Immune monitoring of cancer immunotherapies: Next generation tools to identify baseline immunocompetence and 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*

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)*

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

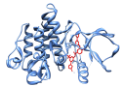
Need: High-dimensional immune monitoring and analysis tools

Challenge to find biomarkers for immunotherapy

Small Molecule Targeted Therapies

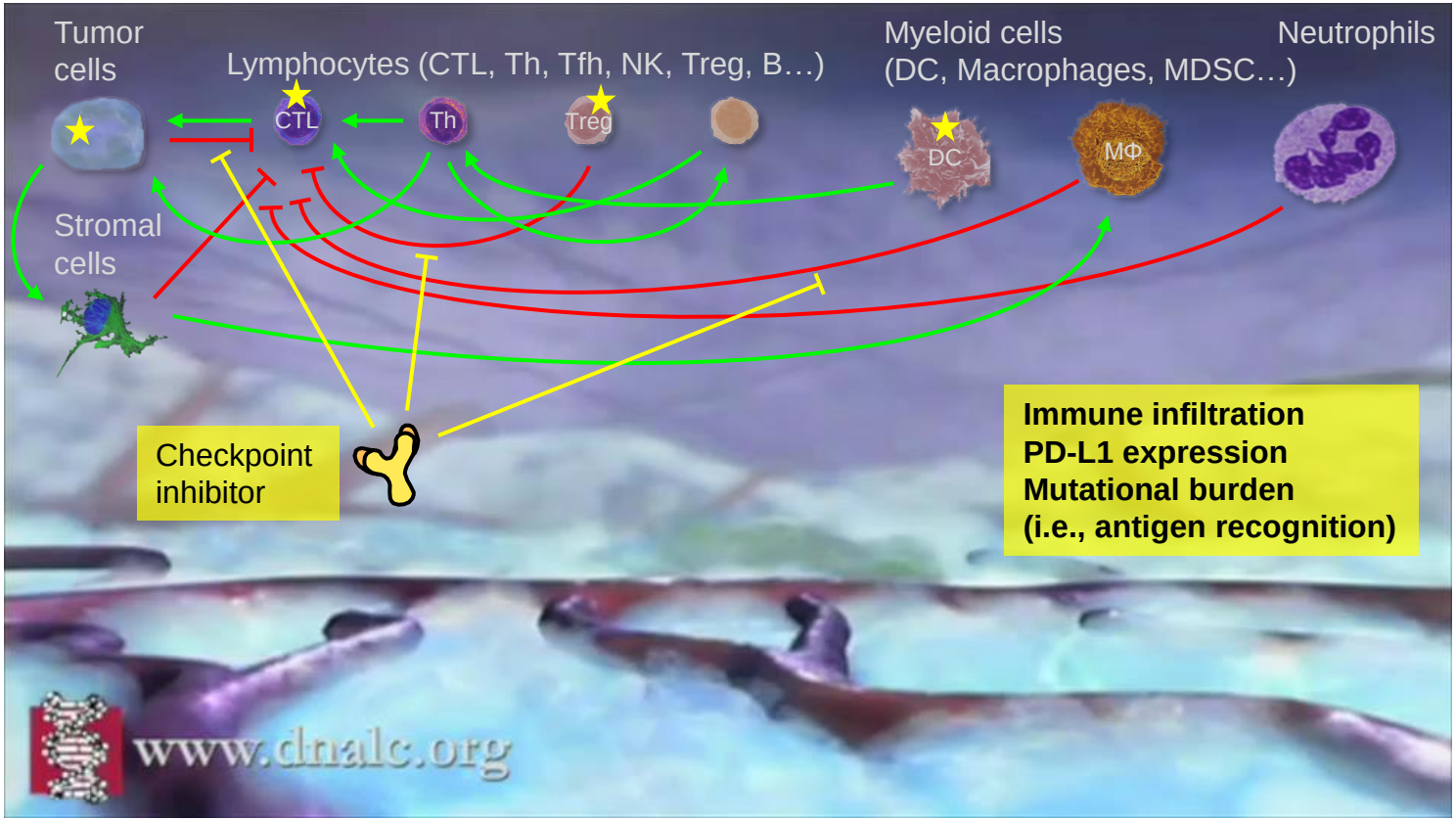
Tumor cells

Mutated Kinase…



Kinase Inhibitor

Immunotherapies

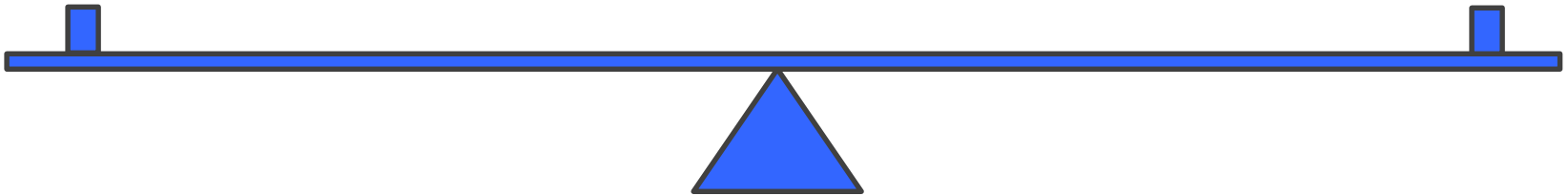


Adapted from Cold Spring Harbor Laboratory's website

The ideal immune monitoring program

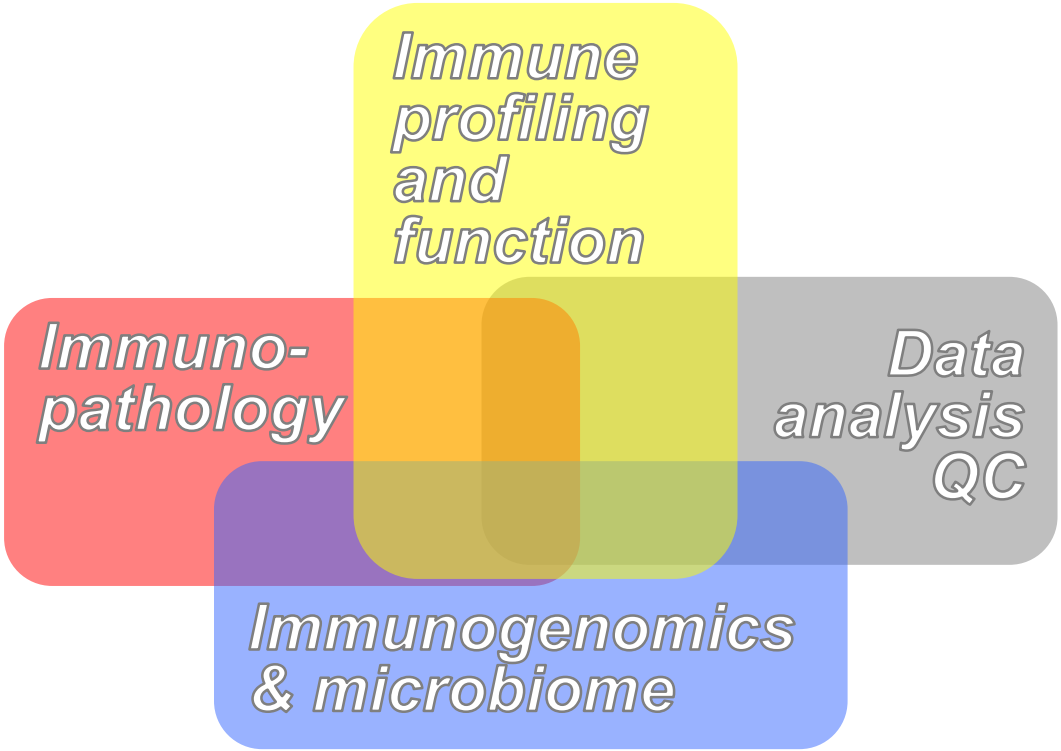
Develop innovative assays to monitor disease-relevant immune signatures and discover new mechanisms, biomarkers and immune targets

