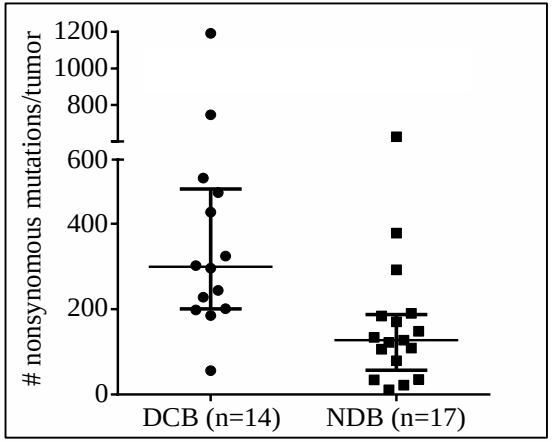
THE MANAFEST ASSAY FOR MONITORING ANTI-TUMOR IMMUNITY

Kellie N. Smith, PhD

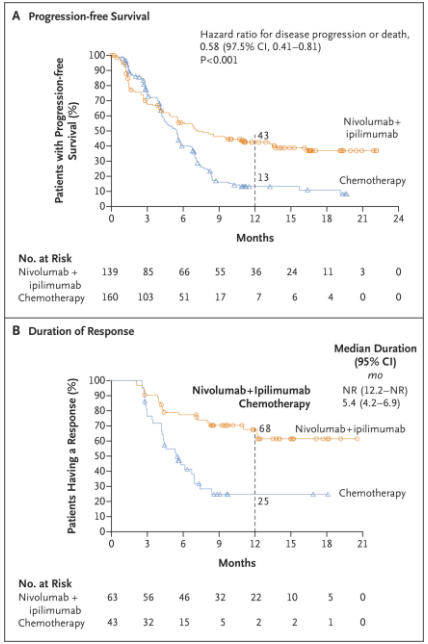
SITC Immuno-Oncology Biomarkers:
State of the Art
May 16, 2018

kellie@jhmi.edu

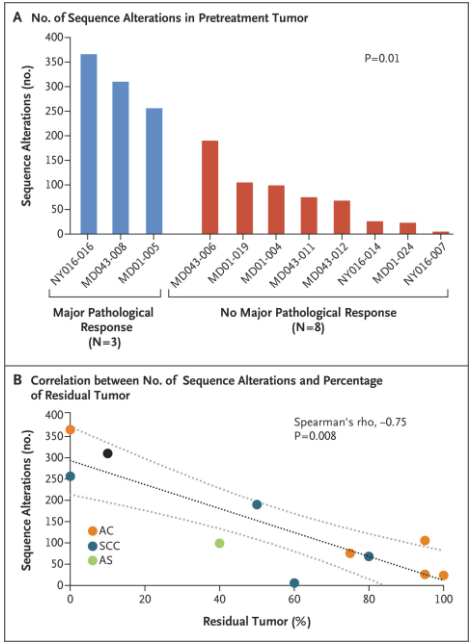
Tumor mutational burden (TMB): a predictive biomarker of response to checkpoint blockade



Rizvi et al, Science, 2015



Hellmann et al, NEJM, 2018



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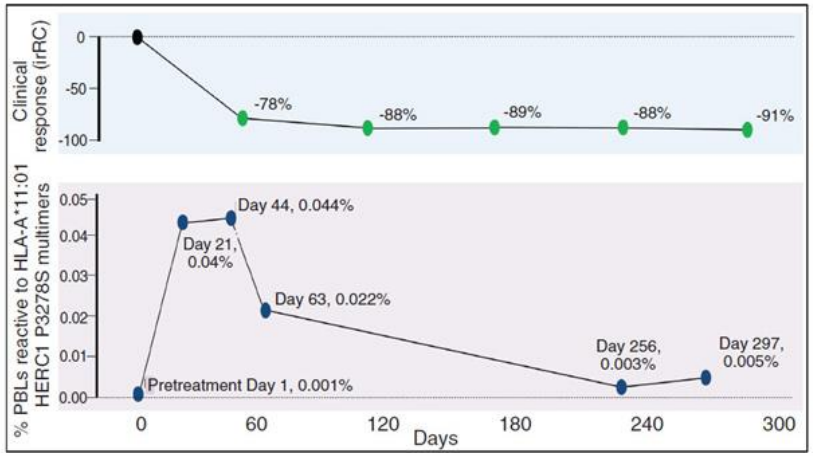
TMB and The Importance of Neoantigens



