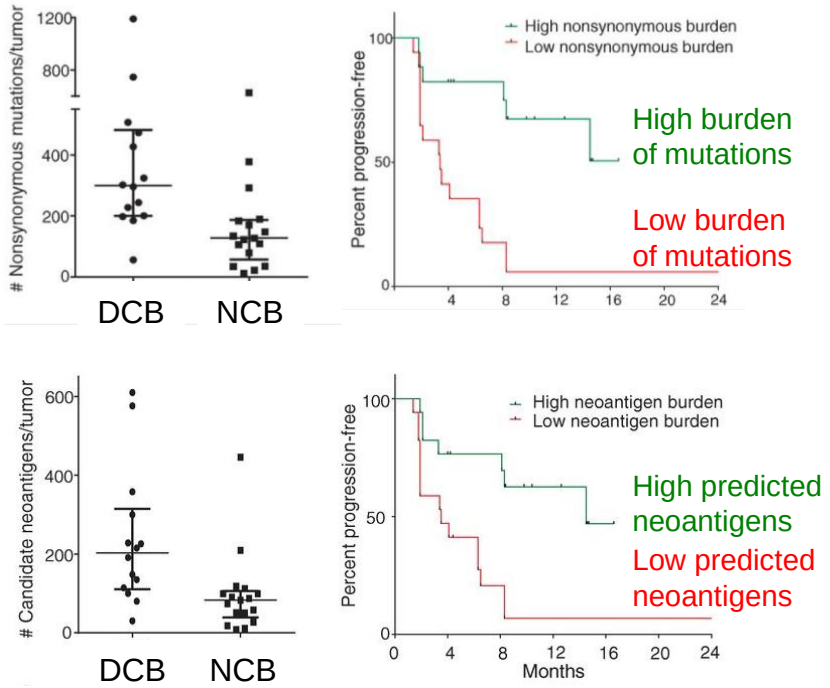
- PD-L1 staining as the only approved companion or complementary test for immunotherapy
- PD-L1^{hi} tumors respond better to PD-1/PD-L1 blockade

But:

- Three tests are approved with only partial overlap in interpretation of their results
- Dynamic changes upon treatment and heterogeneity are difficult to capture
- Some patients experiencing benefit in PD-L1 low tumors, and many patients have no response despite PD-L1 high tumors.

Tumor mutation burden (TMB) as biomarker

TMB correlates with response to PD-1 blockade in NSCLC



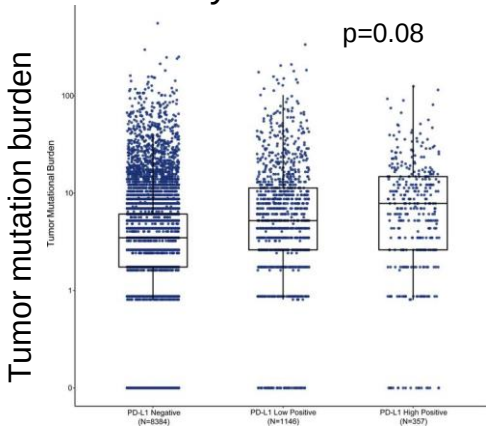
DCB = durable clinical benefit
NDB = no durable clinical benefit

Rizvi *et al*. Science. 2015. 348-124

- Multitude of studies indicating high TMB correlates with better response to immune checkpoint blockade (cutoff for high TMB typically at ≥ 10 mut/mb)
  - CheckMate-227 trial
  - CheckMate-568 trial – independently of PD-L1 expression
- Meta-analysis in NSCLC treated with anti-PD-1/PD-L1 (n=1290) showed TMB ≥ 20 mut/mb associated with increased OS and clinical benefit (JAMA. 2019;321:1391-1399)
- Rationale based on higher likelihood of creating neoepitopes for T cell-mediated tumor rejection
- Trials have been proposed based on high TMB as a biomarker
- However, recent withdrawal of TMB ≥10 mut/mb as a supplemental biologics license FDA application for frontline combination ipilimumab/nivolumab in NSCLC

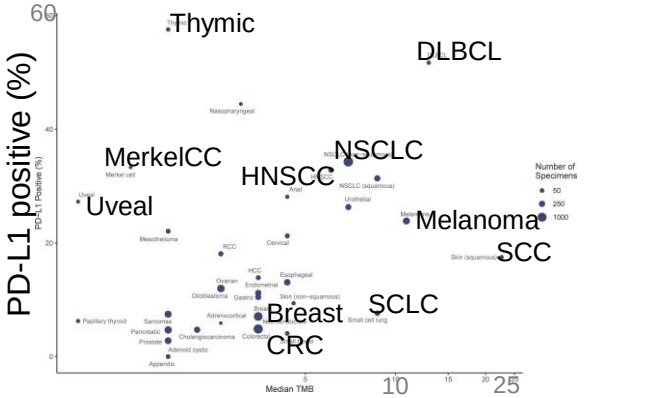
PD-L1 IHC and mutational load as independent biomarkers

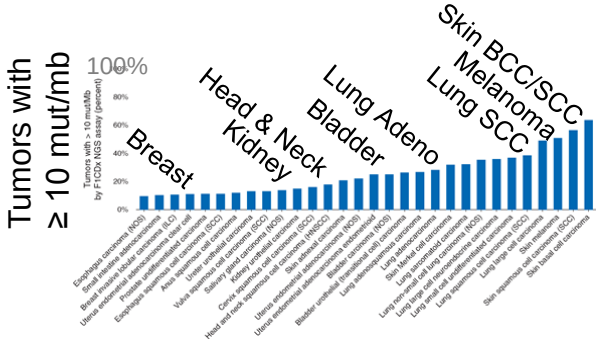
Meta-analysis in various tumor types (n=9887)



Parameter	WES	FM NGS (F1CDx)
No. of genes	~22 000 gene coding regions	324 cancer-related genes
Types of mutations captured	Coding missense mutations in tumor genome	Coding, missense, and indel mutations per Mb of tumor genome
Germline mutations	Subtracted using patient-matched normal samples	Estimated via bioinformatics algorithms and subtracted

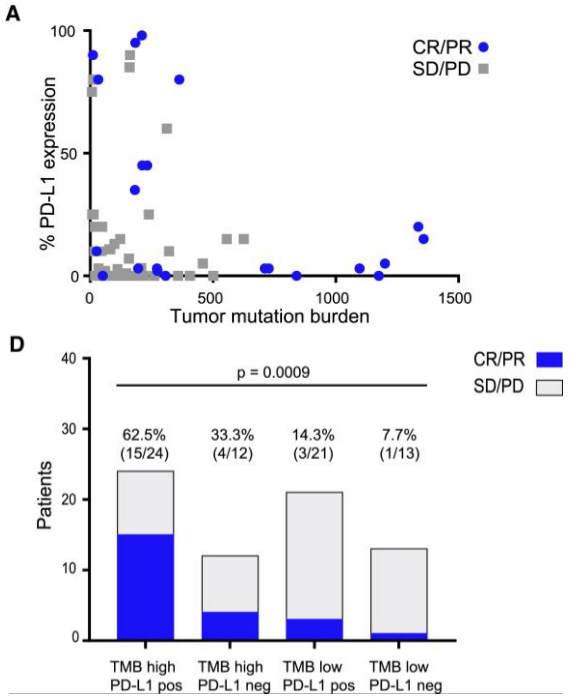
Yarchoan, …, Jaffee. JCI Insight. 2019





Chan *et* al. Annals Oncol. 2019:30;44–56,

Combined predictors of best clinical benefit in NSCLC (n=75)