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

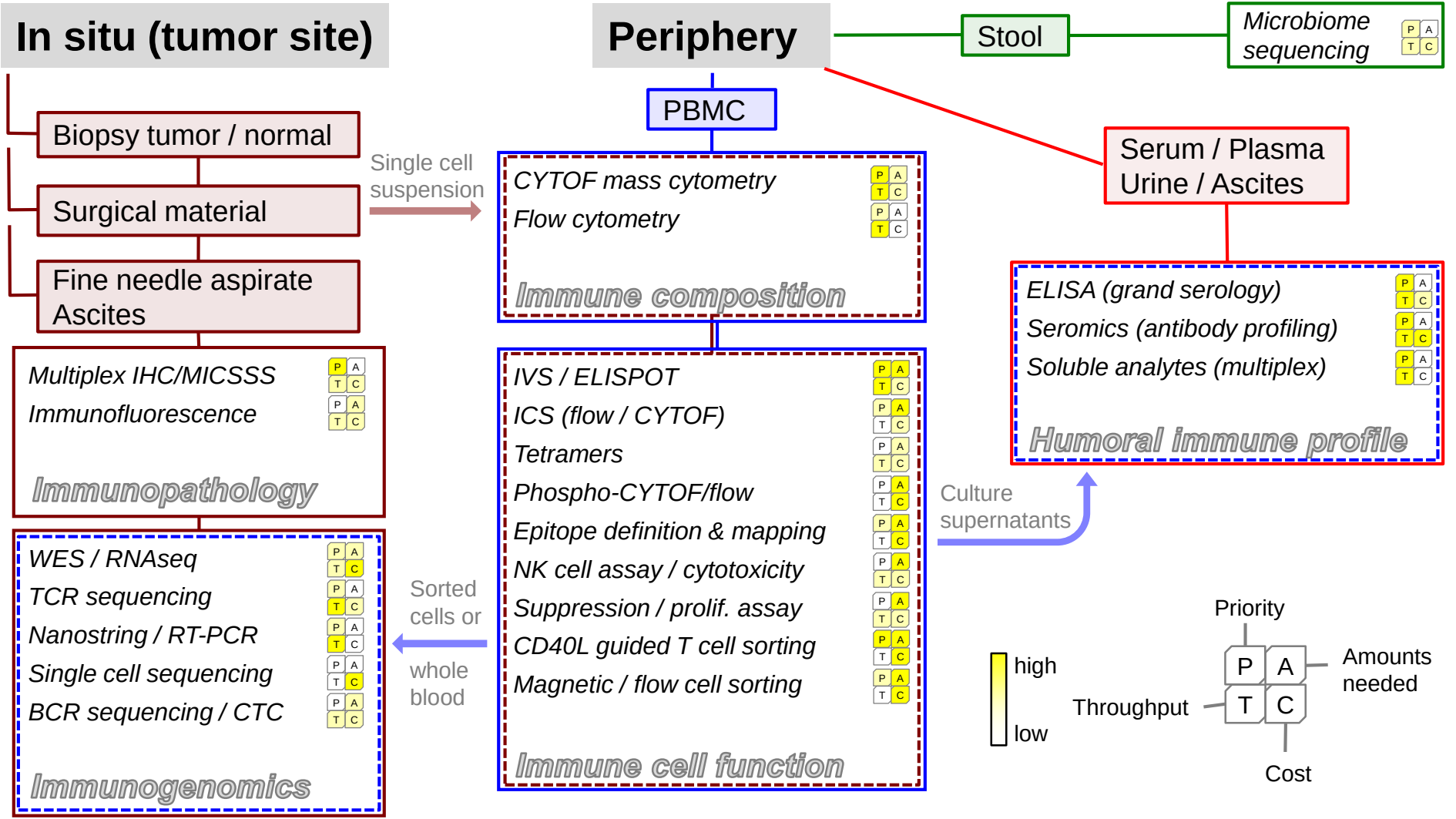


Balancing innovation and standardization.

A multidisciplinary high-dimensional approach



All using analytically validated assays and procedures


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
Priority
high
low
Throughput
P
A
T
C
Amounts needed
Cost

Example of budget prioritization plan for assays

CITN-09

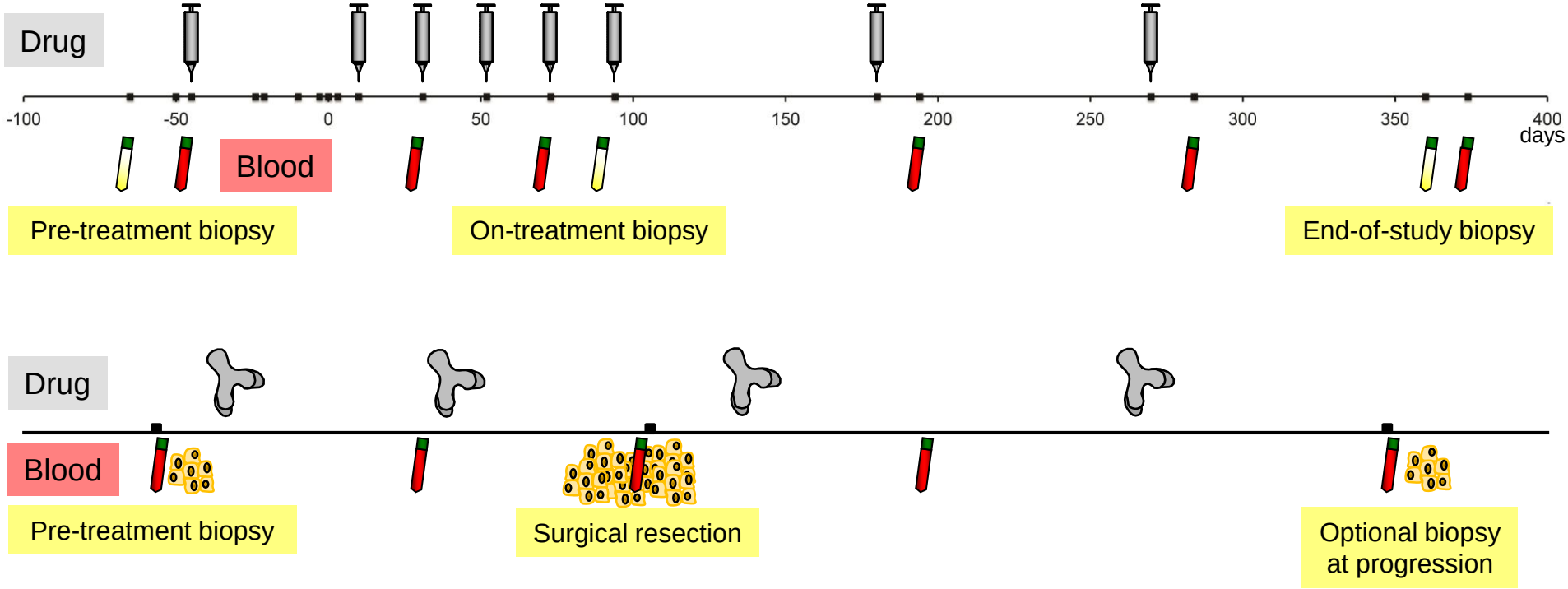
Pembrolizumab in Merkel Cell Carcinoma.

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	$	

Planning ahead for sample collection



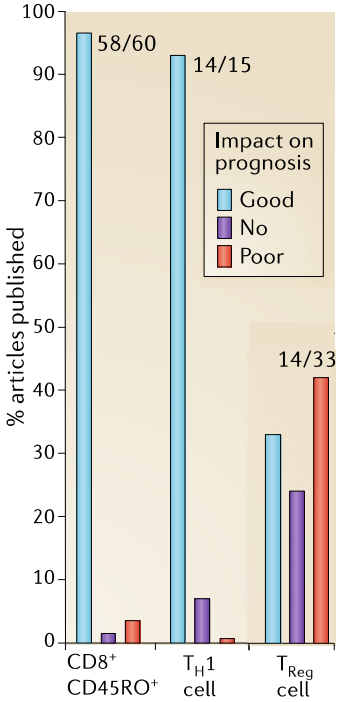
- Blood collected before or after each treatment
- Tumor tissue biopsies or surgical material collected throughout treatment
- Clinical annotations

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

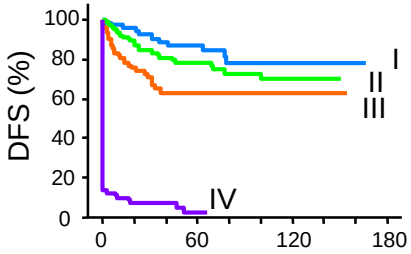
T cell tumor infiltration as a prognostic marker in various tumors and a predictive biomarker of PD-1 response in melanoma

Meta-analysis of 124 articles (20 cancer types)

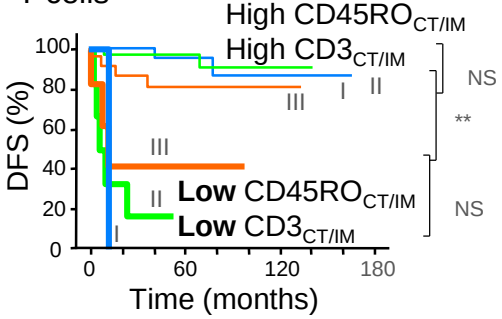


Immunoscore
(colorectal cancer)

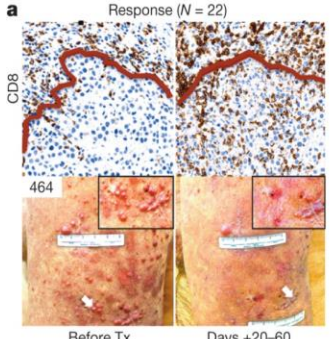
TNM classification



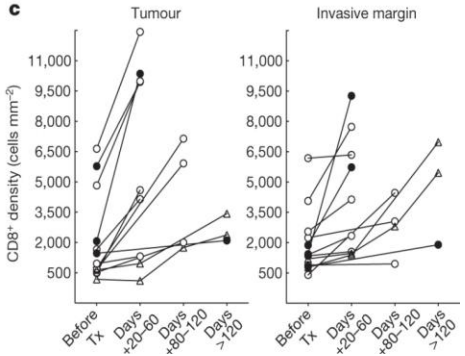
T cells



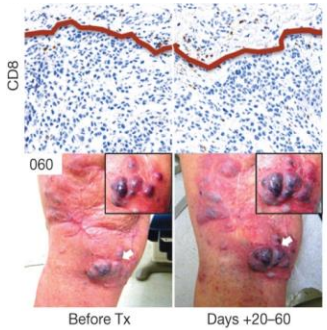
Responders (n=22)



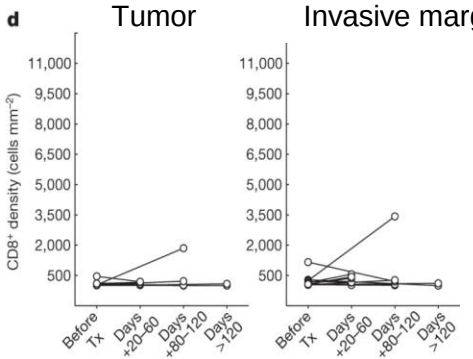
Tumor / Invasive margin



Progressors (n=24)