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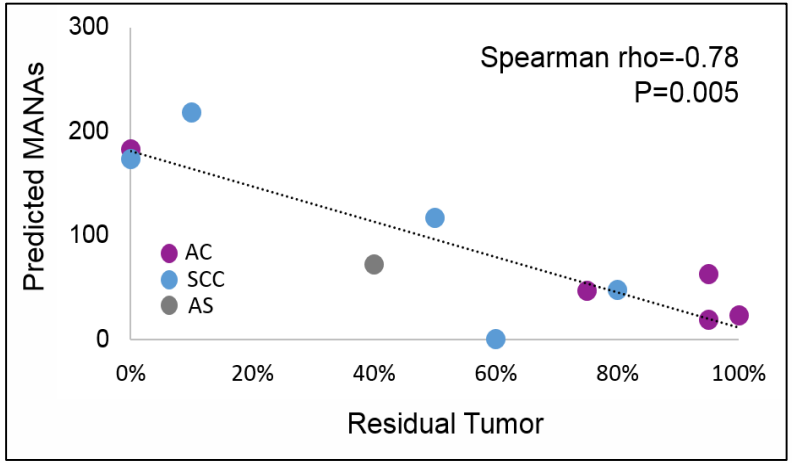

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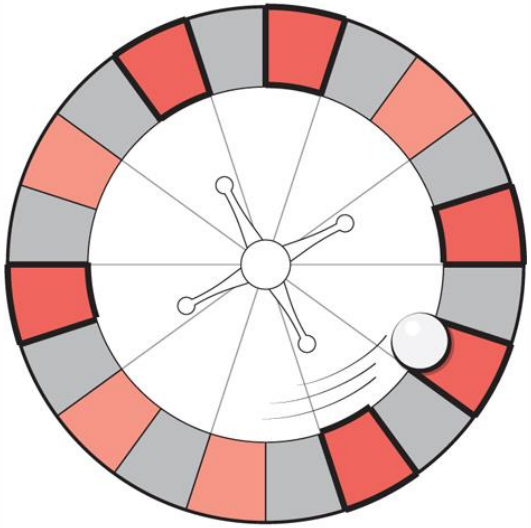
TMB and The Importance of Neoantigens

Responders
Cancer patients with more mutations have a higher chance of responding to therapy



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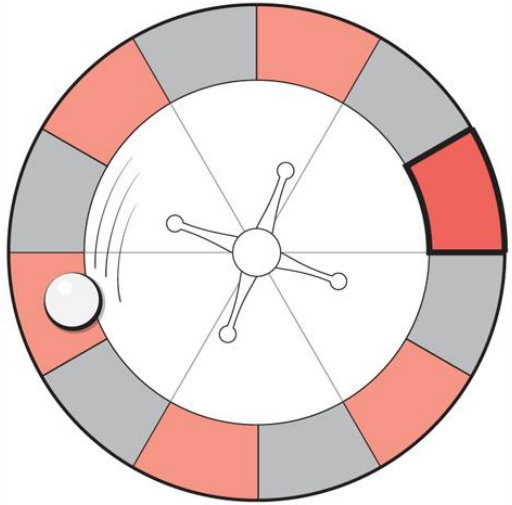

Mutations Ipilimumab-responsive mutation

Gubin MM and Schreiber RD, Science, 2015

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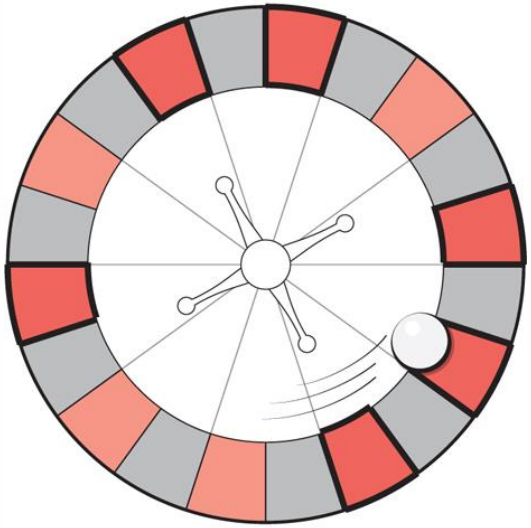

