Immune monitoring of cellular and humoral responses in immunotherapy

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Immune Design: *Research Support*
Janssen R&D: *Research Support*
Third Rock Ventures: *Consulting Fees (e.g., advisory boards)*

Basic immunology is transforming cancer clinical care

- Accelerating FDA approval of a series of immune agents for the treatment of cancer, including first combinations
  - Anti-CTLA-4 for advanced melanoma (BMS 03/2011)
  - Anti-PD-1 for advanced melanoma (Merck 09/2014)
  - Anti-PD-1 for advanced melanoma (BMS 12/2014)
  - Bi-specific antibody for ALL (Amgen 12/2014)
  - Anti-PD-1 for NSCLC (BMS 03/2015)
  - Anti-PD-1 for PD-L1^{+} NSCLC (Merck 10/2015) with companion test
  - Anti-PD-1 + Anti-CTLA-4 for BRAF^{WT} melanoma (BMS 10/2015)
  - Anti-CTLA-4 for adjuvant melanoma (BMS 10/2015)
  - T-VEC for melanoma (Amgen 10/2015)

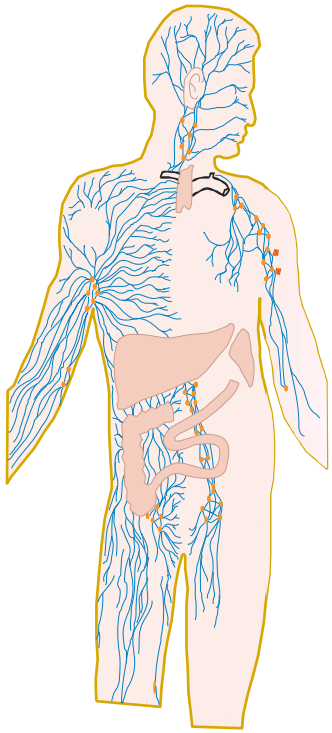
- Novartis invests $20 million in CAR therapy and JUNO therapeutics raises $265 million

- *Mode of action is immunological, prompting intense search for correlative markers to understand and improve efficacy*

Immune monitoring goals

- To provide a comprehensive assessment of the immune status in patients
- To discover immune profiles of disease, leading to new biomarkers of diagnosis, prognosis, and response to therapy
- To quantitate immune responses to help optimize dose, delivery, schedule, and combinations and to identify immunomodulatory effects of novel drugs

Human immuno-monitoring



BIOTHERAPEUTICS

Single cell level

BLOOD AND TISSUES

Comprehensive

CELL COMPOSITION
FLOW CYTOMETRY / MASS CYTOMETRY

SPECIFICITY & ENUMERATION
ANTIGEN-SPECIFIC T CELL ASSAYS
SERUM PROFILING OF ANTIBODIES

FUNCTION & QUALITY
ELISPOT, INTRACELLULAR CYTOKINES, TETRAMERS, SORTING, AVIDITY, POLYFUNCTIONALITY, ISOTYPE

TRANSCRIPTOMICS
OF PURIFIED POPULATIONS
TCR SEQUENCING

PROTEOMICS
MULTIPLEX (antibody or aptamer-based)
SEROMICS and ELISA for autoantibodies
PHOSPHO-CYTOMETRY

TISSUE ANALYSIS & IMAGING
IMMUNOSCORE, CONTEXTURE, MULTIPLEX IMMUNOHISTOCHEMISTRY

DATA ANALYSIS
BIOINFORMATICS, DATA MANAGEMENT, HARMONIZATION, QUALITY CONTROL

Biomarker of disease
Target identification

Case study: neoadjuvant therapy trial design

Hypothetical study of the immunomodulatory effect of a new drug administered prior to surgery followed by checkpoint blockade

At the tumor site

- Fresh biopsies, surgical material
  ➔ *Mass cytometry profiling of tumor composition, cell sorting for expansion or functional characterization*
- Frozen material
  ➔ *Genomic analyses of microenvironment, immunofluorescence*
- Paraffin-embedded blocks
  ➔ *Multiplex immunohistochemistry for immunoscore-type analyses, TCR sequencing*

In the periphery

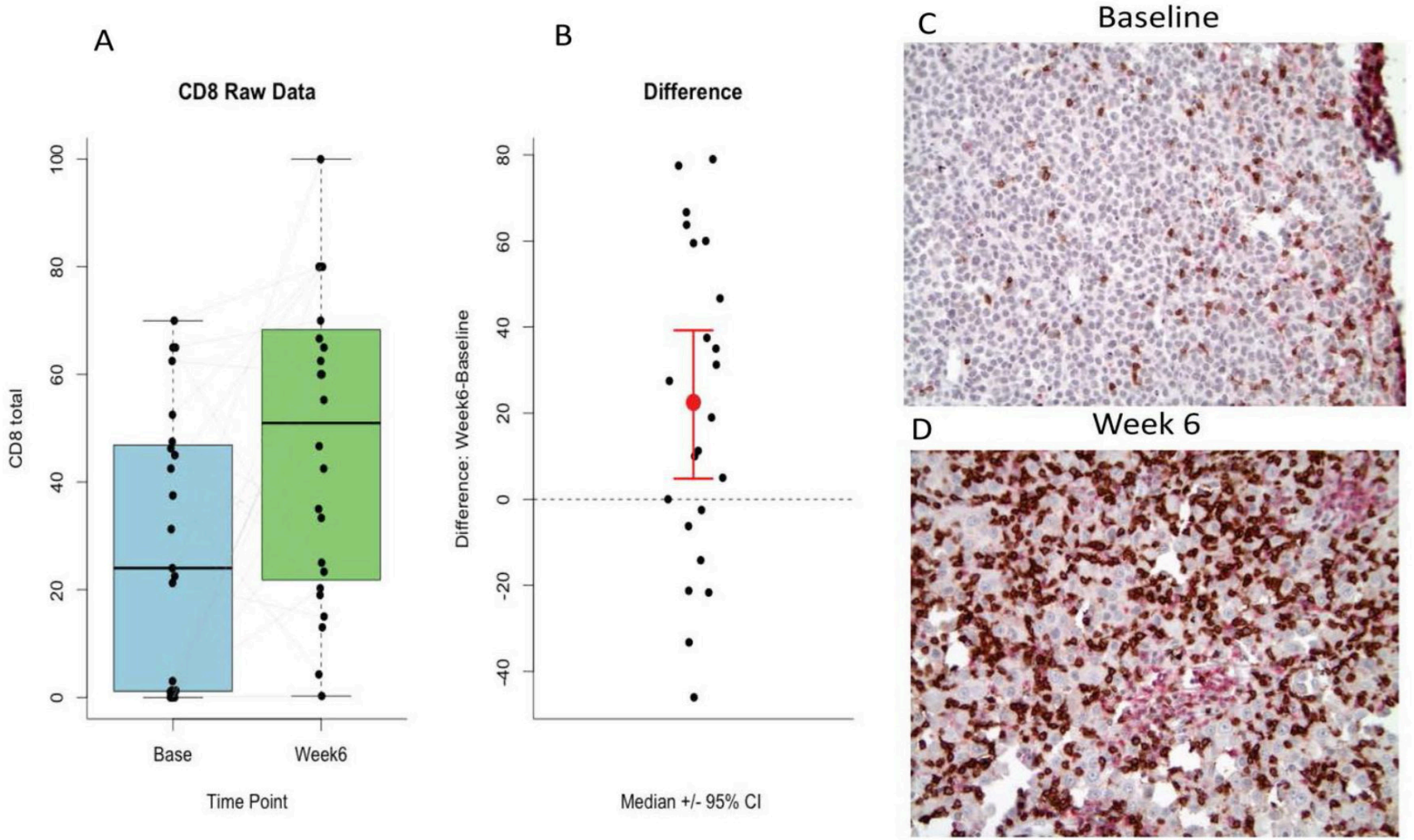
- Serially collected serum or plasma
  ➔ *Antibody profiling*
- Serially collected peripheral blood mononuclear cells (PBMC)
  ➔ *Mass cytometry profiling of phenotypic and functional changes, antigen-specific T cell characterization, TCR sequencing*