Tumor / Invasive margin



The immune contexture of the tumor influences prognosis

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

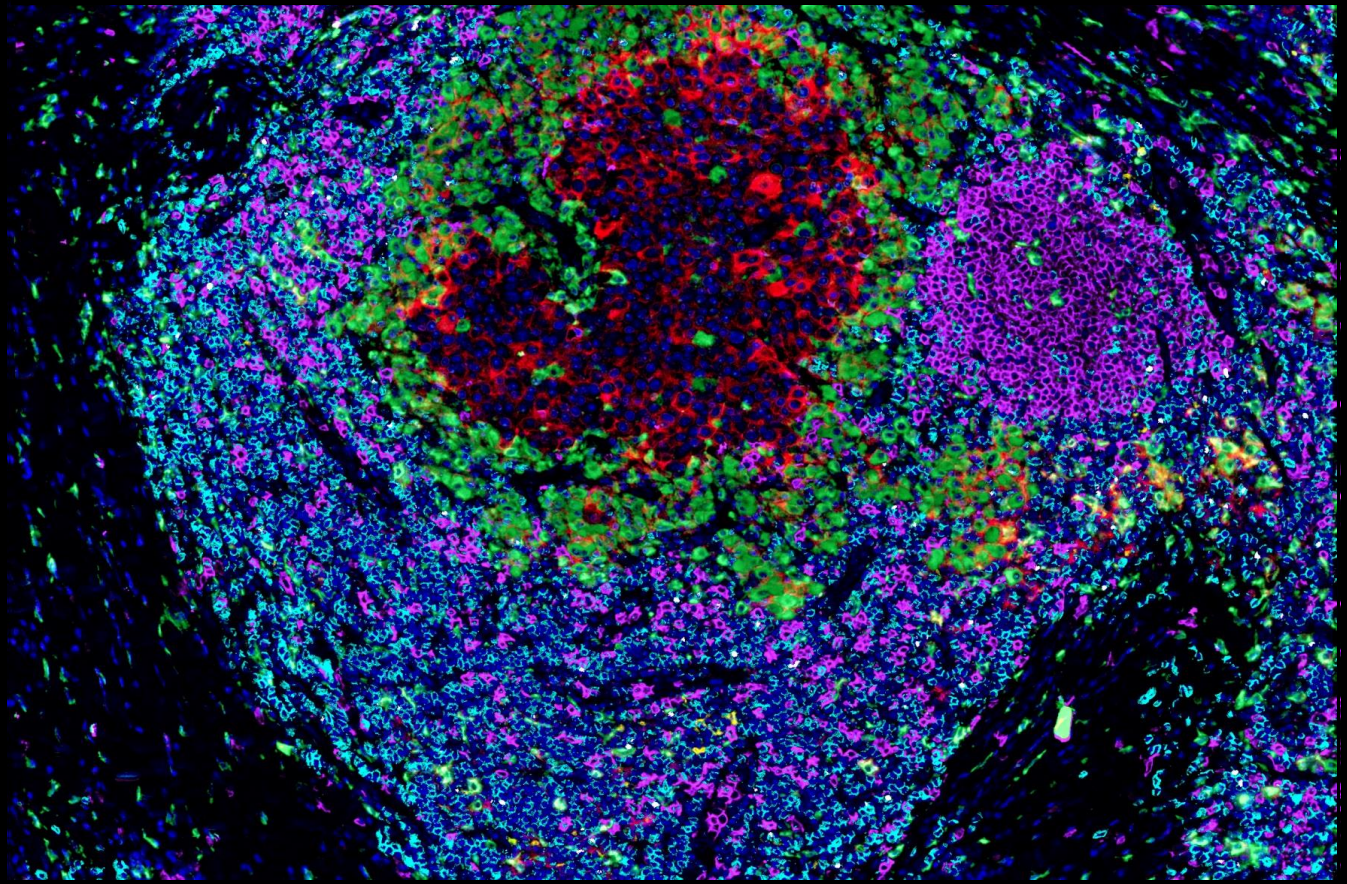
Galon et al., *Science*, 2006;
Pagès et al., *J Clin Oncol*, 2009

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

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

Applying multiplex IHC to query effect of checkpoint blockade

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



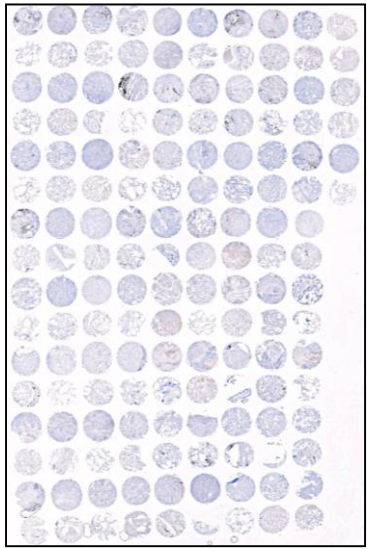
Melanoma lesion biopsied after CTLA-4 treatment (1 FFPE slide) **x100**

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

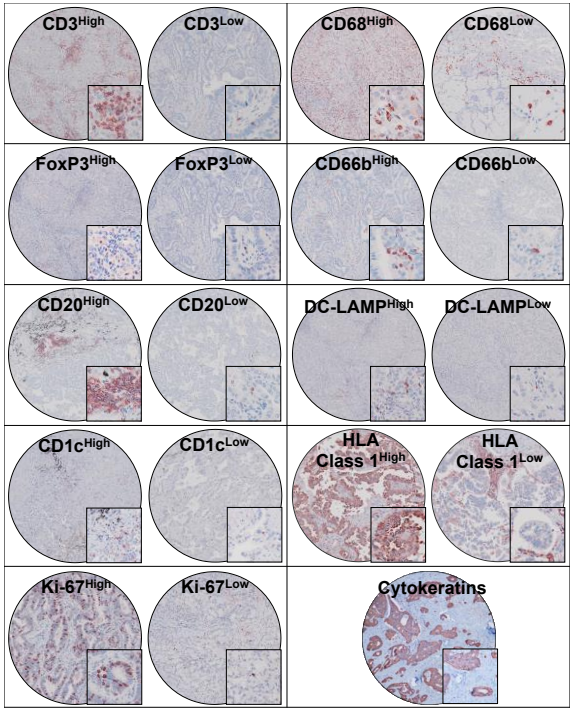
Multiplex IHC on tissue microarrays to identify prognostic biomarkers

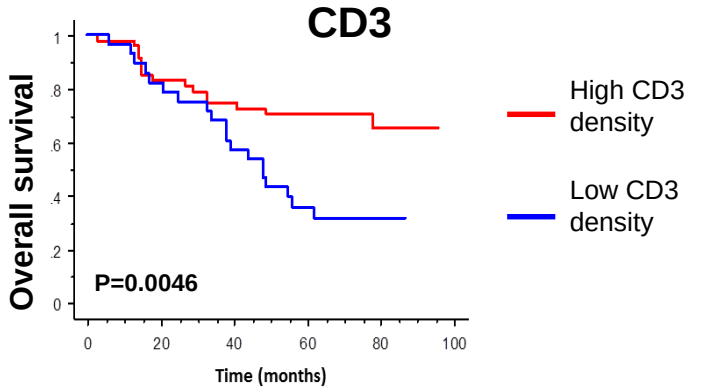
Heterogeneity of immune markers in non-small cell lung cancer (NSCLC)

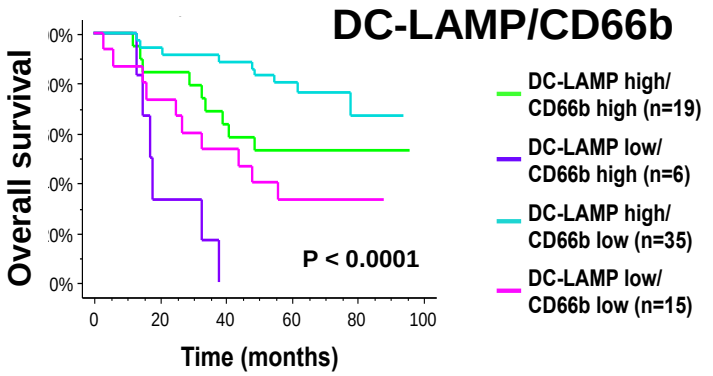
NSCLC tissue microarray



n=75 patients





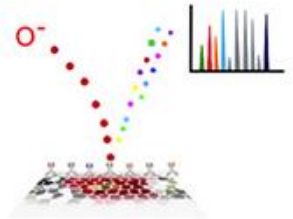


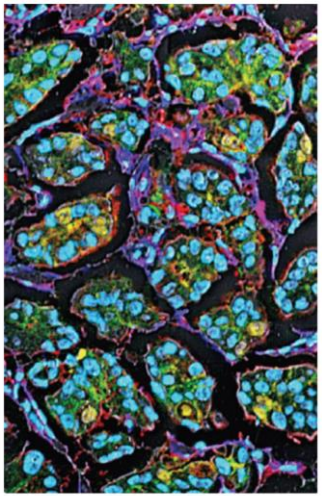
Remark R, Merghoub T, Grabe N, Litjens G, Damotte D, Wolchok JD, Merad M, Gnjatic S. *Science Immunology; 1:aaf6925 (2016).*

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

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

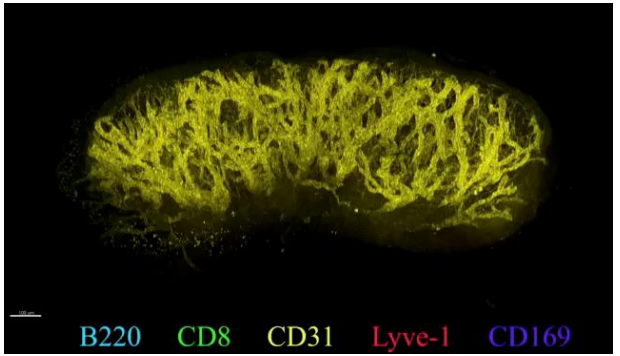
More than 40 markers using metal-conjugated antibodies
And an ion-beam or CYTOF for mass cytometry analysis