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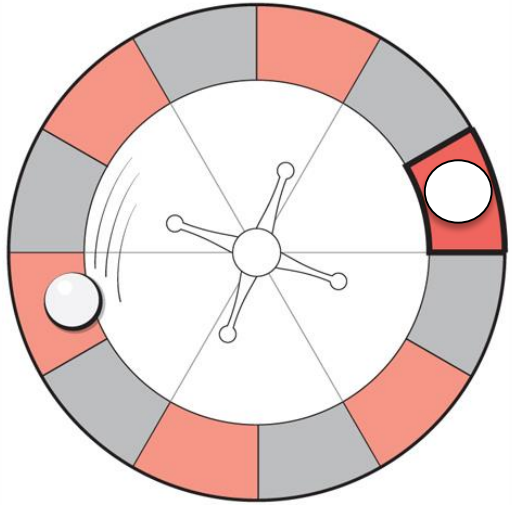

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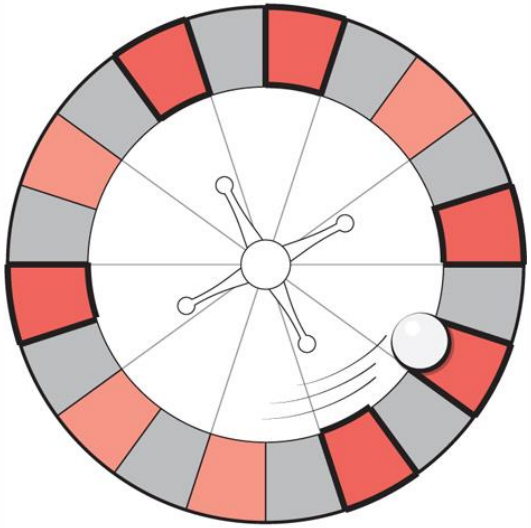

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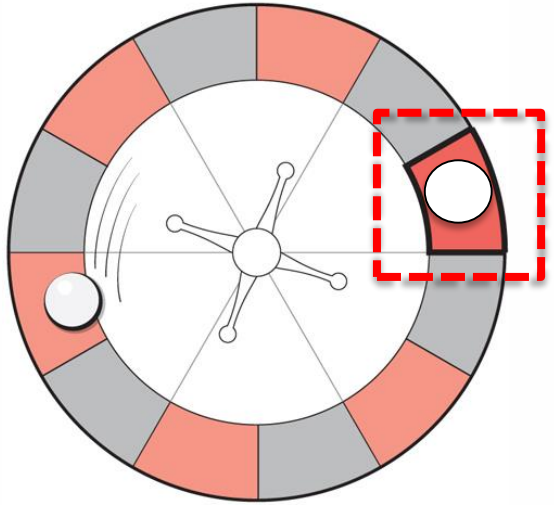

Identifying these patients = predictive power

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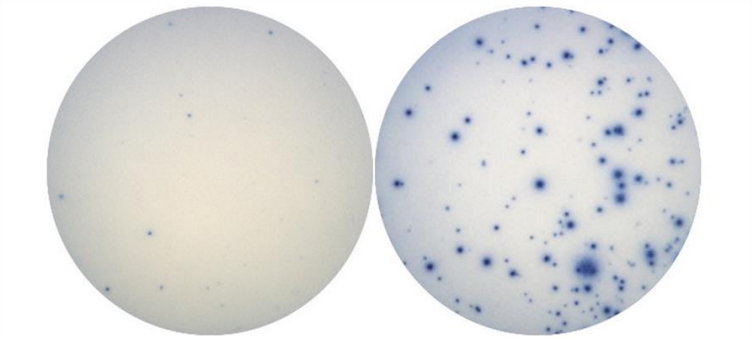
ELISpot: The gold standard in immunological monitoring



Adapted from ImmunoSpot

Pitfalls:

- Can’t track response without repeating assay
- Can’t link with FFPE specimens
- Can’t track geographic distribution
- Background after culture/treated patients
- Cytokine-dependent
- No clonal dynamics