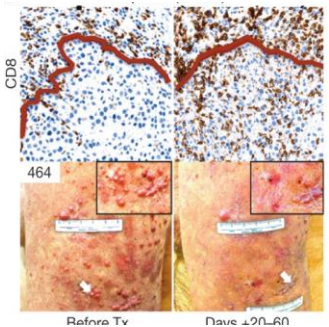


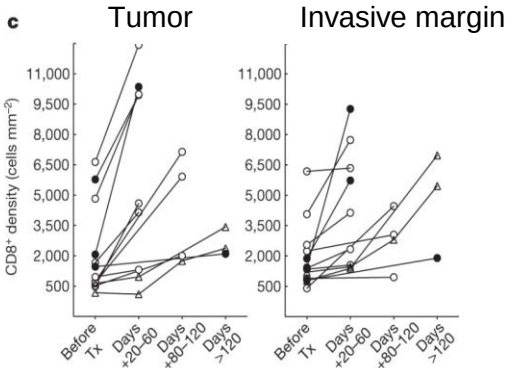
Hellman, …, Wolchok. Cancer Cell. 2018;33:843-852

Density and localization of immune infiltrate matters…

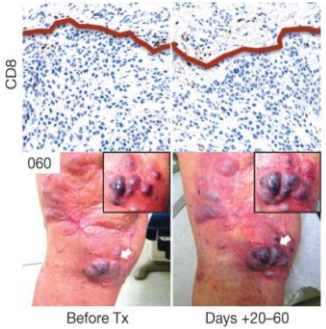
CD8 T cell infiltration before and during pembrolizumab in advanced melanoma.

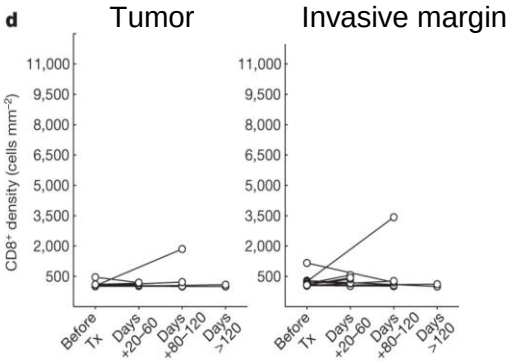
Responders (n=22)





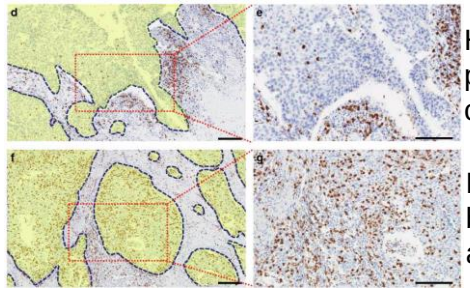
Progressors (n=24)





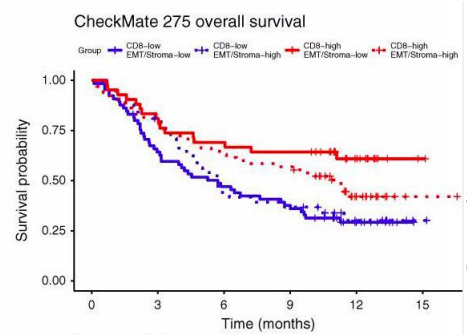
PC Tumeh *et al. Nature* 515, 568-71 (2014)

Metastatic urothelial carcinoma (n=214) treated with nivolumab



High peritumoral T cell infiltration

High T cells in both stroma and tumor

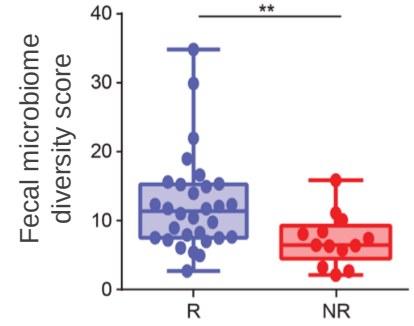


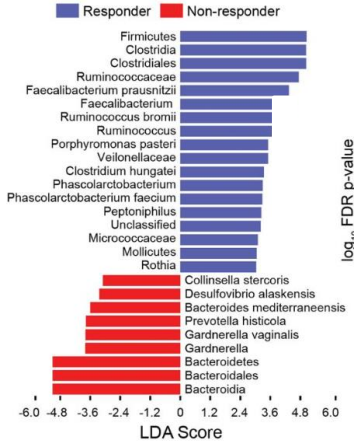
Best response in CD8 high stroma low Independently of PD-L1 IHC

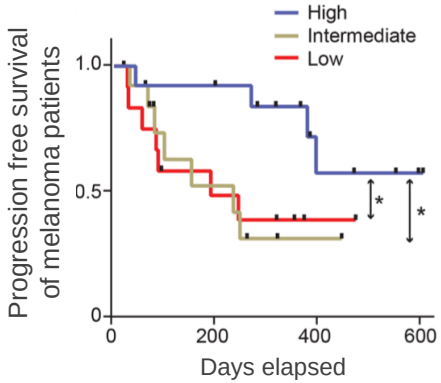
Wang, …, Galsky. Nat Commun. 2018;9:3503.

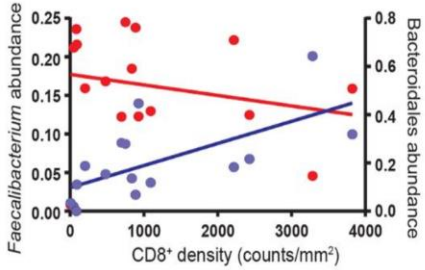
Emerging biomarkers with novel methods may matter…

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



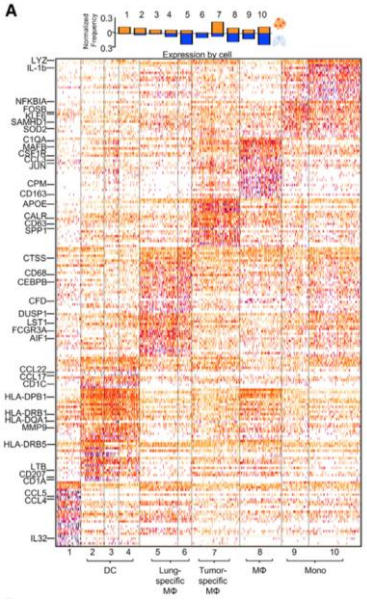


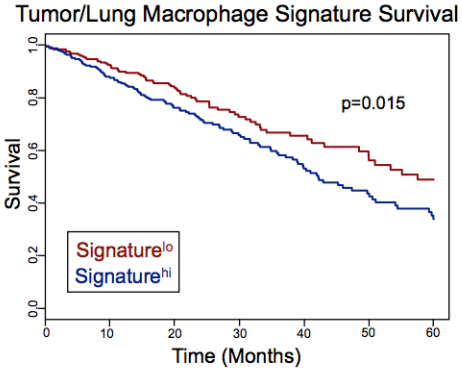


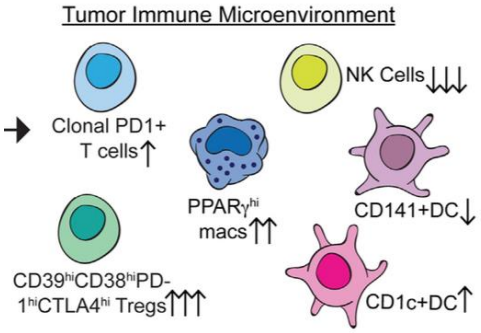


Matson, V. et al. Science 359, 104–108 (2018).
Gopalakrishnan, V. et al. Science 359, 97–103 (2018).