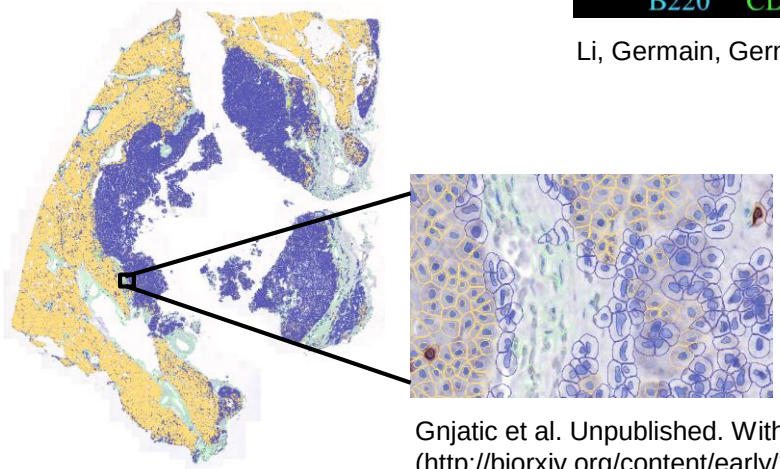


From Stanford.edu

3D imaging of lymph node



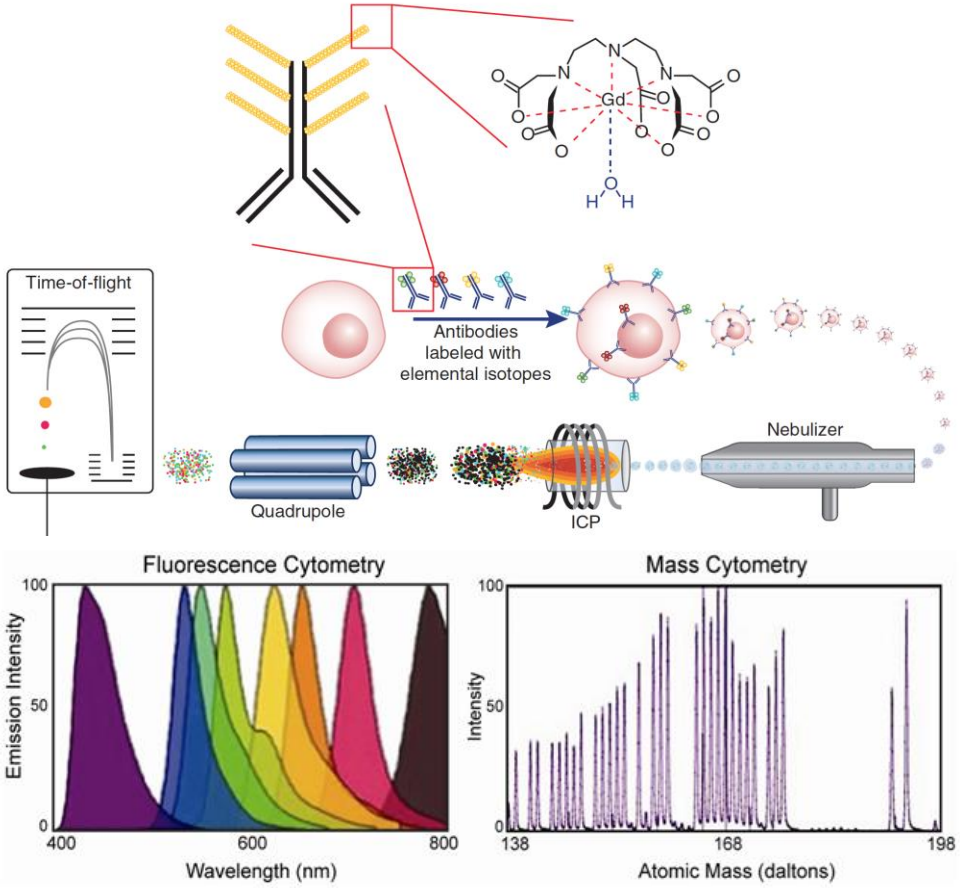
Li, Germain, Gerner. PNAS 2017; 114:E7321



Normal pancreas
Stroma
Neuroendocrine pancreatic tumor
CD3 lymphocytes

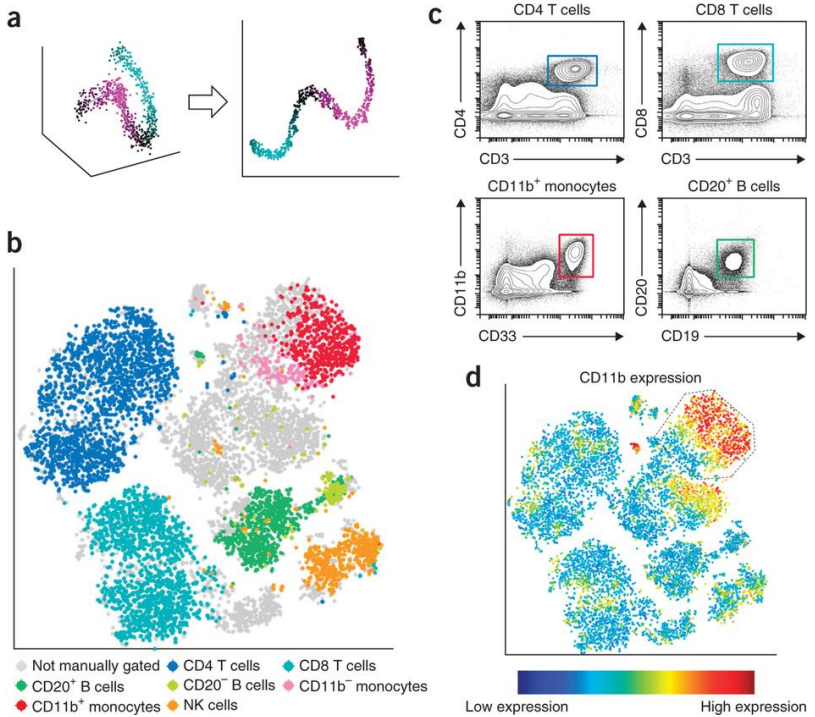
Gnjatic et al. Unpublished. With QuPath software
(http://biorxiv.org/content/early/2017/01/12/099796)

Mass cytometry (CyTOF) to explore phenotypic and functional composition in high dimensions



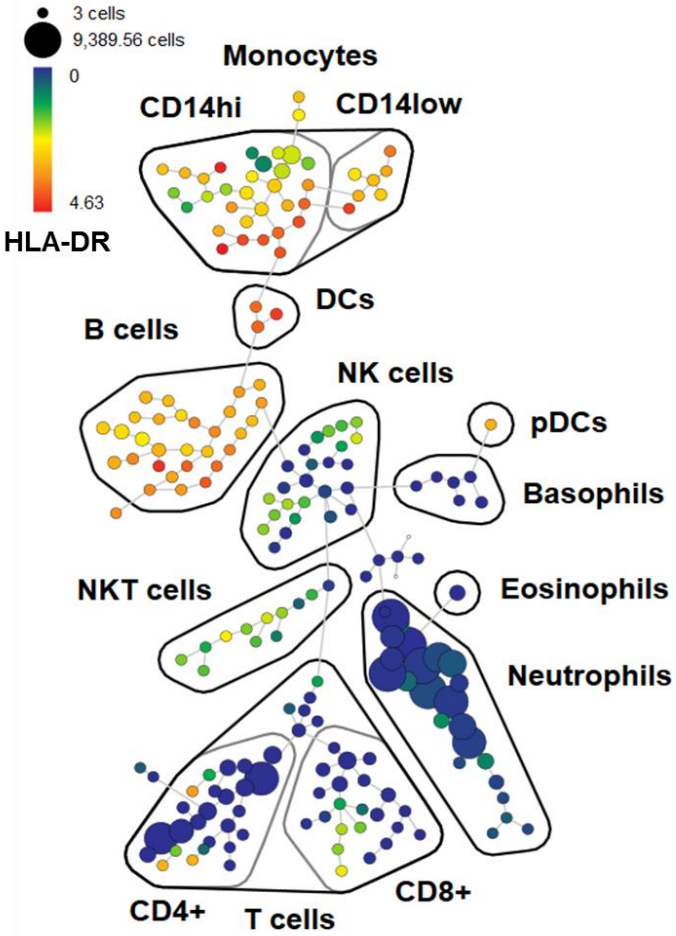
Bendall & Nolan. *Nature Biotechnology* 2012;**30**:639–47

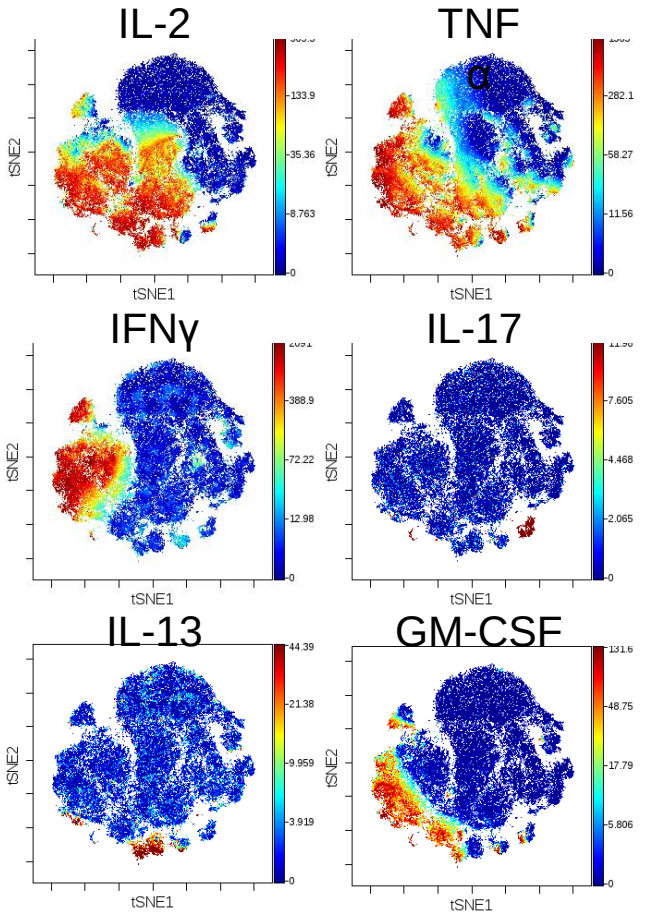
35 markers can be routinely analyzed using dimensionality-reduction algorithm (viSNE, etc.)



Amir El-Ad et al. *Nat Biotechnol*. 2013;**31**:545-52.