Scope

In situ immune monitoring

Immunotherapy can make tumors “hot”

Measuring T cell infiltration by immunohistochemistry
after neoadjuvant ipilimumab treatment in melanoma patients

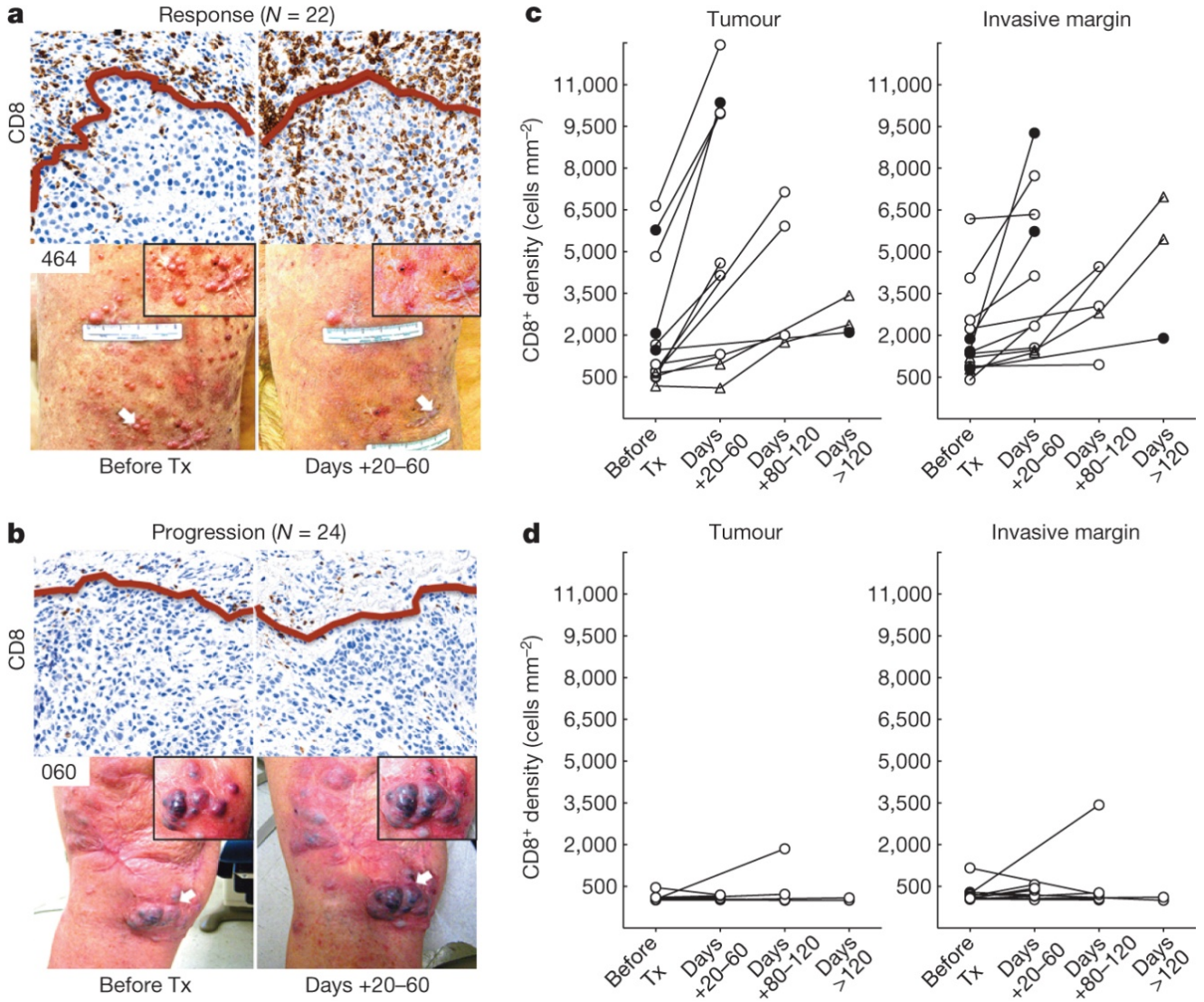


Tarhini AA et al. PLoS One. 2014;9:e87705

CD8 tumor infiltration as a biomarker

Predictive value of immunocytic infiltration in the context of immunotherapy

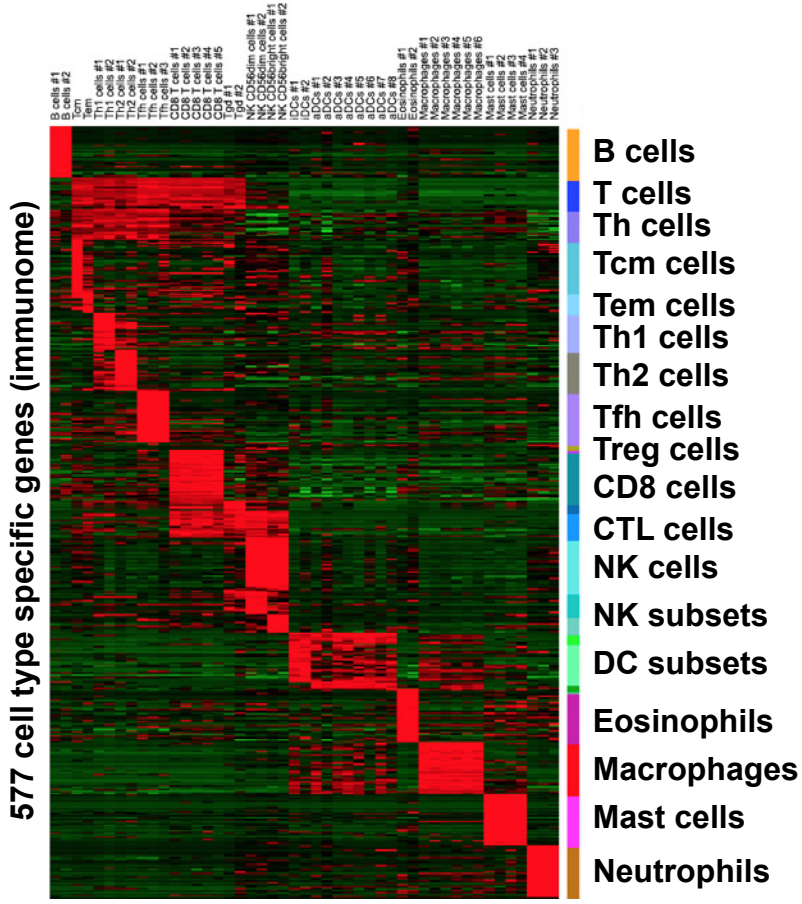
Immunohistochemical analysis of $CD8^+$ T cells in samples obtained before and during pembrolizumab treatment in advanced melanoma.