Signature of tumor macrophages by scRNAseq associated with poor outcome in early NSCLC



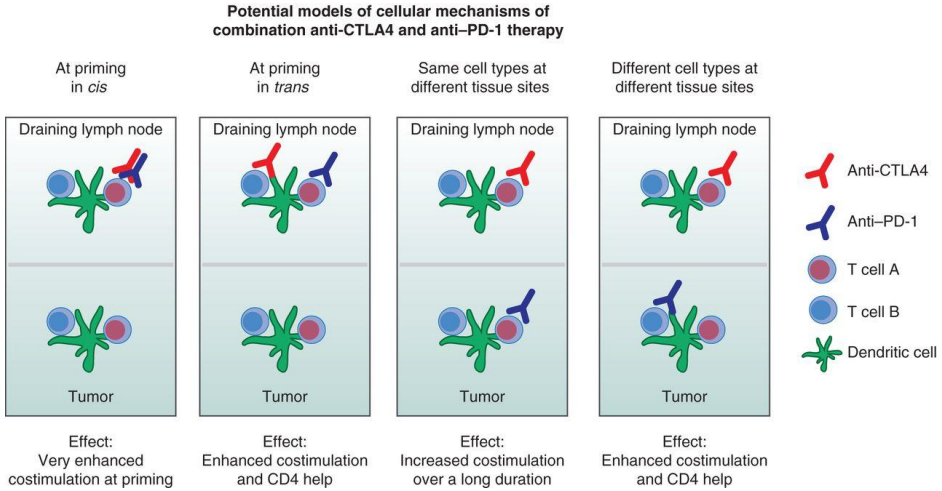




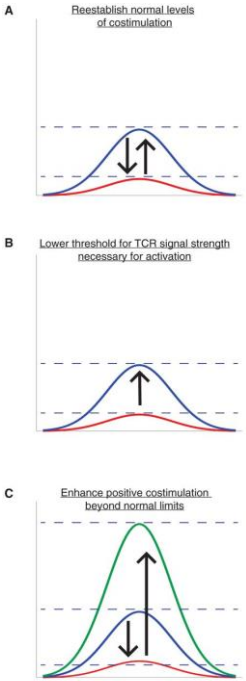
Routy al. Science. 2018

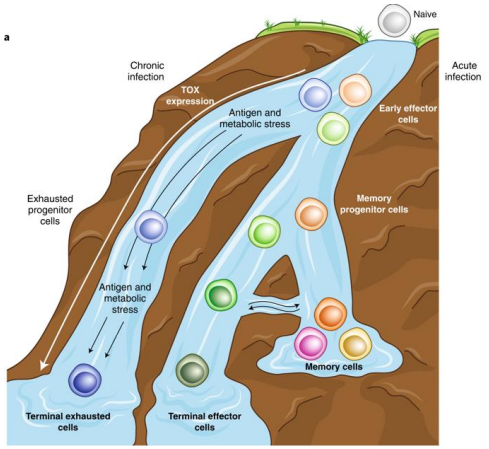
Lavin, Rahman, Gnjatic, Merad, Cell 2017;169:750-765

T cell states, exhaustion, and metabolism

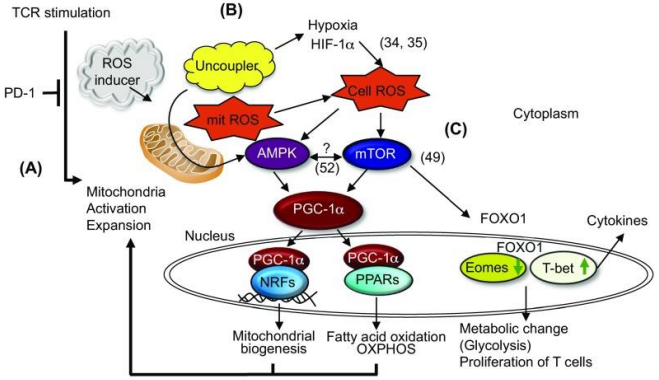


Wei et al. Cancer Discov. 2018





Mann & Kaech, Nature 2019



Chamoto, PNAS, 2019

TOX, TOX2, and NR4A cooperate and contribute to T cell exhaustion (but also TCF1, PTPN2, CD226…)

Khan…Wherry, Nature 2019; Seo…Rao, PNAS 2019;
Alfei et al, Nature 2019; Scott et al, Nature 2019,
Manguso et al, Nature 2017

https://www.cancerresearch.org/blog/september-2019/cicon19-day-2-t-cell-exhaustion-tumor-microenviron

Cancer Immune Monitoring and Analysis Centers and Cancer Immunologic Data Commons

The CIMAC-CIDC Network: A Cancer Moonshot Initiative (U24)

The CIMAC-CIDC network will provide a standing infrastructure of bioassays and data commons for correlative studies in NCI-funded trials involving immunotherapy ($50M+)

- 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 / 2) immunotherapy trials in the CTEP Trial Networks and Grant-supported trials
- 500 patients / multiple timepoints / year for comprehensive profiling
- Many additional patients from industry and non-NCI trials through Partnership for Accelerating Clinical Trials (PACT, $220M)

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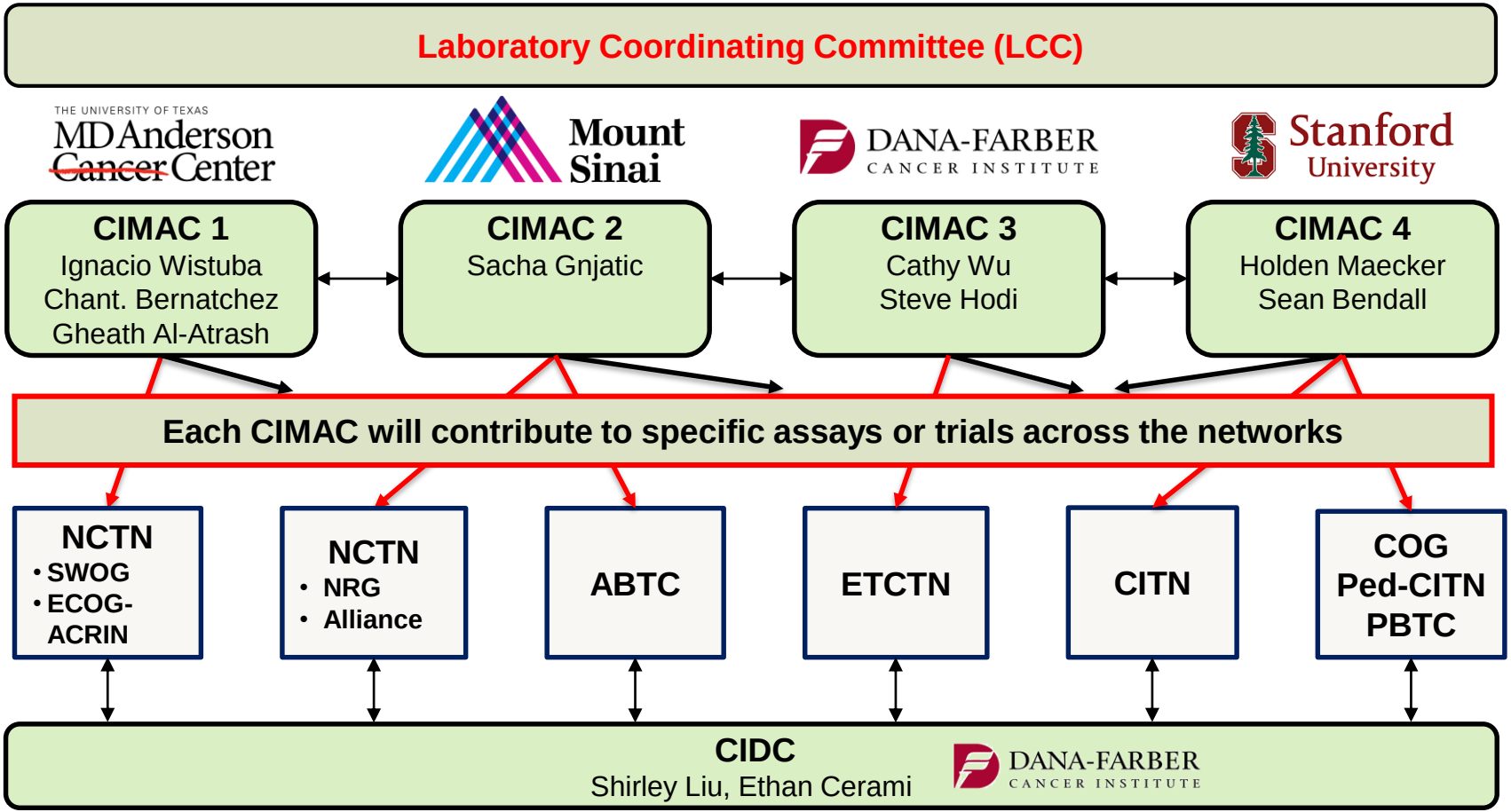
CIMAC-CIDC Immuno-Oncology Biomarkers Network