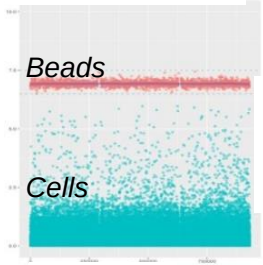
Functional applications of CyTOF mass cytometry



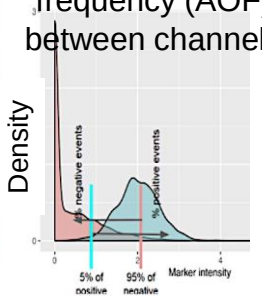


Optimization of CyTOF with beads and lyophilized panels

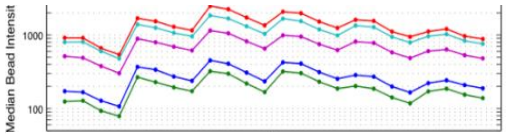
Beads for QC



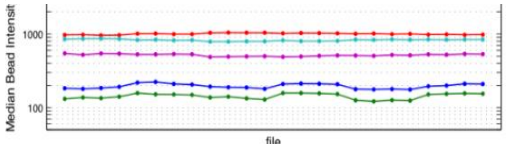
Average overlap frequency (AOF) between channels



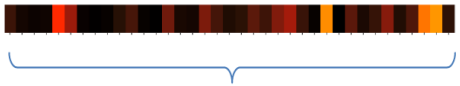
Bead intensity before normalization



Bead intensity after normalization

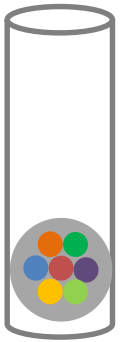


AOF for all antibody channels



Overall staining quality score

Core set of consensus markers to identify major immune cell subsets



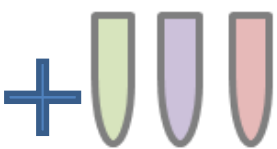
Core Panel	
CD45	CD19
CD3	CD27
CD4	CD56
CD8	CD16
CD45RA	CD14
CD38	CD123
HLADR	CD11c

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

Cross-tissue immune profiling by mass cytometry in NSCLC patients

(Yonit Lavin, Adeeb Rahman, Christian Becker, Sacha Gnjatic, Miriam Merad, Cell 2017;169:750-765)

Resection tissue
Stage I/II adenocarcinoma

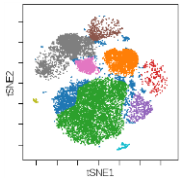
Barcode with tissue-specific CD45 isotopes

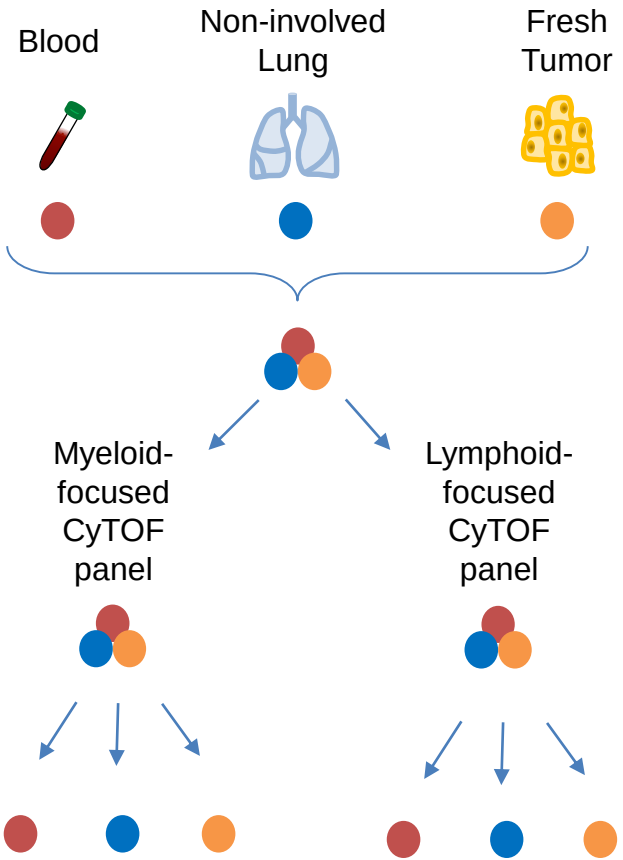
Pool

Stain

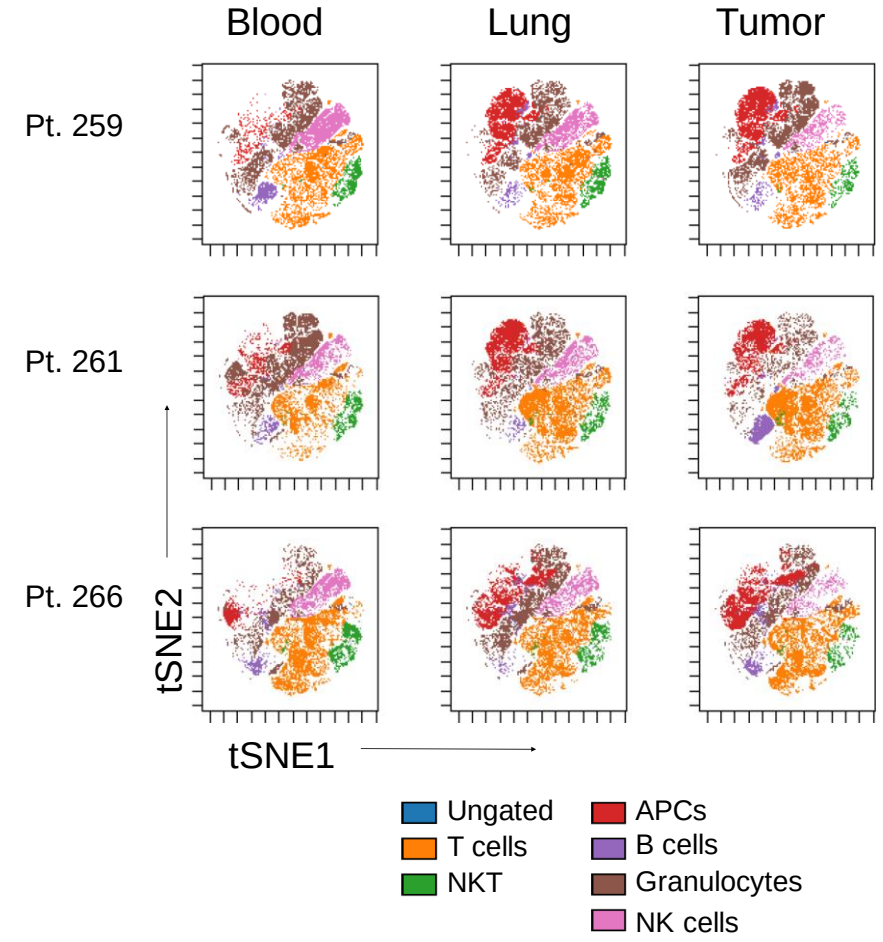
Acquire on mass cytometry

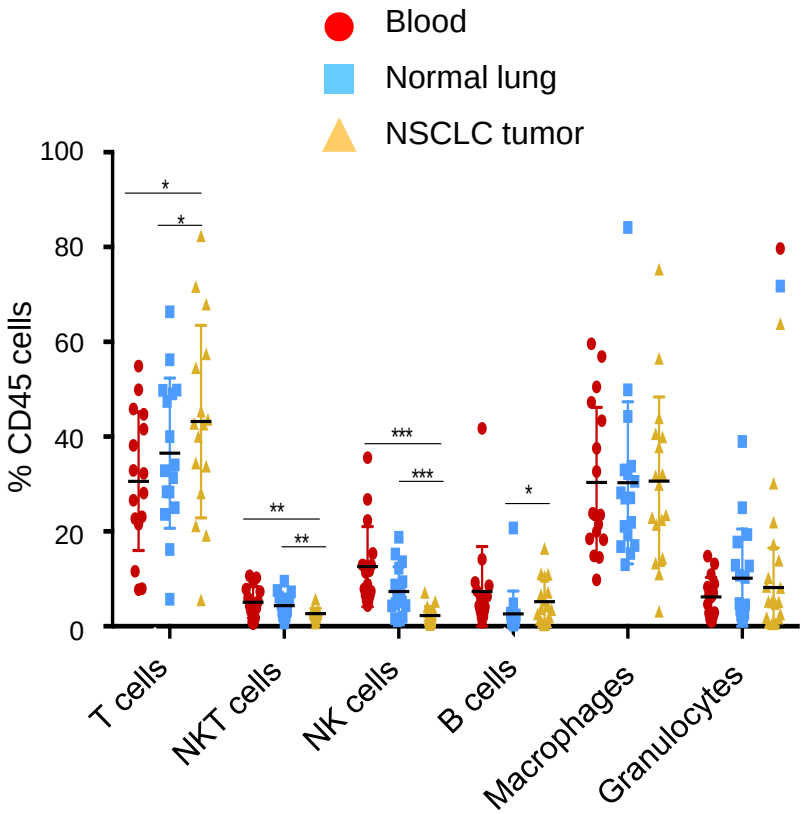
Deconvolute barcodes and analyze





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





Automated analysis pipeline for CyTOF

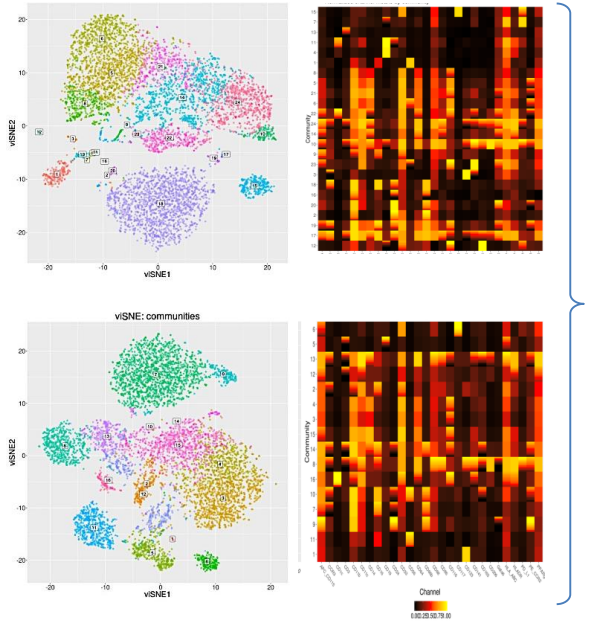
Unbiased identification and characterization of cell populations in individual CyTOF samples