PC Tumeh *et al. Nature* 515, 568-571 (2014)

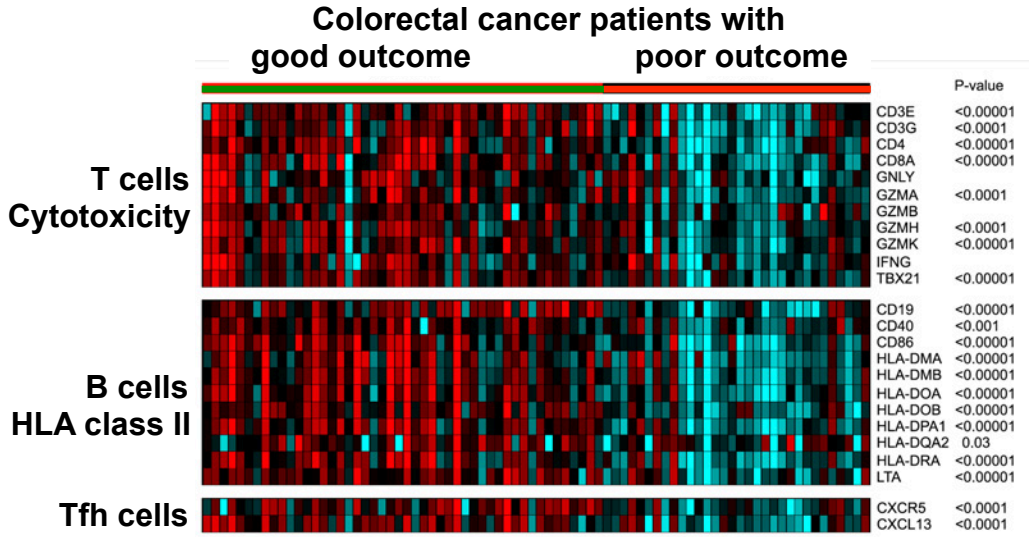
Immunomics. Establishing an immune landscape using genomic or transcriptomic immune signatures of tumors

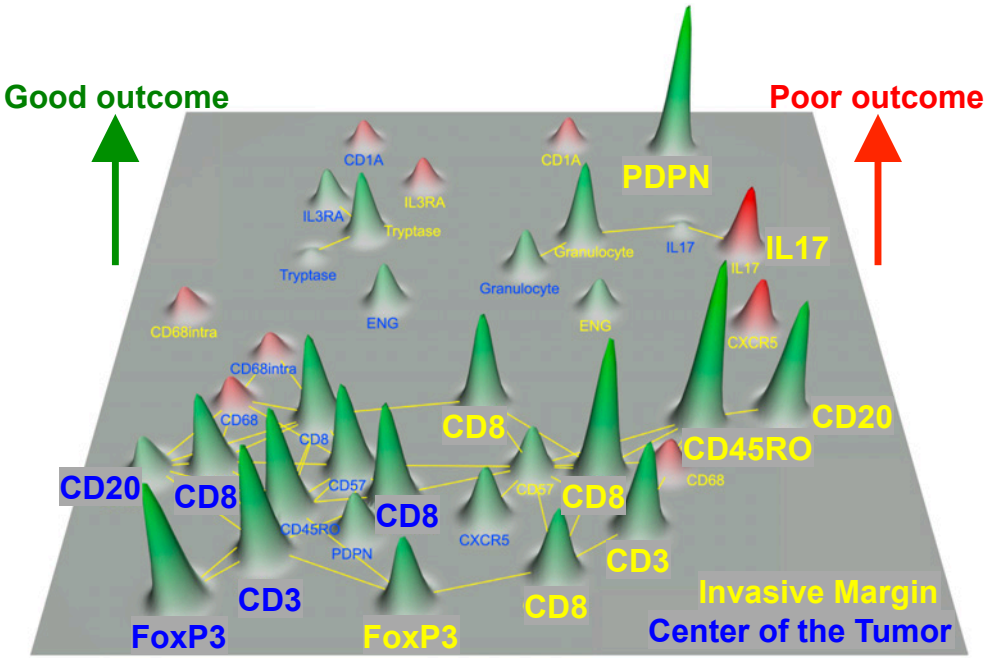
Genomic data from public data of purified immune cells and subsets used to define specific signatures



Adapted from Bindea et al., *Immunity*, 2013;39:782-95

Colorectal cancer patients with





Validation by immunohistochemistry of CRC tumors

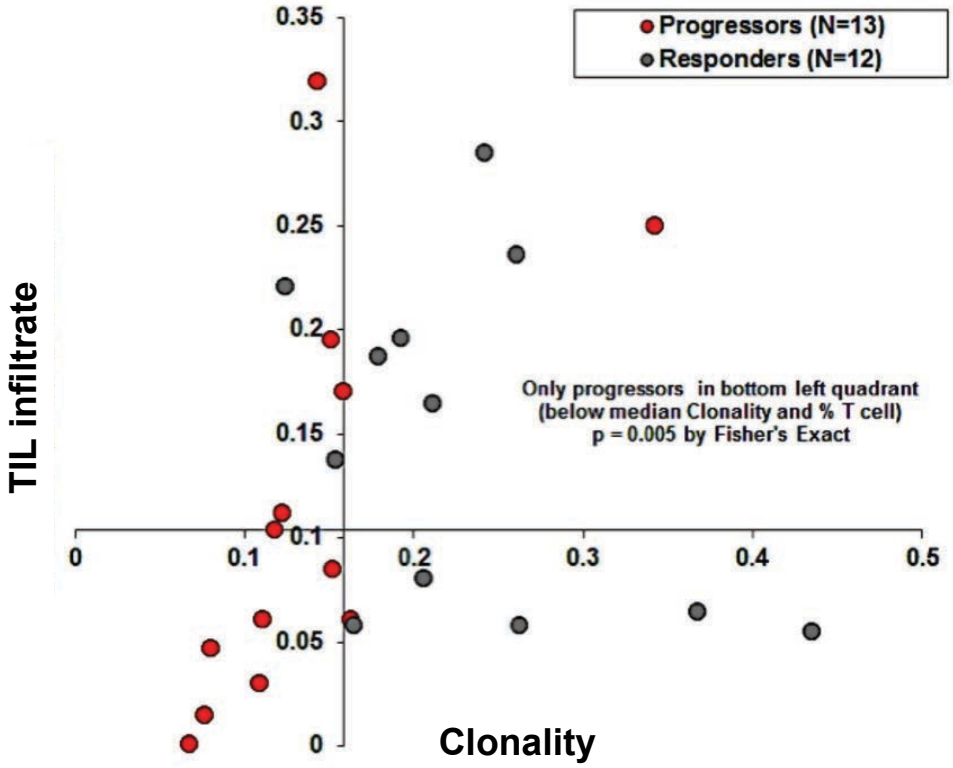
Identifying T cell repertoire and functionality in tumors