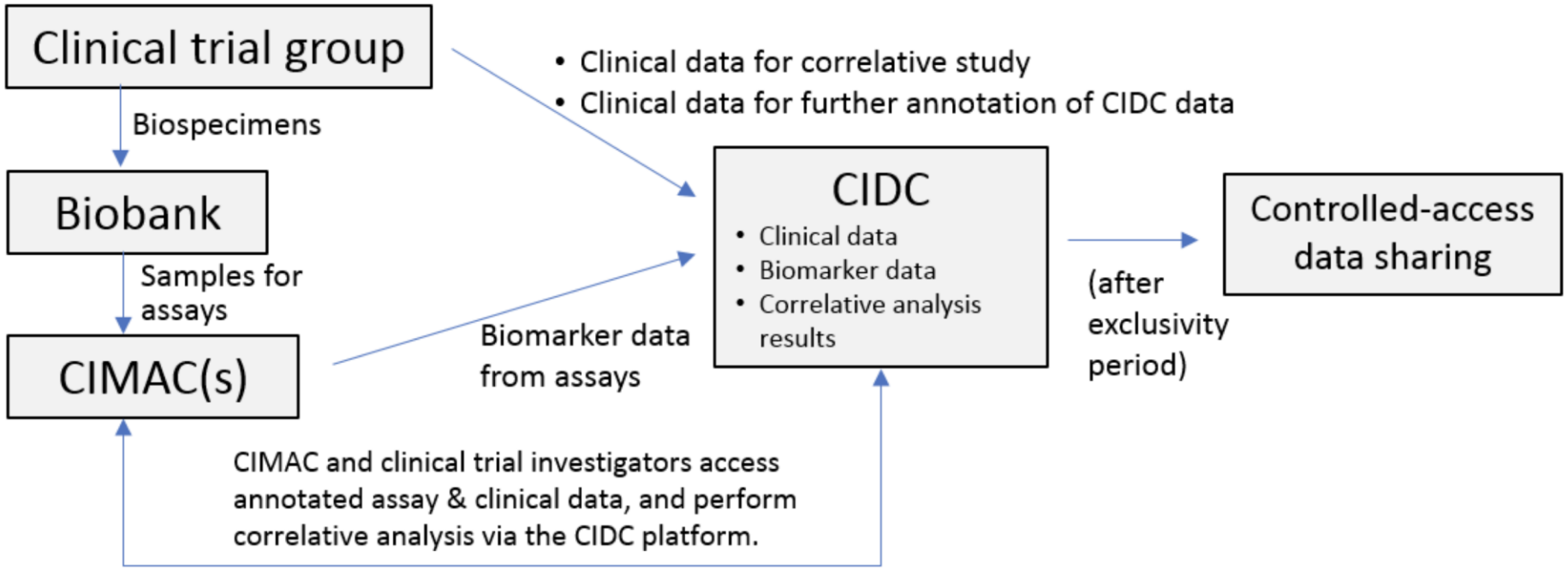
FNIH Foundation for the National Institutes of Health

AbbVie, Amgen, Boehringer Ingelheim, Bristol-Meyers Squibb, Celgene, Genentech, Gilead Sciences, GlaxoSmithKline, Janssen, Novartis, Pfizer