T cell receptor (TCR) sequencing

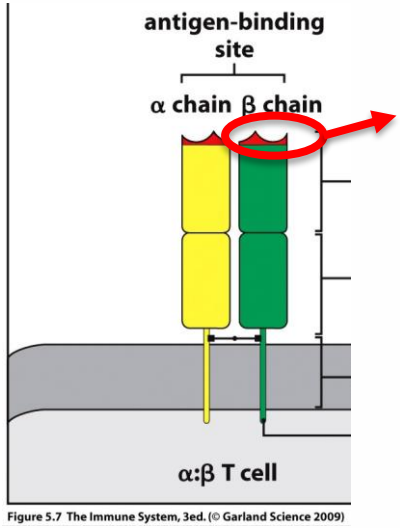
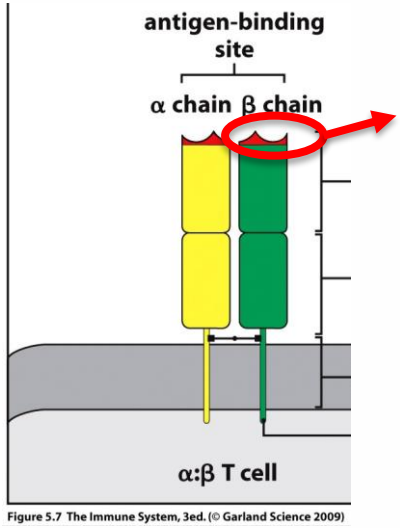


Figure 5.7 The Immune System, 3ed. (© Garland Science 2009)

TCR Vβ CDR3 sequencing

Adaptive biotechnologies

T cell receptor (TCR) sequencing



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Sample types:

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Flash frozen

Viably frozen

Cultured cells

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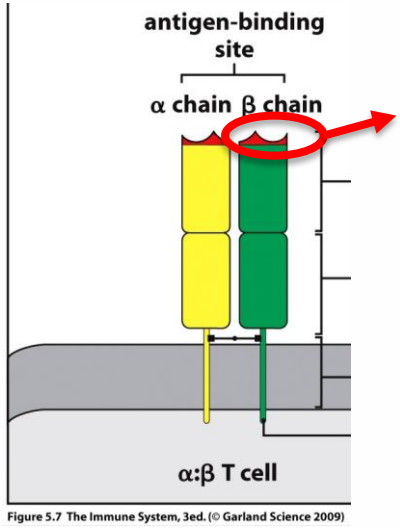

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Adaptive biotechnologies

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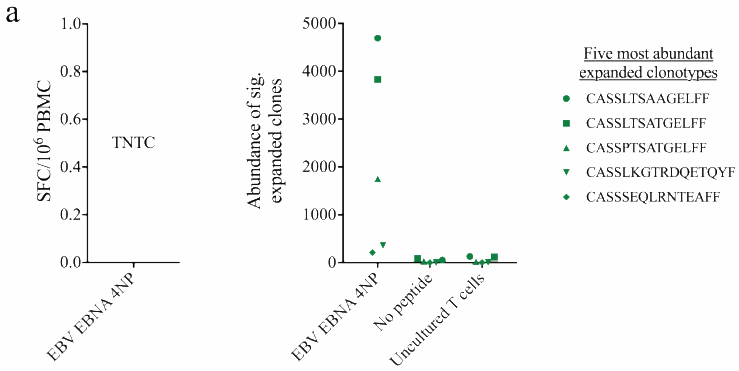
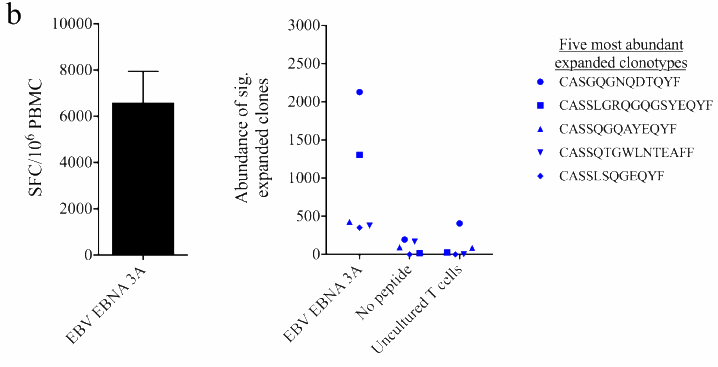
Cultured cells

(01) 0 9501101 53000 3 (17) 140704 (10) AB-123

If you can get DNA, you can track it!