From fresh/frozen tissues:

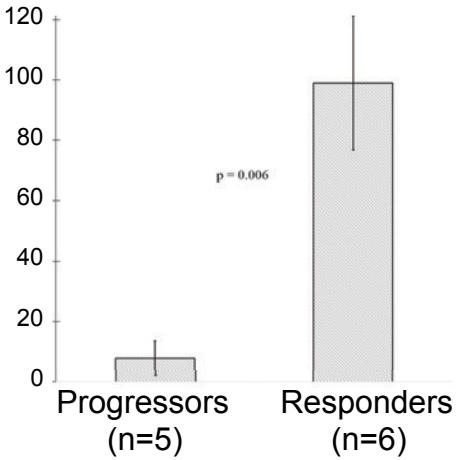
- Use of CD154, 4-1BB, IFN-γ capture, etc. to sort cells following stimulation with antigen or tumor
- RNASeq of sorted populations

From paraffin-embedded tissue:

- Multiplex IHC with functional markers, needs development:
  - better tools to identify functional status of cells, including exhaustion markers and metabolic stress
  - better tools to assess master regulators of T cell differentiation and lineage: STAT1, T-bet, GATA-3, RORγt, etc.
- TCR sequencing to look for clonality status at baseline and diversification of repertoire after treatment



Number of significantly expanded T cell clones

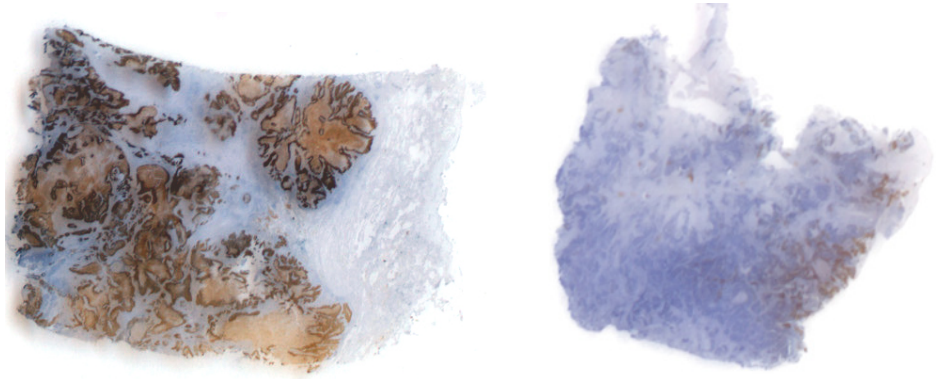


PC Tumeh *et al. Nature* 515, 568-571 (2014)

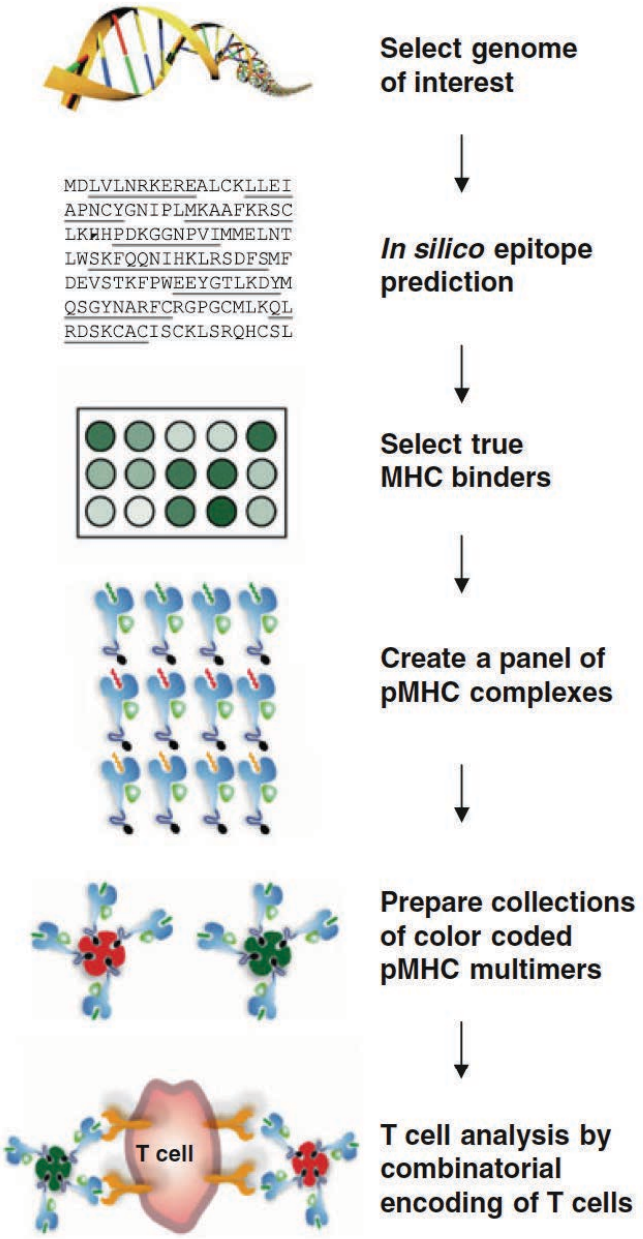
Identifying antigens recognized at the tumor site

- 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)
  ➔ Mutational load linked to clinical response rate in checkpoint blockade

- Immunohistochemistry and RT-PCR to confirm presence of known tumor antigens

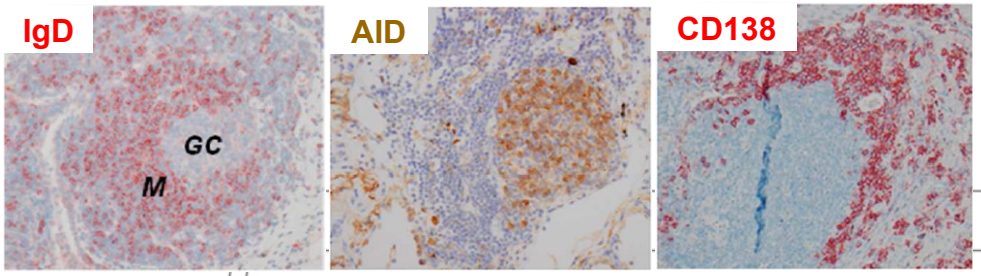


- Serology to quickly screen for immunogenic target antigens, as a surrogate for T cells, possible from fresh tissue after expansion