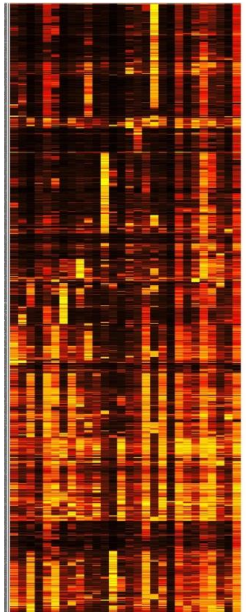
Automated meta-clustering of populations across multiple samples

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

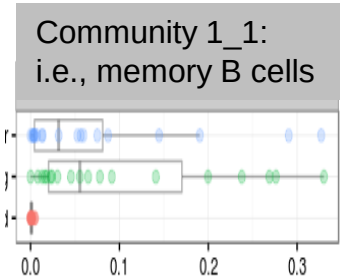
Sample 1

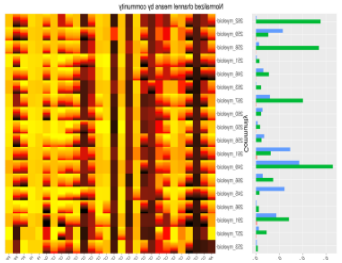
Sample 2



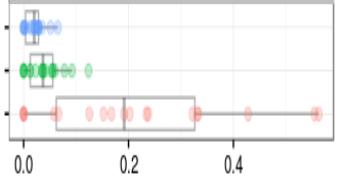


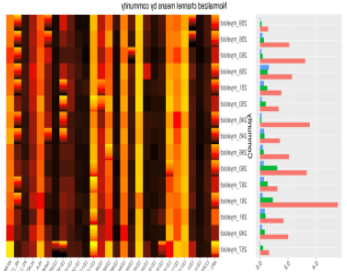
Community 1_1:
i.e., memory B cells





Community 1_4:
i.e., Tregs



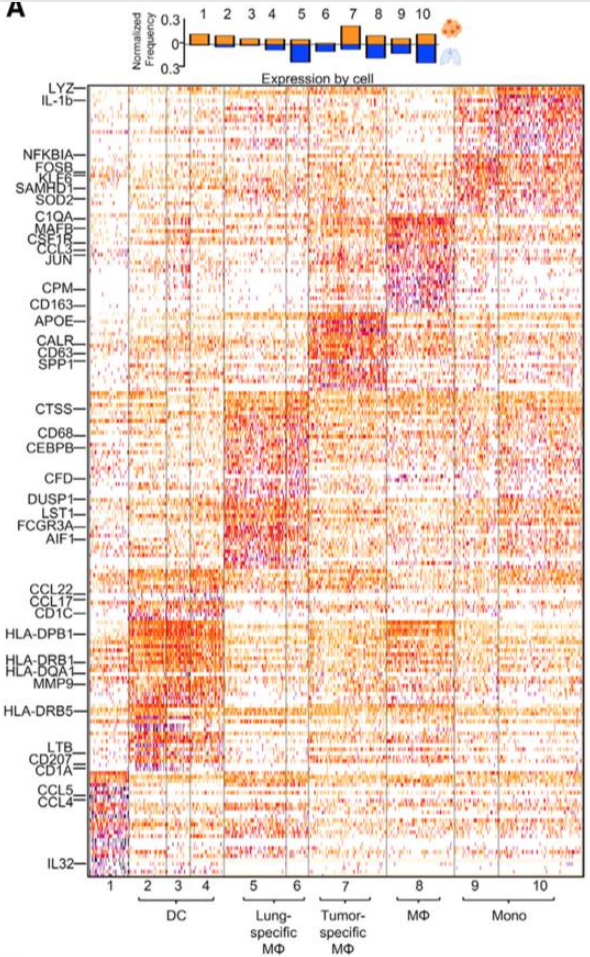


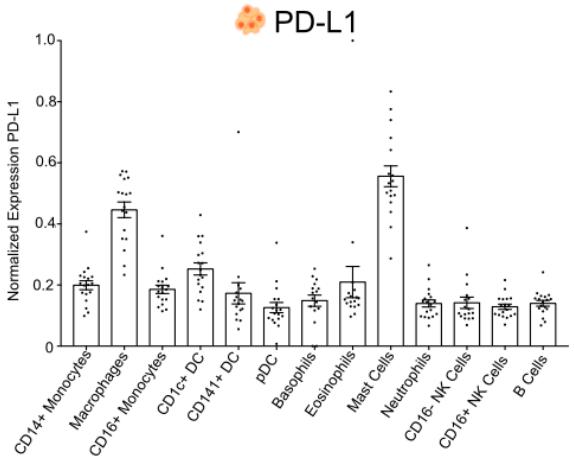
… multiple samples

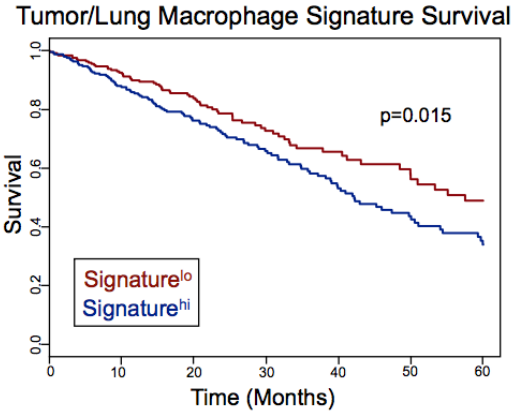
… multiple differing features across samples

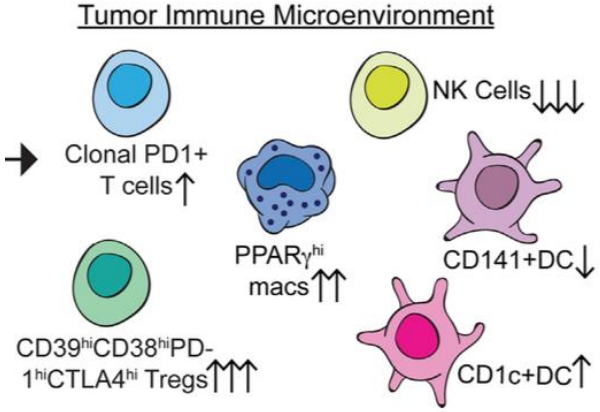
Rahman et al. Unpublished

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





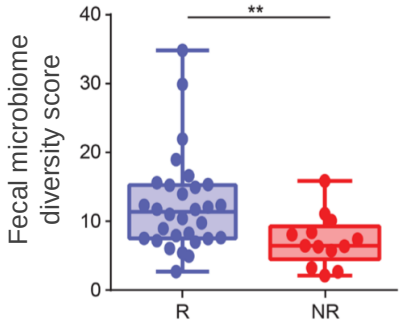


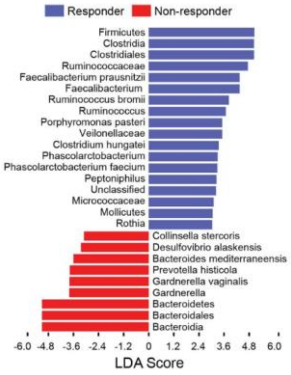


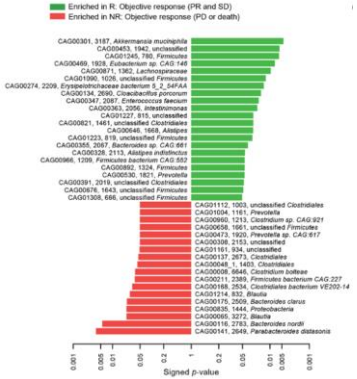
Lavin, Rahman, Gnjatic, Merad, Cell 2017;169:750-765

Role of microbiome as a tumor extrinsic factor contributing to immune recognition during immunotherapy (Jenq+Wargo, Kroemer+Zitvogel)

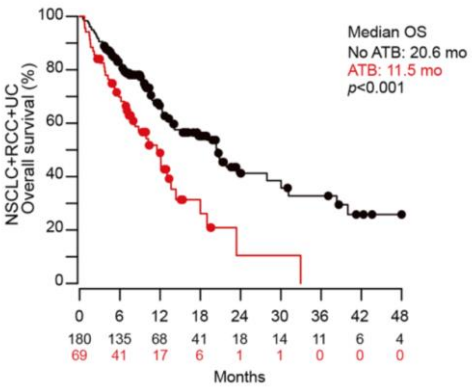
Cancer patients treated with PD-1 blockade, sequenced for gut bacteria by 16S or shotgun metagenomics