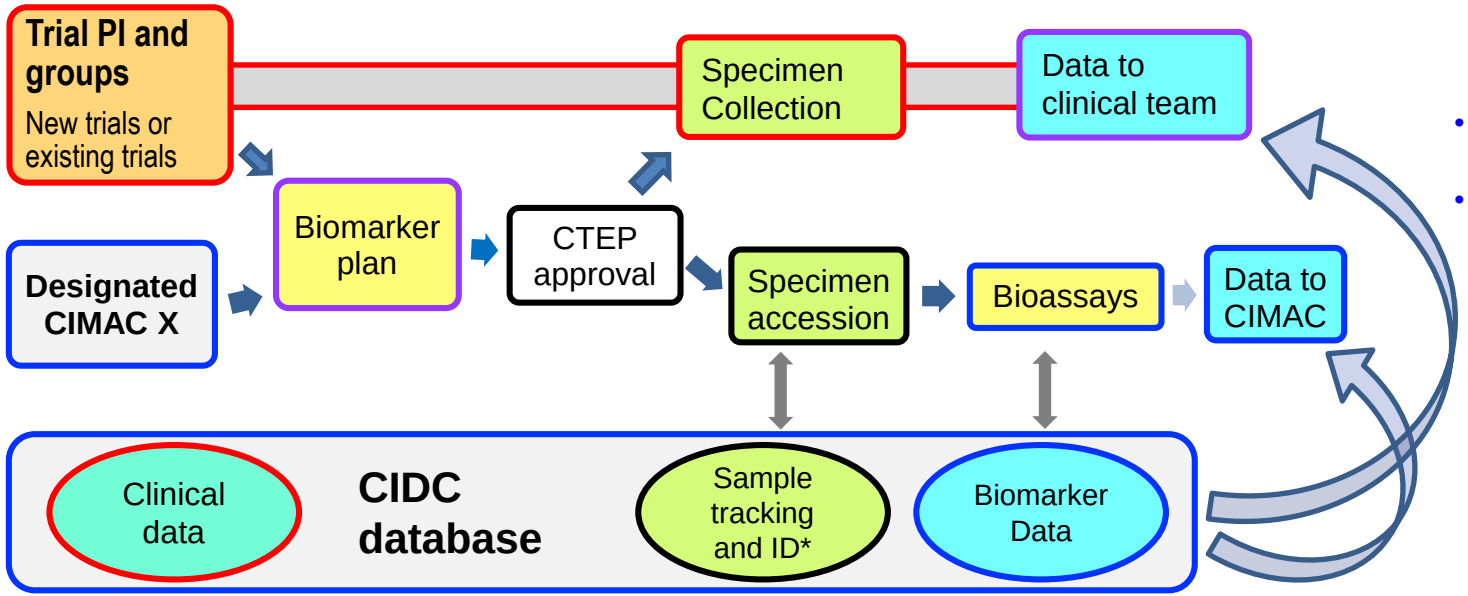
CIMACs-CIDC Network Structure



CIMACs-CIDC Network Structure



Workflow with the clinical networks



- Joint primary analysis
- Secondary analysis

Collaborative effort between study PI, CIMAC, biobank, and NCI

Clinical Trial Coordination Working Group

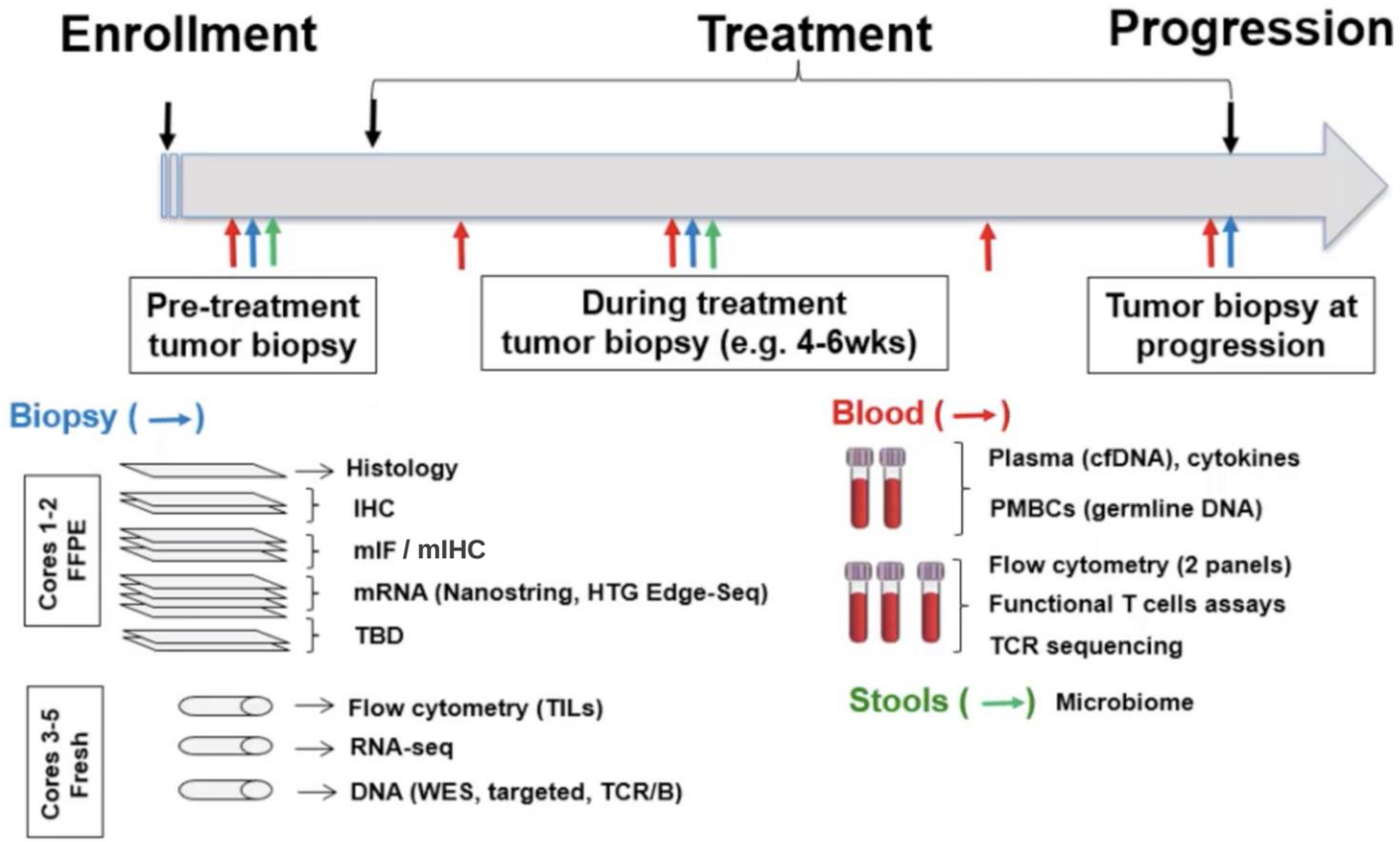
Assay Platforms Working Group

Biobank Working Group

Software Database Working Group

Bioinformatics Biostatistics Working Group

Biomarker discovery strategy



B. Sanchez –Espiridion et al, MD Anderson Cancer Center CIMAC

Assays and platforms for CIMACs

Tier 1 assays
(recommended for most trials)

- **Whole Exome Sequencing (WES)**
- **RNA-seq**
- **IHC singleplex**
  - **PD-L1 FDA approved assay**
- **IHC/IF multiplex**
- **CyTOF**
- **Olink**
- **Nanostring IO panel**

Tier 2 assays
(trial-dependent)

- Microbiome
- TCR repertoire
- Single-cell-seq
- ctDNA-seq
- MIBI
- ATAC-seq
- scRNA-TCR

Other Assays:

ELISA, B cell response, T cell functional assays (ELISPOT, multimers)

A prospective CIMAC Umbrella Protocol for collections and processing of specimens has been developed.

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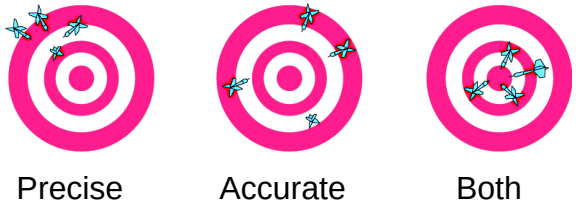
Slide Courtesy of Holden Maecker, PhD, Stanford University CIMAC

CIMAC-CIDC Immuno-Oncology Biomarker Network

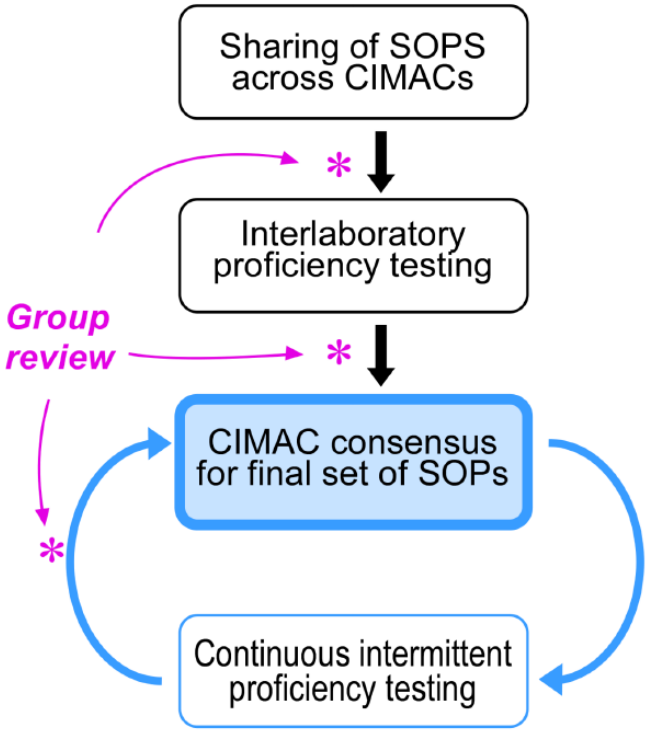
Analytical validation procedure and data

- accuracy
- precision
- analytical sensitivity
- analytical specificity including interfering substances
- reference intervals (normal values) with controls and calibrators
- standardization, harmonization, reproducibility and ruggedness
- establishment of appropriate quality control and improvement procedures
- any other performance characteristics required for assay performance



Sharing and comparing SOPs
Specimen collection umbrella protocols with QC parameters
Harmonizing instrument, assay, and analytical procedures
Proficiency panels – sending around samples for testing and analysis
Agreeing on interpretation even when platforms differ (multiplex IHC…)

Harmonization and proficiency panels



Courtesy: DFCI Tissue Biomarker Laboratory

MDACC: Michael Tetzlaff, Edwin Parra, Jack Lee
DFCI: Evisa Gjini, Ana Lako, Donna Neuberg, Scott Rodig
Mt Sinai: Guray Akturk, Sacha Gnjatic

Phase 0: Harmonization of algorithms and of scanning for singlet IHC

- DFCI shared pre-stained H&E slides from human tissues, scanned centrally and redistributed for local scanning: Normal tonsil (n=3), Melanoma (n=3), Colorectal adenocarcinoma (n=3), Head and Neck Squamous cell carcinoma (n=3), with KI67, CD68, CD3.