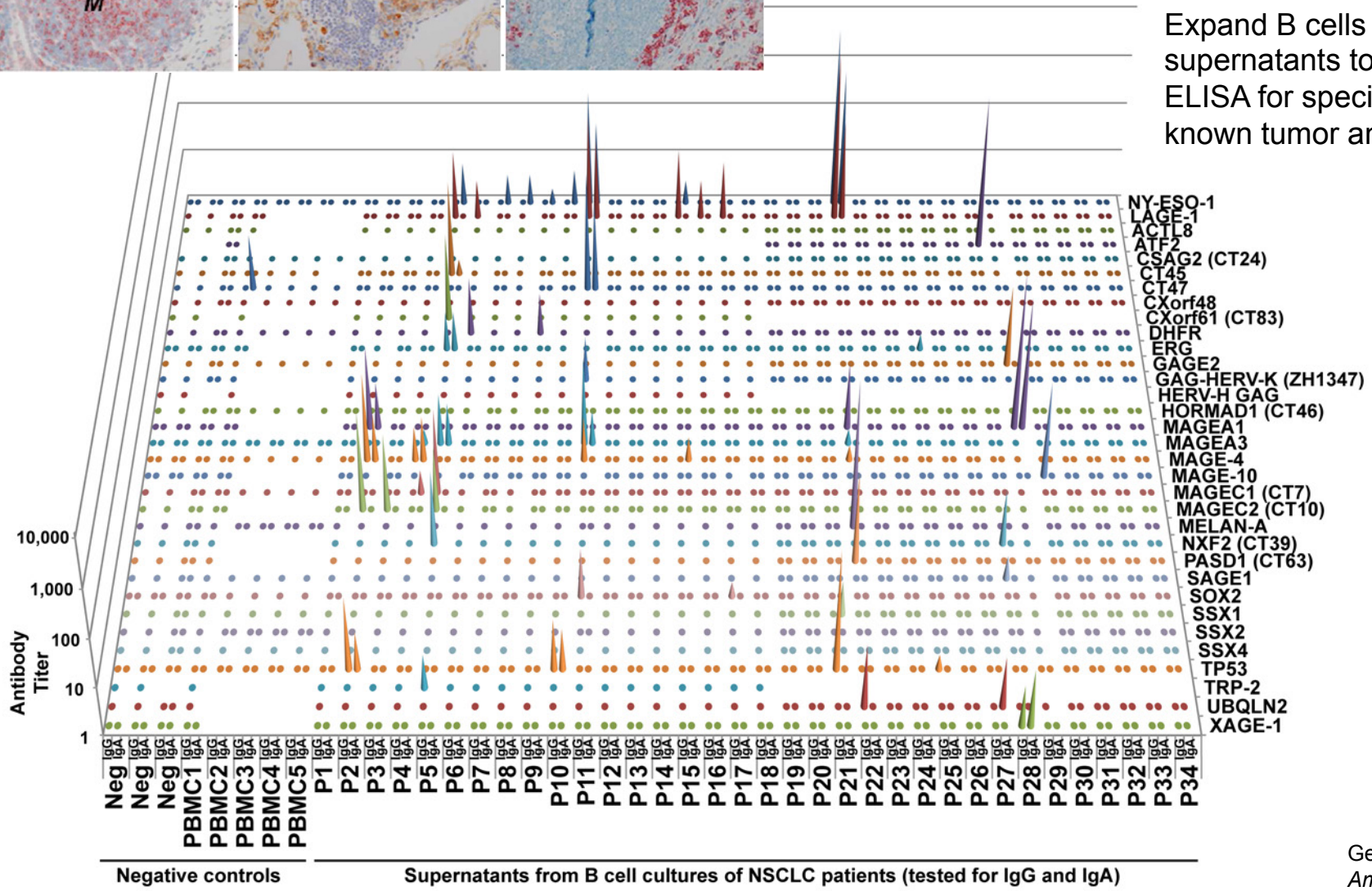
Cancer Immunol Immunother (2010) 59:1425–1433

Screening supernatants from tumor-infiltrating B cells in NSCLC



Presence of tertiary lymphoid structures, germinal centers, and plasma B cells at the tumor site

Expand B cells and collect supernatants to test by ELISA for specificity to known tumor antigens



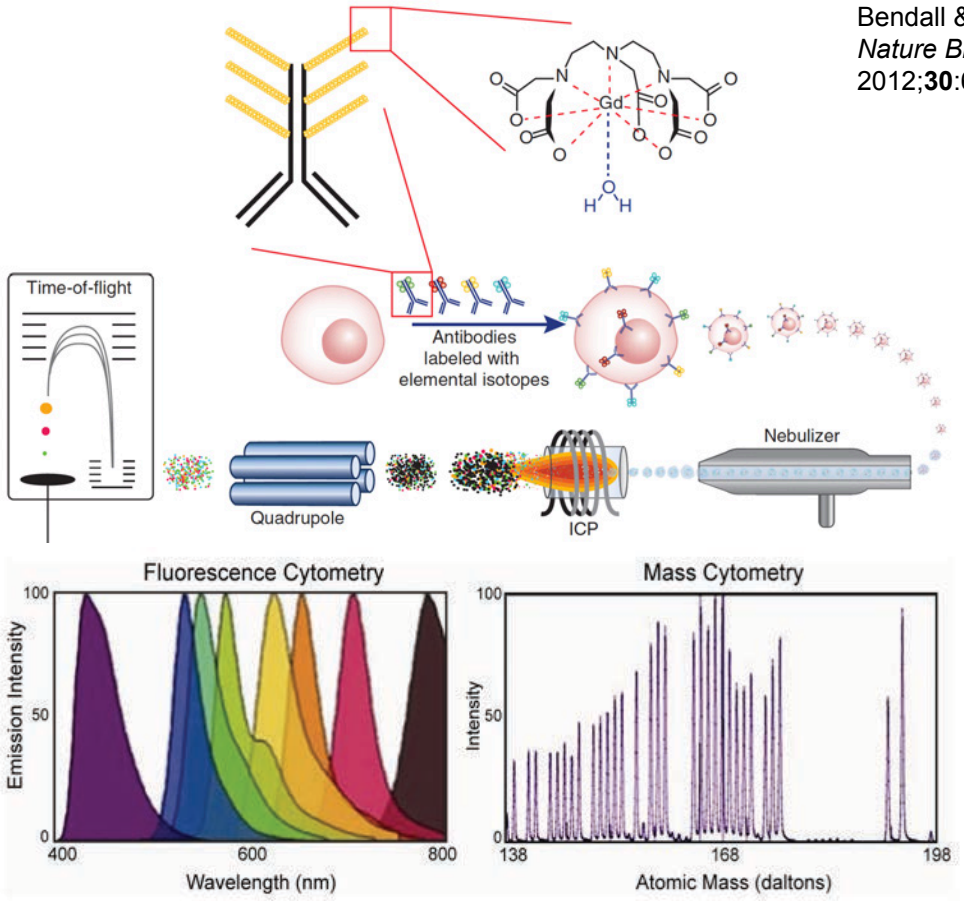
→ **Production of tumor-specific antibodies by intratumoral plasma cells**