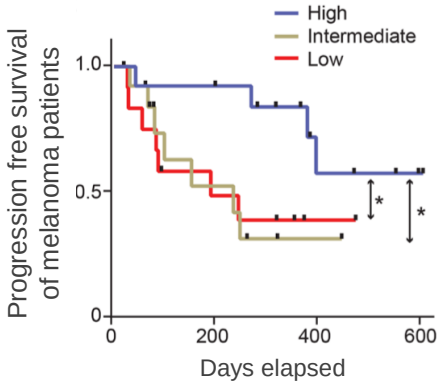


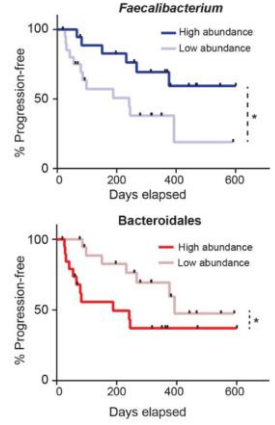


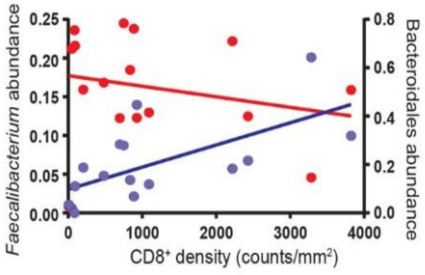


Effect of antibiotics…

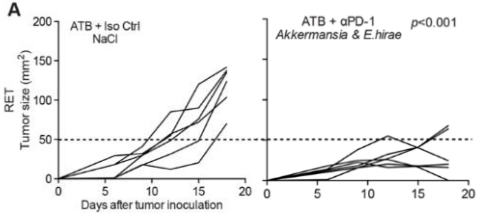








… and hope for fecal transplants

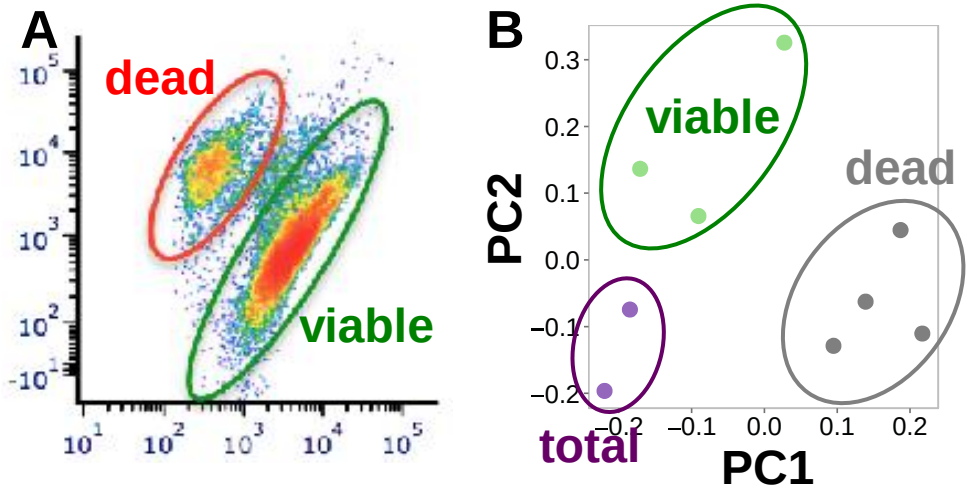


Gopalakrishnan, Spencer, Nezi et al. Science. 2017, in press

Routy al. Science. 2017, in press

Improving microbiome analyses with shotgun metagenomics and live cell detection (Clemente, Faith)

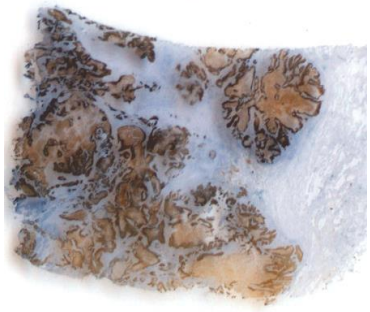
Importance of distinguishing live from dead bacteria for QC aspects



Identifying antigens recognized at the tumor site

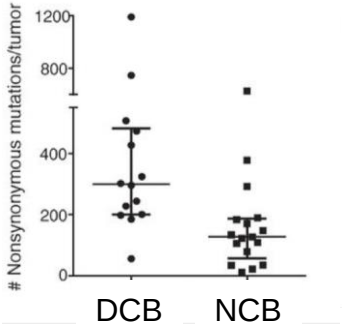
- Whole exome sequencing and RNASeq to identify **tumor-specific mutations** that may give rise to neoepitopes, followed by high-throughput tetramer screening or cytokine production of T cells (Schumacher et al)

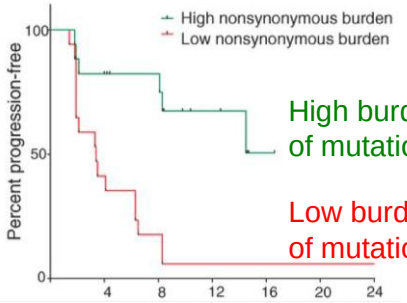
- Immunohistochemistry and RT-PCR to look for presence of known tumor antigens, such as **cancer/testis antigens**

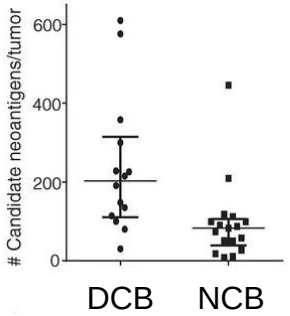


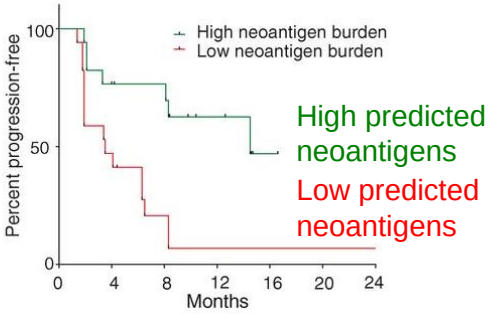
- **Serological assays** to quickly screen for immunogenic target antigens

Mutational burden correlates with response to PD-1 blockade in NSCLC







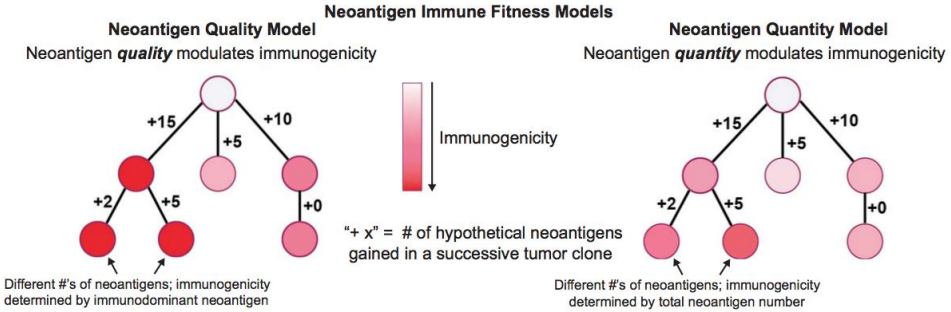


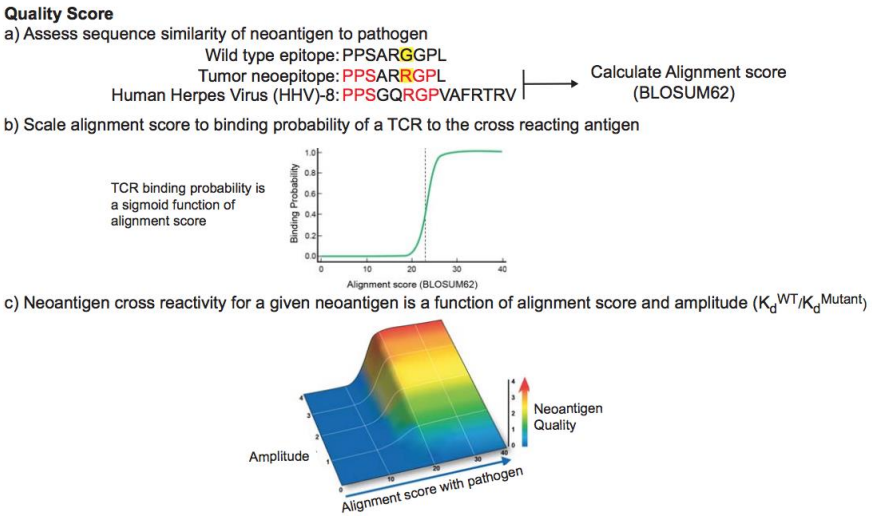
DCB = durable clinical benefit
NDB = no durable clinical benefit

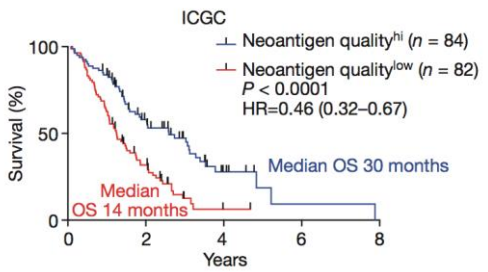
Rizvi et al. Science. 2015. 348-124

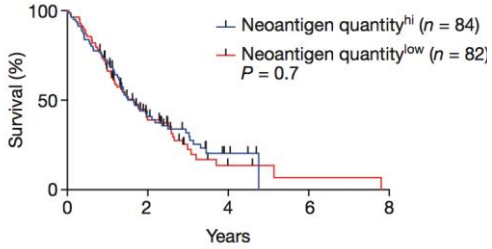
In silico modeling improves prognostic value of neoantigens by assessing their quality

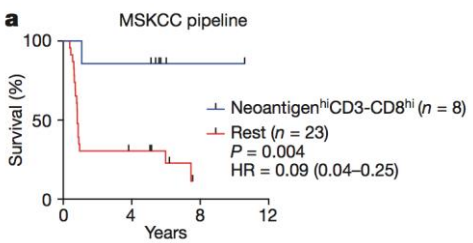
Pancreatic cancer patients, including subset with long-term survival