Phase I: Harmonization of algorithms for scoring mIF

- Two samples of head and neck SCC (HN-SCC) stained at one site (DFCI) and only ROI-Tiffs were scored in a multi-site manner using identically stained and scanned images, with CD3, CD8, PD-1, PD-L1, CytoK

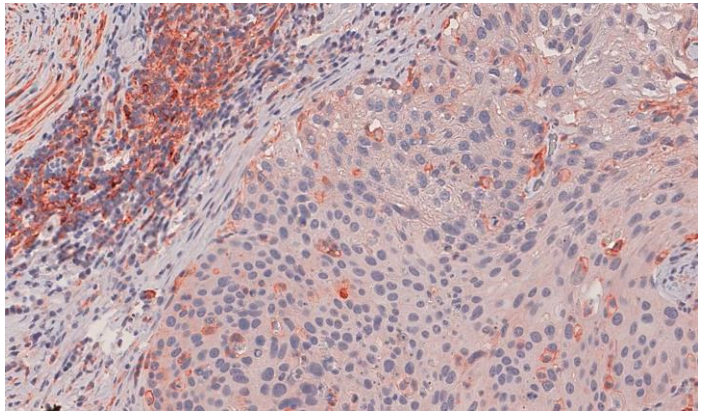
Phase II: Harmonization of staining and algorithms for scoring mIF and mIHC

- Sequential unstained slides from the above 2 HN-SCCs were distributed to all sites
- Staining was performed for CD3, CD8, PD-1, PD-L1, CytoK at all sites, with site-specific protocols, site-specific platforms and site-specific antibody clones
- Image scoring was performed on ROI-Tiffs generated in each of the sites

Phase III: Expanded Harmonization of Staining and Algorithms for scoring mIF and mIHC

- Same as above with unstained serial slides from TMAs (n=27 tissues)

Challenge: harmonizing different methods of staining and quantification

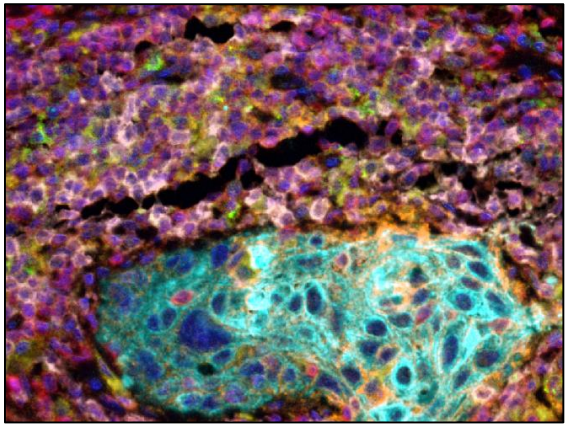


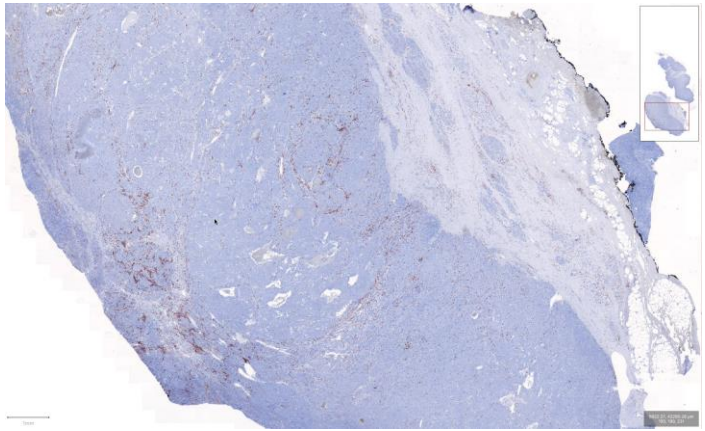
PD-L1
CD8
CD3
PanCK
Fibronectin
CD68
FAP
DC-LAMP
CD11b

Mount Sinai

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

DANA-FARBER CANCER INSTITUTE



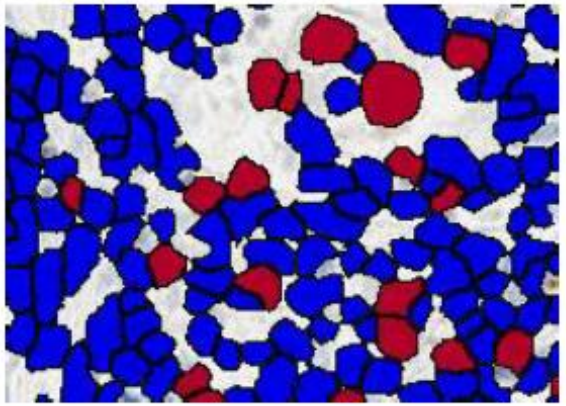


Red: Tumor
Green: Stroma
Orange: Normal Pancreas
Purple: Immune Cells / TLS

QuPath software

KI67

InForm software



Multiplex immunohistochemistry (mIHC) chromogen staining on a single slide (MICSSS)

Multispectral immunofluorescence (mIF) staining with TSA dyes

Challenges: Different antibody clones, platforms, and visualization

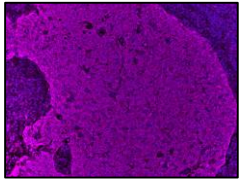
1. Different antibody clones
2. Different IHC/IF protocols
3. Multiplex panels uniquely built at each institution
4. Different platforms for staining, scanning and/or quantification
5. Different geographic regions of the tissue stained

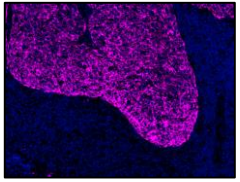
Multiplex IF and multiplex IHC			
	DFCI	MDACC	Mt Sinai
Platform	Leica Bond	Leica Bond	Leica Bond
Scanner	Vectra	Vectra	Hamamatsu
Image analysis	Inform 2.4.2 PE	Inform 2.4.2 PE	QuPath 0.1.3
CD3	A0452 Dako	A0452 Dako	2GV6 Ventana
CD8	C8/144b Dako	C8/144b Thermoscientific	C8/144b Dako
PD-1	EH33 Cell Signaling	EPR48772 Abcam	NAT105 ABCAM
PD-L1	405.9A11 Cell Signaling	E1L3N Cell Signaling	E1L3N Cell Signaling
Keratin	AE1/AE3 Dako	AE1/AE3 Dako	AE1/AE3 Dako

Strategy:

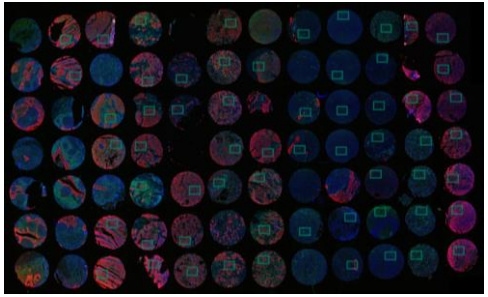
Select same regions of interest (ROIs) from a tissue microarray (TMA) to harmonize

Different geographic regions of the same tumor change the values – even when carefully selecting the ROI.