Germain et al. *Am J Respir Crit Care Med.* *2014*;189:832-44

Scope

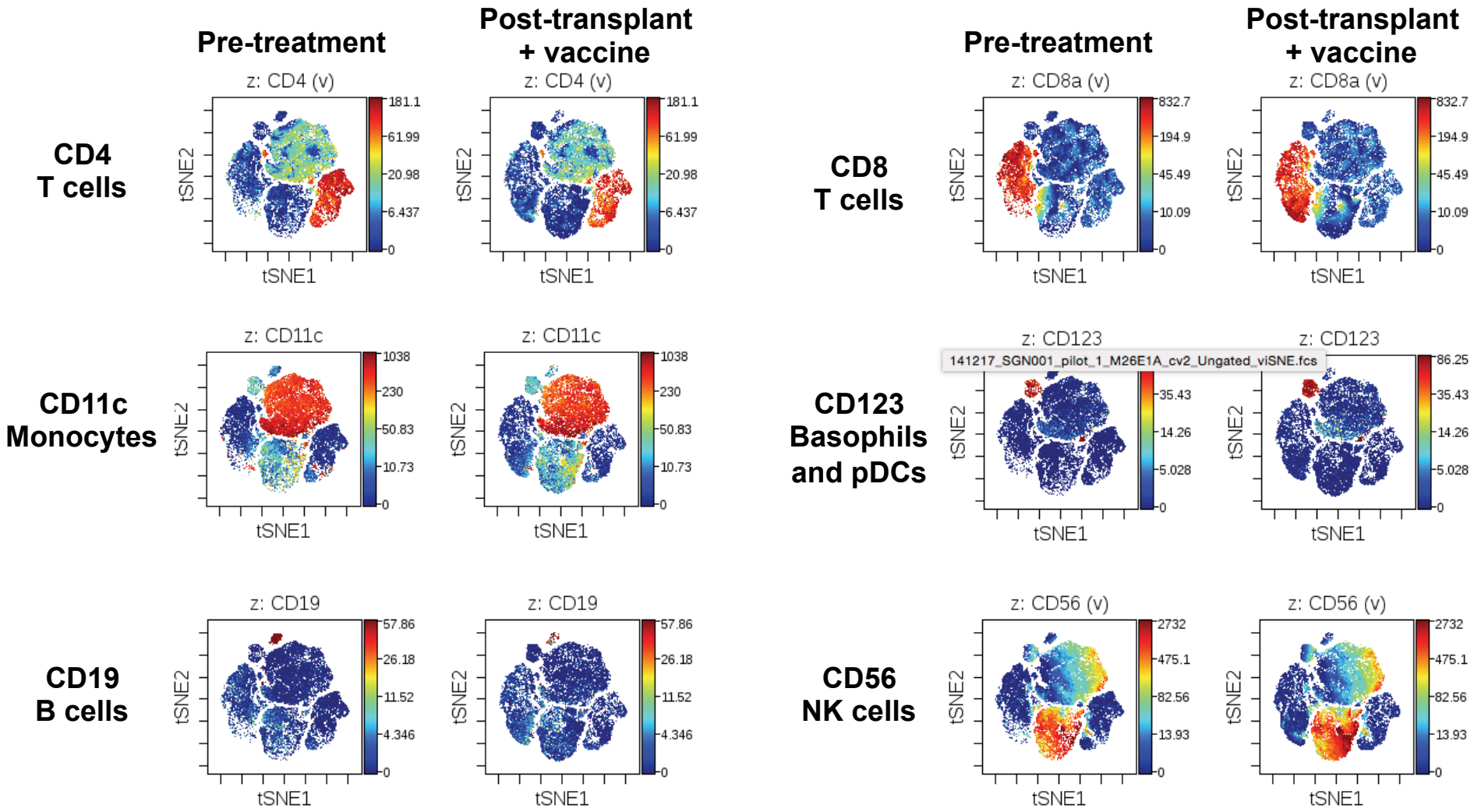
Peripheral immune monitoring

Mass cytometry to explore phenotypic changes during treatment



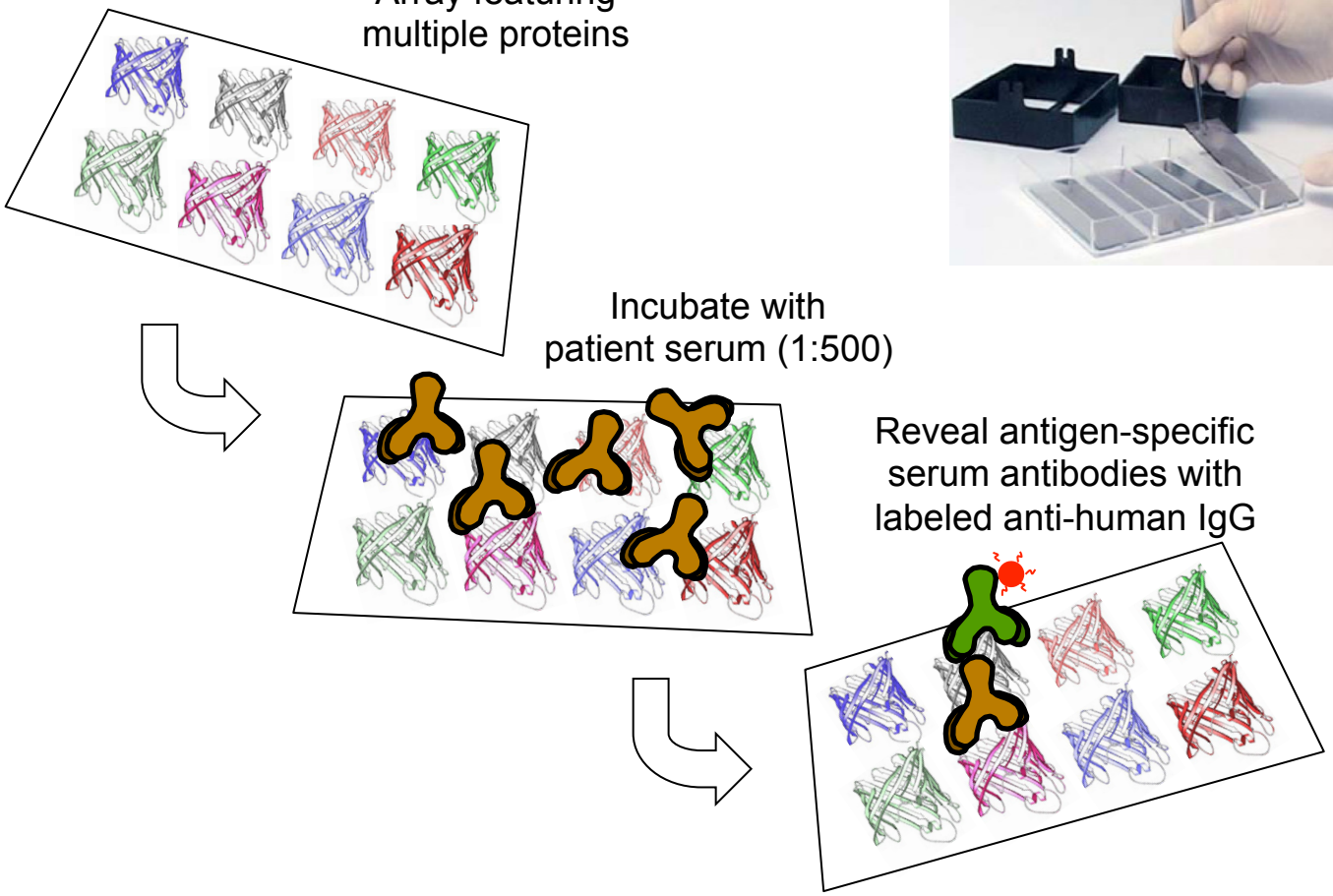
Bendall & Nolan.
Nature Biotechnology
2012;**30**:639–47