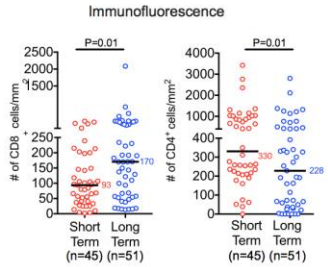


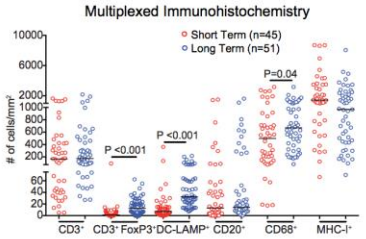


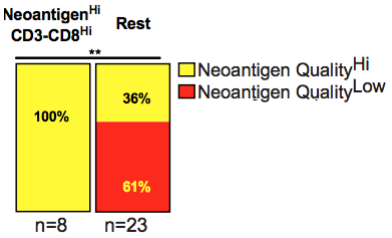






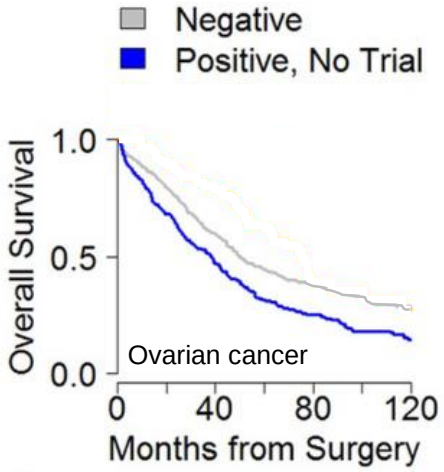




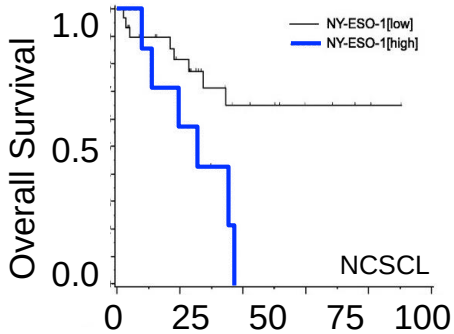


Balachandran V et al. Nature. 2017. In press

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

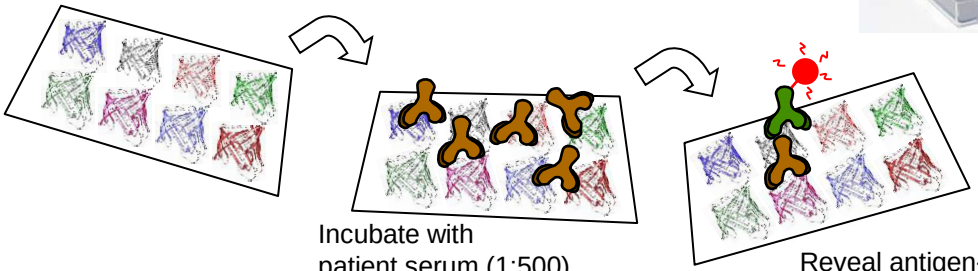
No Clinical Benefit:
POD: Progression of Disease (includes MR)
DOD: Dead of Disease

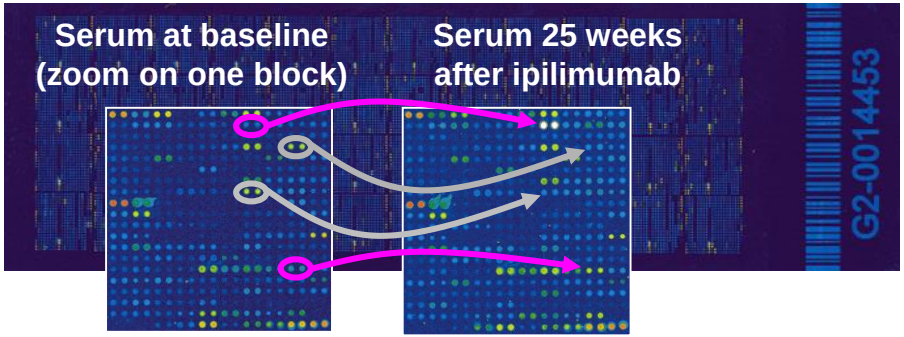
Fisher's exact test (two-tailed):
P value 0.0481
RR = 1 .8 (1.1-2.9)

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

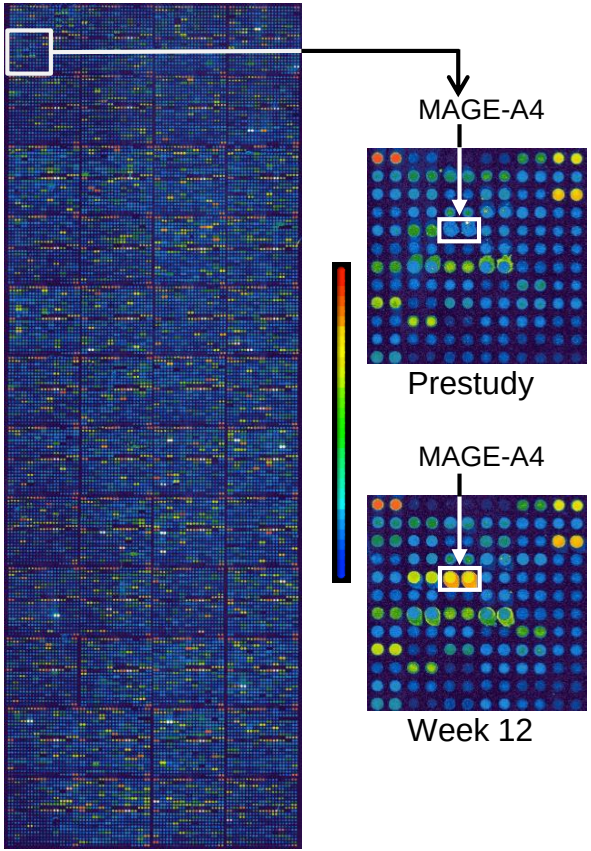
Seromics detects antigen-specific changes in autoantibody profiles during treatment (H. Wada, Osaka JP; H. Shiku, Mie JP; unpublished)

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





for biomarkers of treatment:

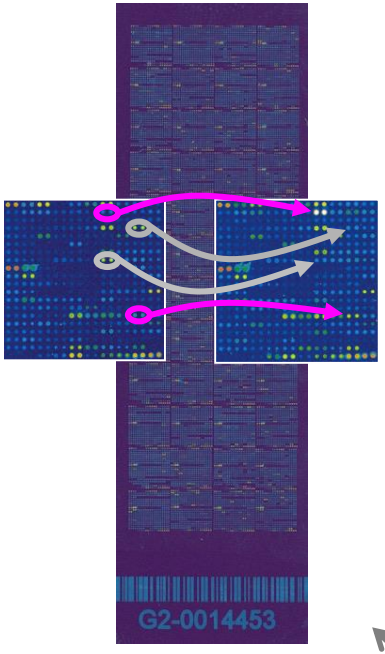


MAGEA4 protein vaccination of esophageal cancer patient

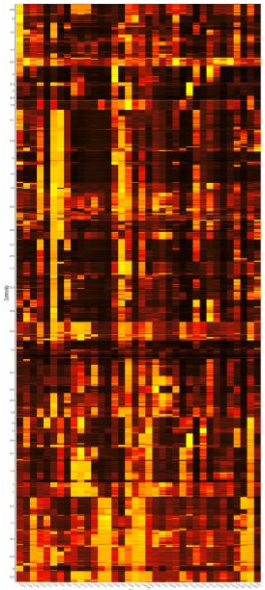
Cross-assay correlations with various data sets

Clinical/demographic patient data

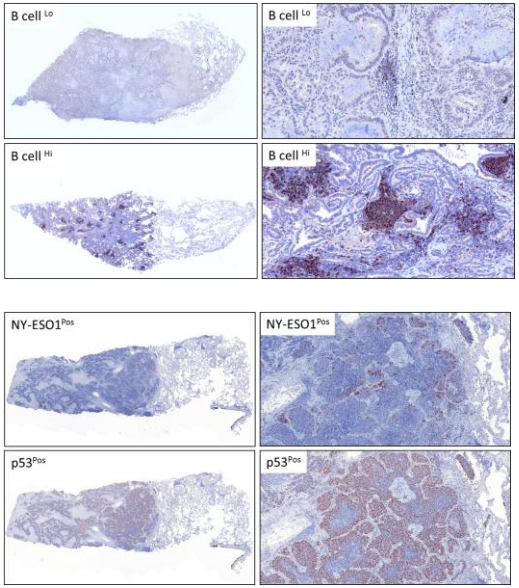
Seromics



Mass cytometry
immune populations

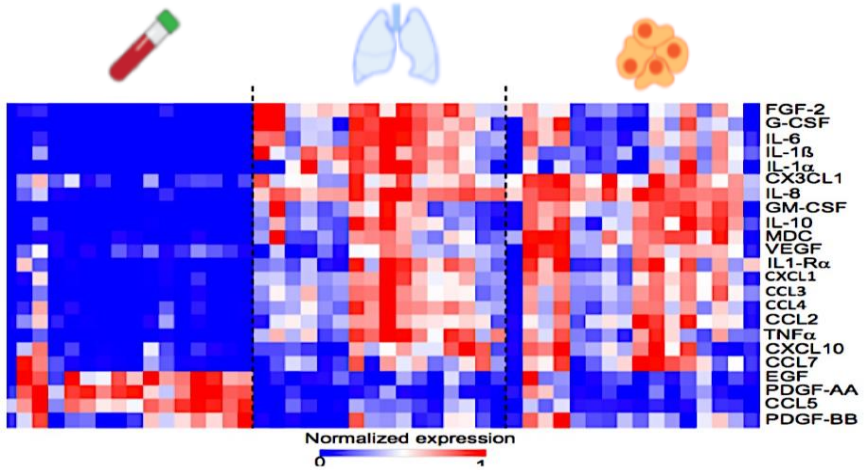


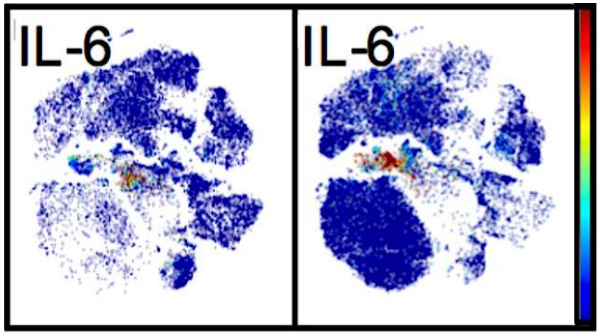
Multiplex IHC

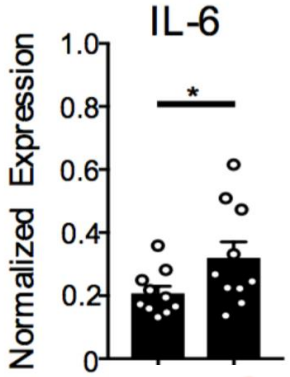


Meta-analyses across studies

Integrating datasets: Luminex, CyTOF, RNAseq







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

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

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