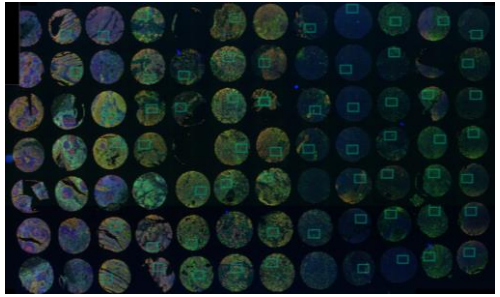




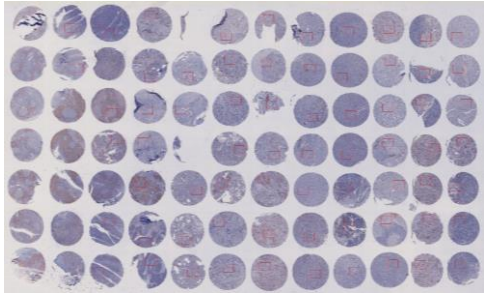
DFCI



MDACC

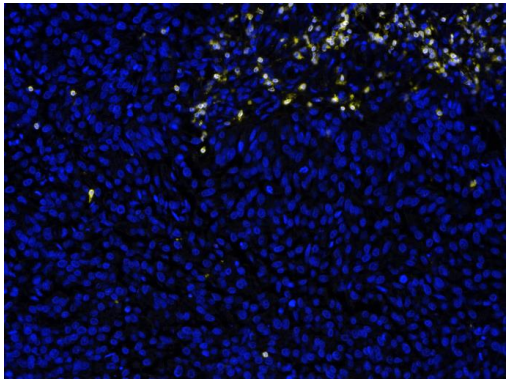
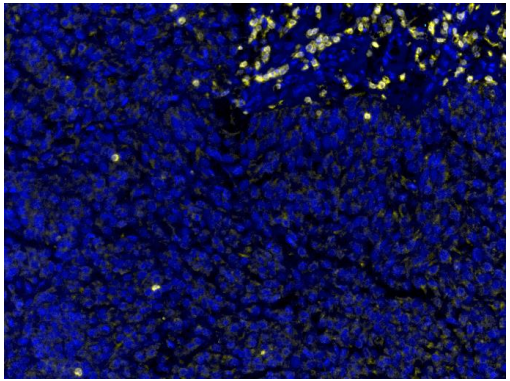
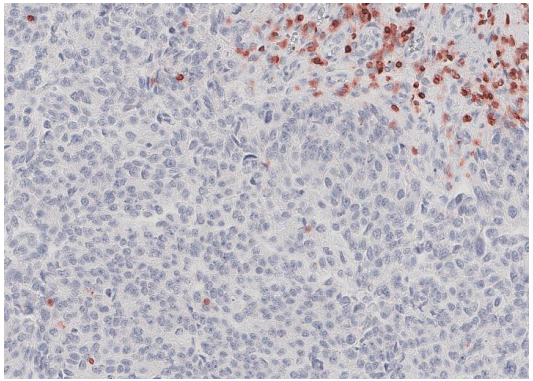
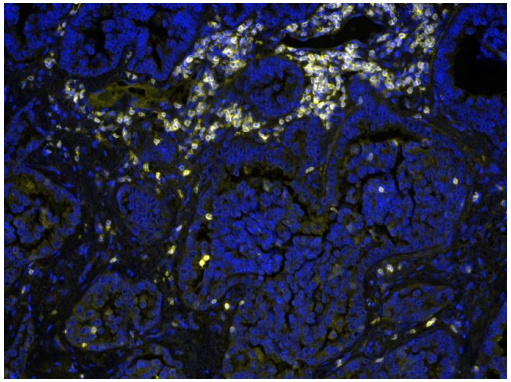
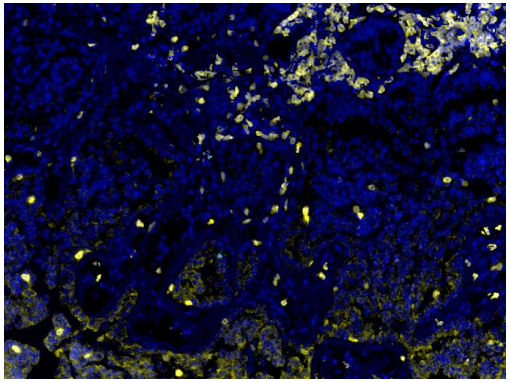
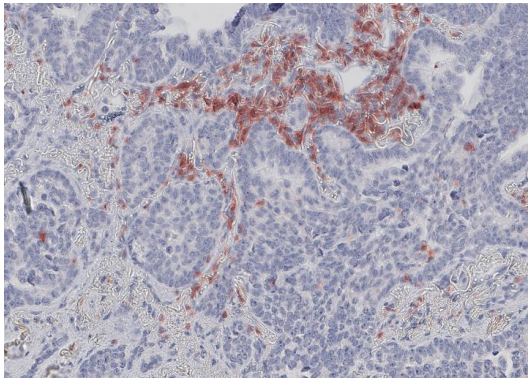


SINAI



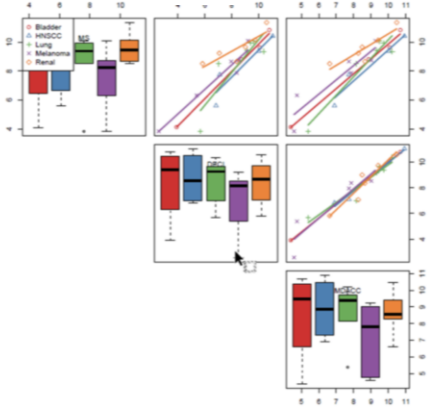
Disease	Patients analyzed
Bladder	4
HNSCC	4
Lung	6
Melanoma	6
Renal	8
Total	28

Comparing CD3 in multiplex IHC/IF

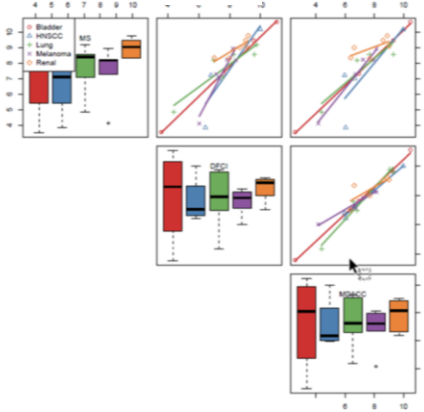
	DFCI	MDACC	SINAI
CD3 staining: Bladder_Patient 3			
CD3 staining: Lung_Patient 1			

Harmonization results from TMA multiplex IHC/IF

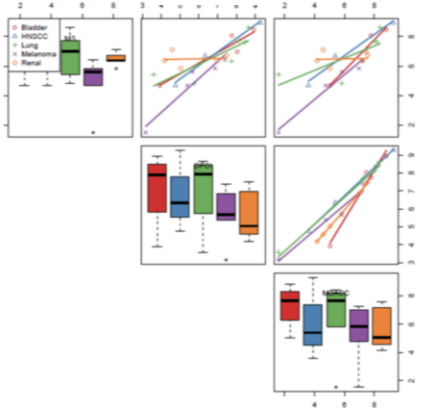
CD3



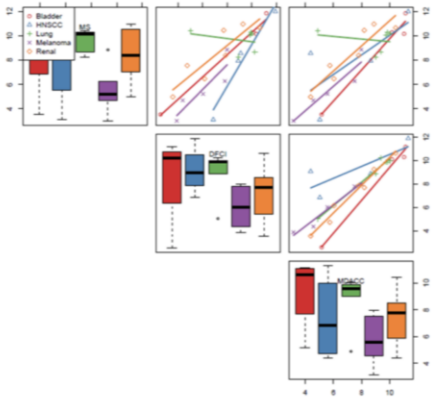
CD8



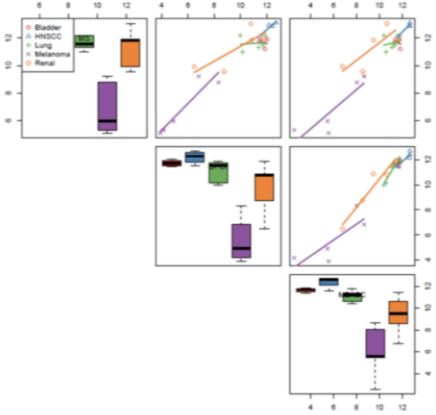
PD-1



PD-L1



Cytokeratin



Legend

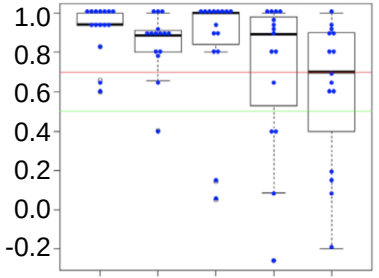
MSSM vs. DFCI

MSSM vs. MDACC

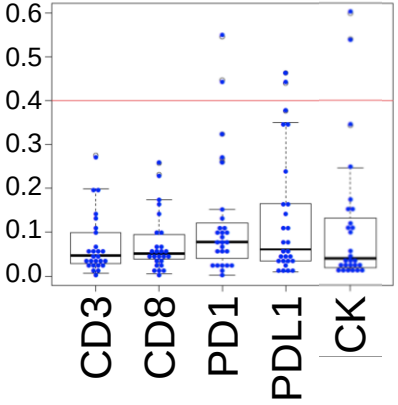
DFCI vs. MDACC

Bladder
HNSCC
Lung
Melanoma
Renal

Spearman r Correlation (>0.7)