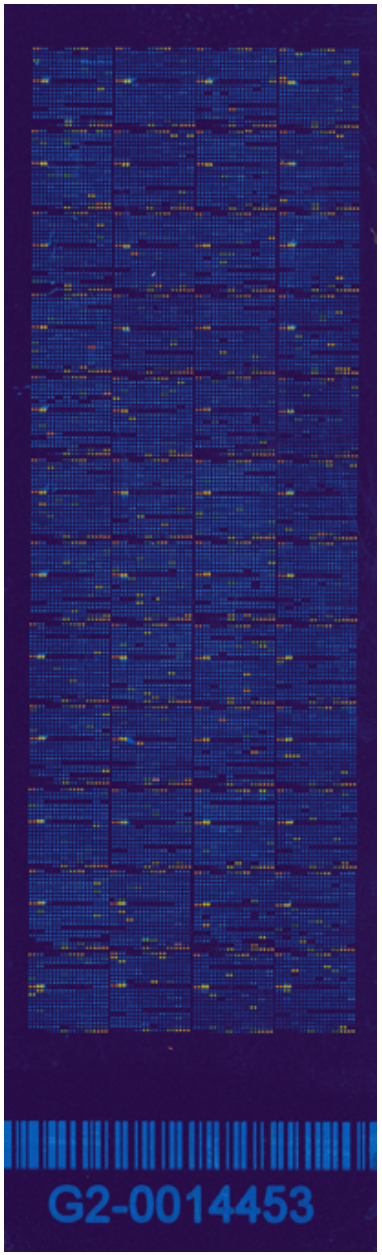
Multi-dimension reduction algorithms and software allow to visualize complex data in 2D plots in an unbiased manner



Mass cytometry allows the analysis, at the single cell level, up to 40 markers simultaneously with minimal signal overlap.

Seromics. Methodology for antibody profiling with protein microarrays



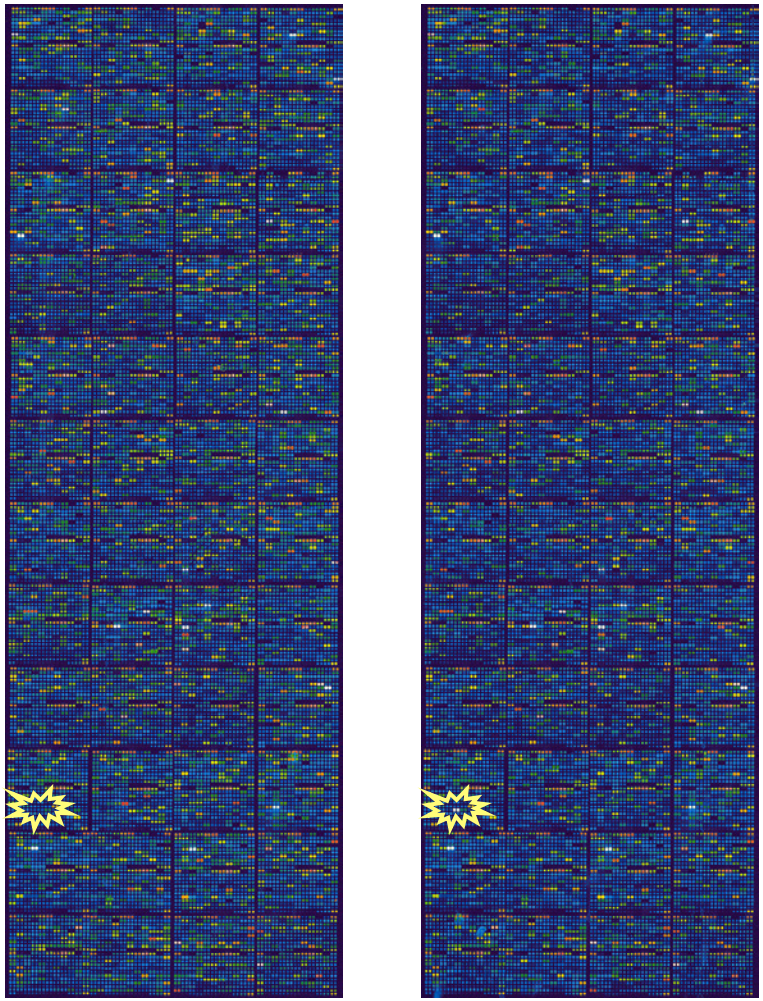


Protein microarrays contain >9000 proteins mostly full-length baculovirus-produced GST-fusion proteins randomly selected, both known and predicted sequences

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

MAGE-A4 **short peptide** vaccine

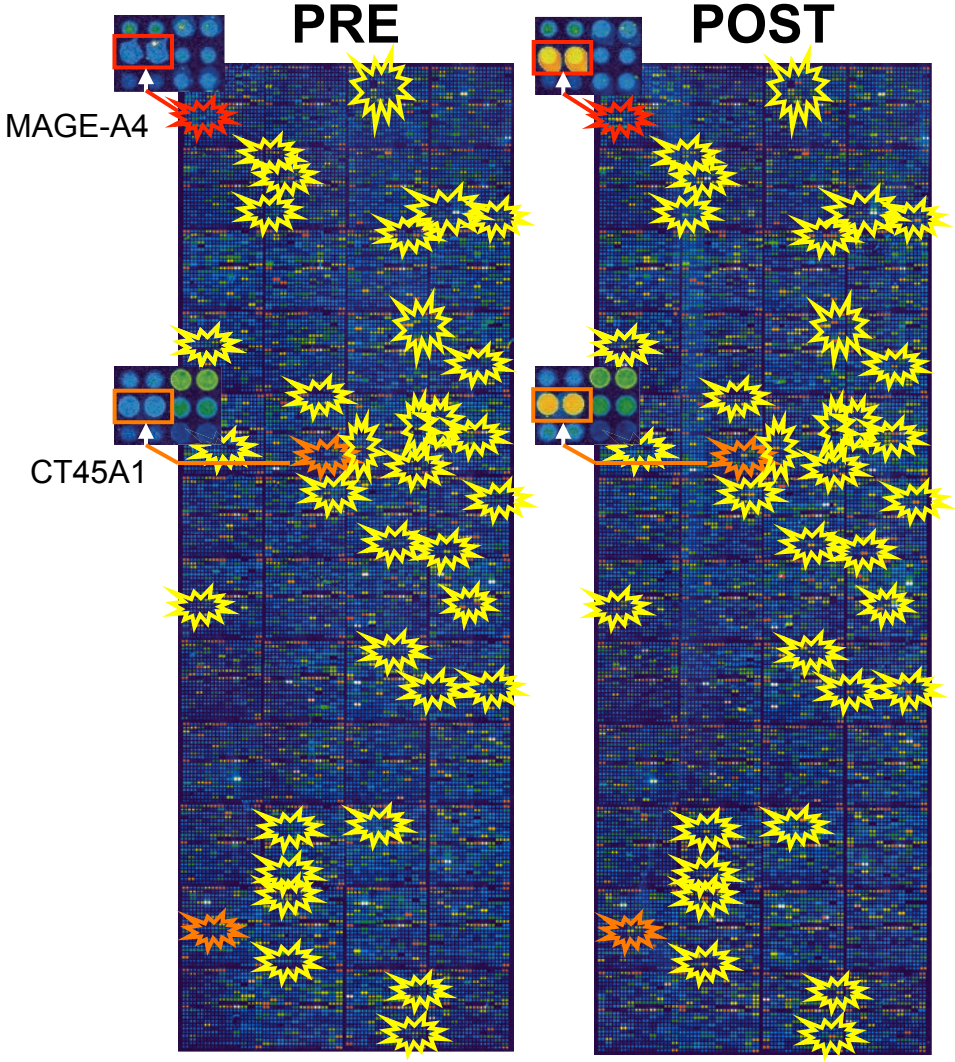
PRE **POST**



Only 1 significant change in antibody reactivity out of 9000 possible proteins

MAGE-A4 CHP-**protein** vaccine

PRE **POST**



40 other non-vaccine related proteins react, including cancer-testis antigens

Correlation of NY-ESO-1 antibody with clinical course following anti-CTLA-4 treatment with ipilimumab

In collaboration with Jedd Wolchok and Jim Allison MSKCC/Ludwig Center and with
Ruth Halaban and Mario Sznol, Yale University - Melanoma sera

Sera from melanoma patients taken at baseline, before CTLA-4 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: mixed response)
DOD: Dead of Disease

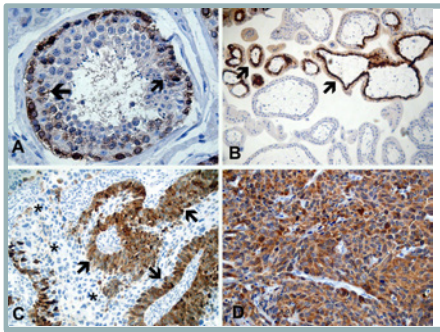
Fisher's exact test (two-tailed):
P value 0.0481
RR=1.8(1.1-2.9)

Yuan, Gnjatic et al. *Proc Natl Acad Sci U S A. 2011*;108:16723

MANIFOLD VARIABLES CLINICAL TRIAL STRATEGY CENTERED ON NY-ESO-1

CVC Trials Network

45 clinical trials of different vaccine combinations and strategies completed or ongoing, involving 950 patients worldwide



Antigen
NY-ESO-1

Cancer Populations

Bladder
Breast
Esophageal
Gastric
Head and Neck
Lung
Melanoma
Ovarian/Peritoneal/Fallopian Tube
Prostate
Sarcoma

Antigen Forms

Peptides – Class I and II
- Short Peptides
- Long Peptides
- Overlapping Peptides

Protein
DNA

Adjuvants

Bacillus Calmette-Guerin
CpG 7909
GM-CSF
Imiquimod
Mixed Bacterial Vaccine
Montanide ISA-51
Poly ICLC
Resiquimod
Streptococcal OK432
AS02B

Modulators of Immunosuppression

Anti-CTLA-4 (ipilimumab)
Cyclophosphamide

Delivery Systems

Viral Vectors
- Vaccinia-NY-ESO-1
- Fowlpox-NY-ESO-1
- Canarypox – ALVAC-NY-ESO-1/TRICOM

Antigen Presenting Cells
- DCs pulsed with NY-ESO-1 peptide

Other
- Cholesteryl-bearing Hydrophobized Pullulan
- ISCOMATRIX

Inoculation Strategy

Method
- Gene Gun
- Intradermal
- Intramuscular
- Subcutaneous
- Topical

Timing
- Serial
- Intensive course
- Prime-boost

Comparative summary of immune responses elicited following various NY-ESO-1-based vaccine trials

Trial, Publication, Comments	Ab	CD8	CD4	Integrated Ab, CD4 and CD8 responses
Vaccinia/Fowlpox PMID: 16984998, Jäger et al. 2006 3 baseline seropositive, various ca.	11/23 (48%)	19/23 (83%)	13/23 (57%)	7/23 (30%)
Protein + CpG PMID: 21163871, Karbach et al. 2011 2 baseline seropositive, prostate ca.	13/13 (100%)	6/13 (46%)	9/13 (69%)	6/13 (46%)
Protein + Montanide + CpG PMID: 17517626, Valmori et al. 2007 No baseline seropositive, various ca.	18/18 (100%)	9/18 (50%)	17/18 (94%)	9/18 (50%)
Protein + CHP PMID: 17441676, Uenaka et al. 2007 2 baseline seropositive, esophageal ca.	9/9 (100%)	7/9 (78%)	7/9 (78%)	5/9 (56%)
OLP+ Montanide + Poly-ICLC PMID: 23032745, Sabbatini et al. 2012 1 baseline seropositive, ovarian ca.	10/11 (91%)	10/11 (91%)	11/11 (100%)	10/11 (91%)

CHP: **Cholesteryl Pullulan delivery adjuvant**
OLP: **Overlapping Long Peptides (30-32mers)**
Ca.: **Cancer**

Induction of immunity by vaccine strategies with different antigen formulations

Vaccine antigen formulation	Ab	CD8	CD4	Recognition of naturally processed antigen
Short HLA Class I peptides	–	+++	–	Rarely
Short HLA Class II peptides	–	+/–	+++	Rarely
rV–NY-ESO-1 / rF–NY-ESO-1	+/–	++/–	++/–	Yes
DNA	–	+/–	++	Yes
Protein	+++	++/–	+++	Yes
Overlapping long peptides	++	+++	+++	Yes

Take home message

Comprehensive immune monitoring strategies to guide and inform future immunotherapy designs

Multiplexing and sample-sparing techniques are becoming critical to address the complexity of immune responses and suppression

Plan ahead: need to consider sampling of tissues and samples carefully

Defining antigen specificity and quality of immune responses is important to validate the mechanism of action of drugs

Era of biomarker discovery for companion diagnostics and patient pre-selection

Future directions

- Microbiome
- Single-cell genomics
- Integration with systems biology and bioinformatics
- Plasticity, ontogeny of immune cells – Variability over time
- In situ specificity (tetramers for IHC, microdissection and functional analyses)

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