Coefficient of Variation (<0.1)



Challenges for combination biomarkers

- Infrastructure required to perform and implement various assays
- Harmonization of multiple assays more difficult to achieve
- Should each assay be considered independently or sequentially?
- Cost of complex assays
- Degree of required personalization – cost/benefit analysis

Deliverables

- SOPs collected for all assays, to be posted on CIMAC website for larger scientific community
- Validation reports generated for most assays by each CIMAC
- Multiple rounds of harmonization and reports – unprecedented concordance
- Umbrella guidelines for specimen collection, to be posted on CIMAC website

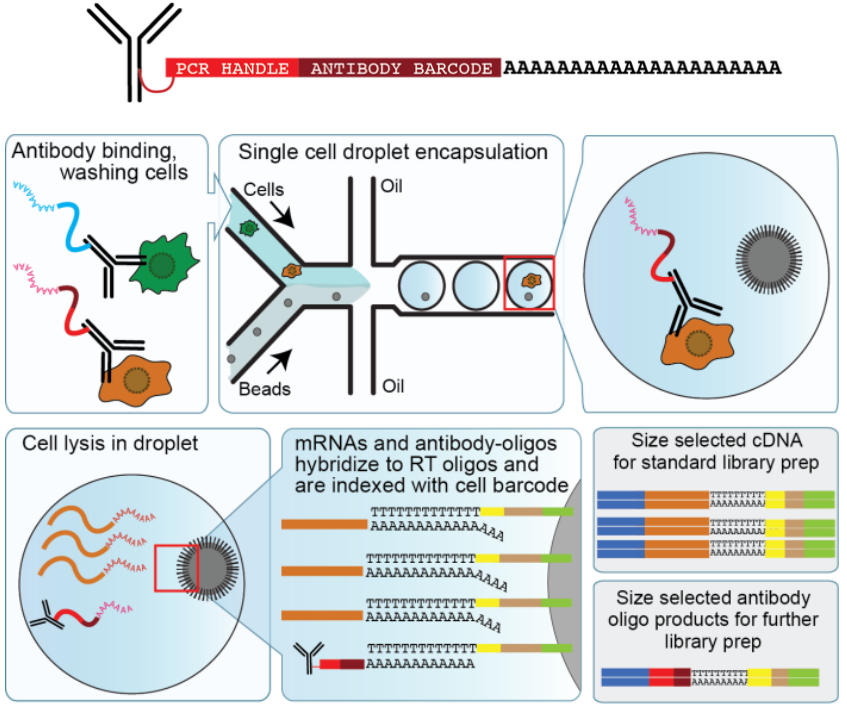
Next: Combing single cell protein and RNA detection

Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-Seq)
Stoeckius et al. *Nature Methods* 14, 865–868 (2017)

Multiplexed quantification of proteins and transcripts in single cells (REAP-Seq)
Peterson et al., *Nat Biotechnol*. (2017)

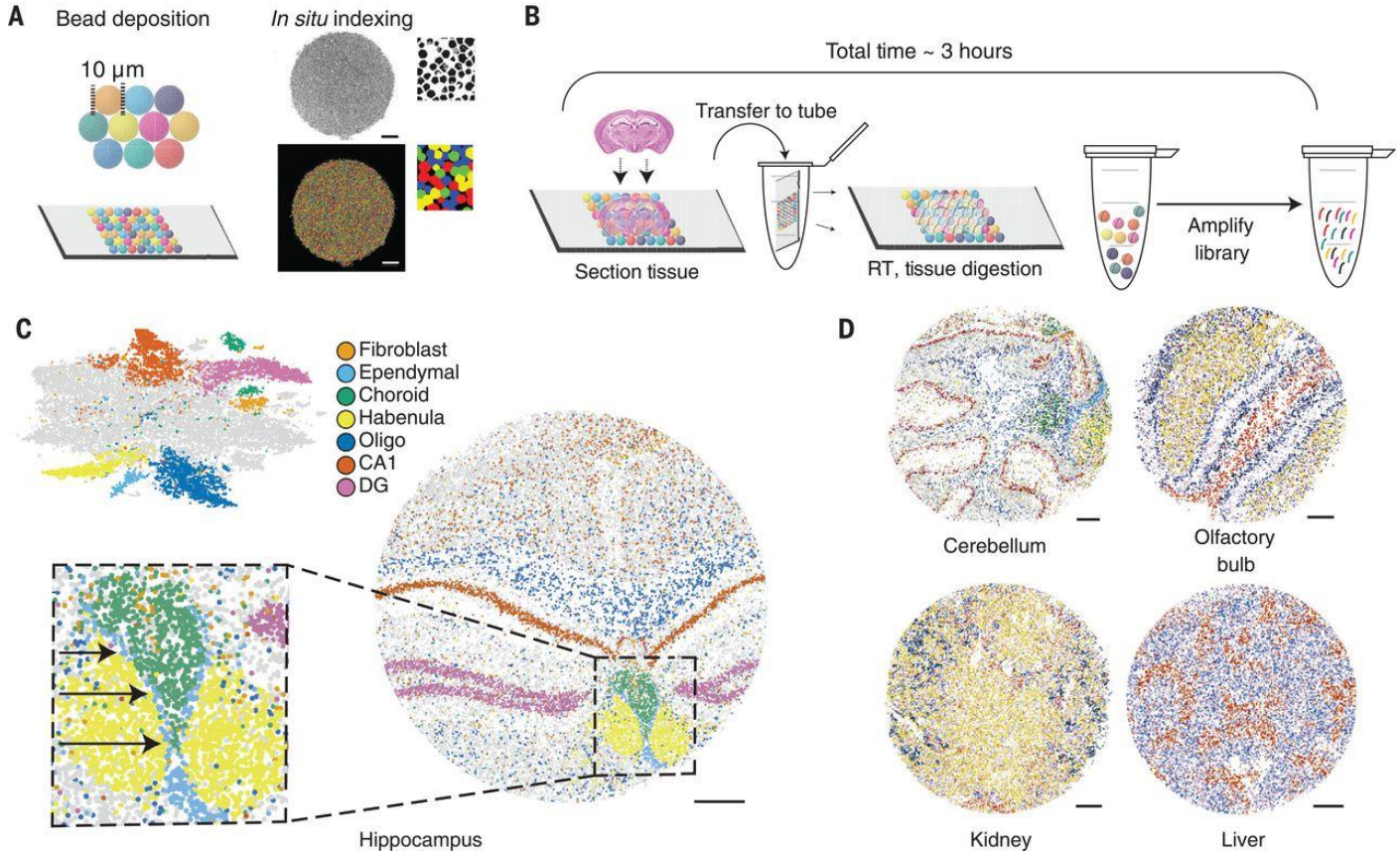
Do you want to study proteins?

…or do you want to study RNA?



Upcoming methods

Slide-seq: near-single cell transcriptomics directly on tissues at 10μm definition



Science 2019:363(6434);1463-1467

Take home message

Importance of validation and harmonization to reliably define baseline characteristics and mechanisms of response or resistance to various immuno-oncology drugs

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

Validation and harmonization efforts should help push biomarker discovery forward

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

Era of personalized combined biomarkers

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