ELISpot May Underestimate the Anti-Tumor Immune Response





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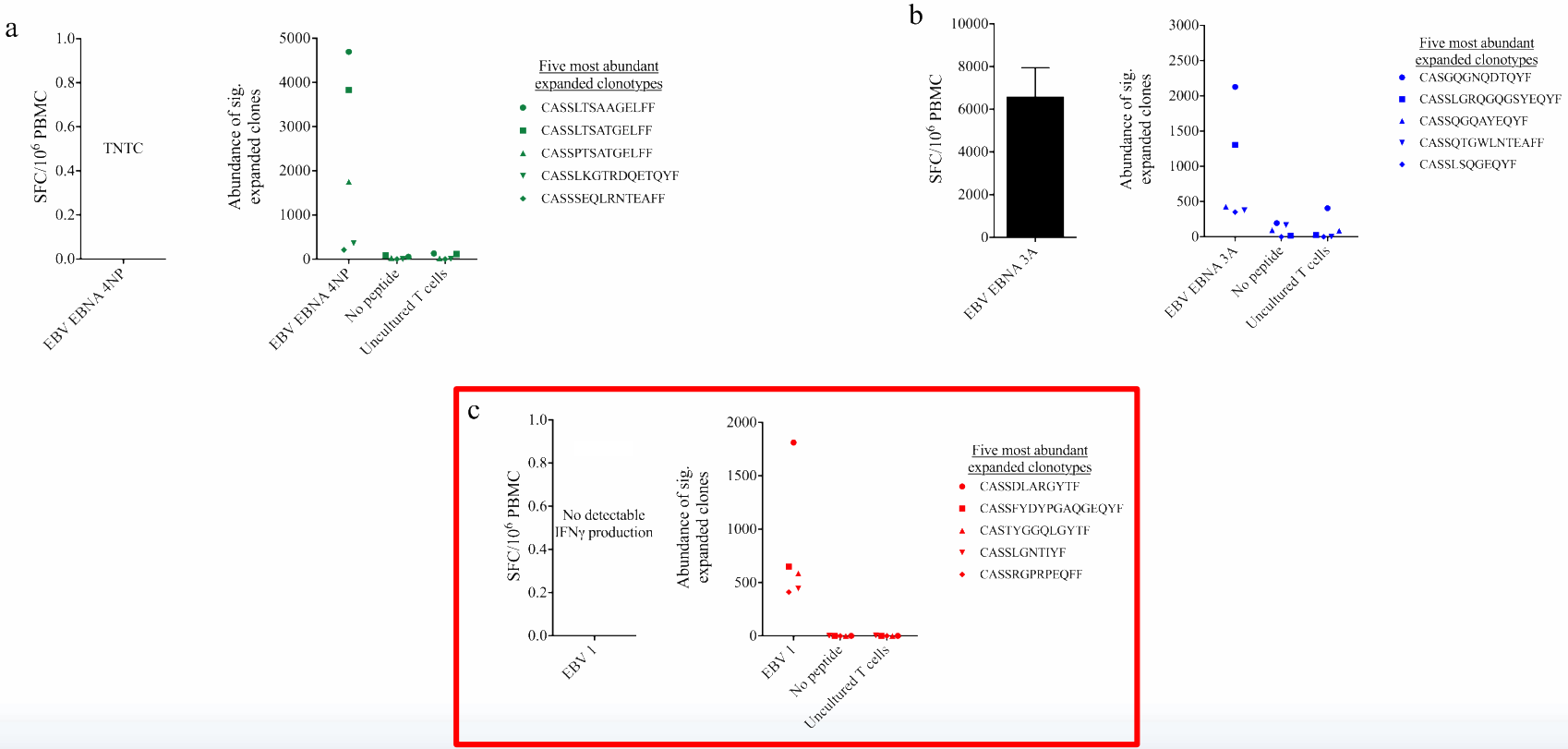
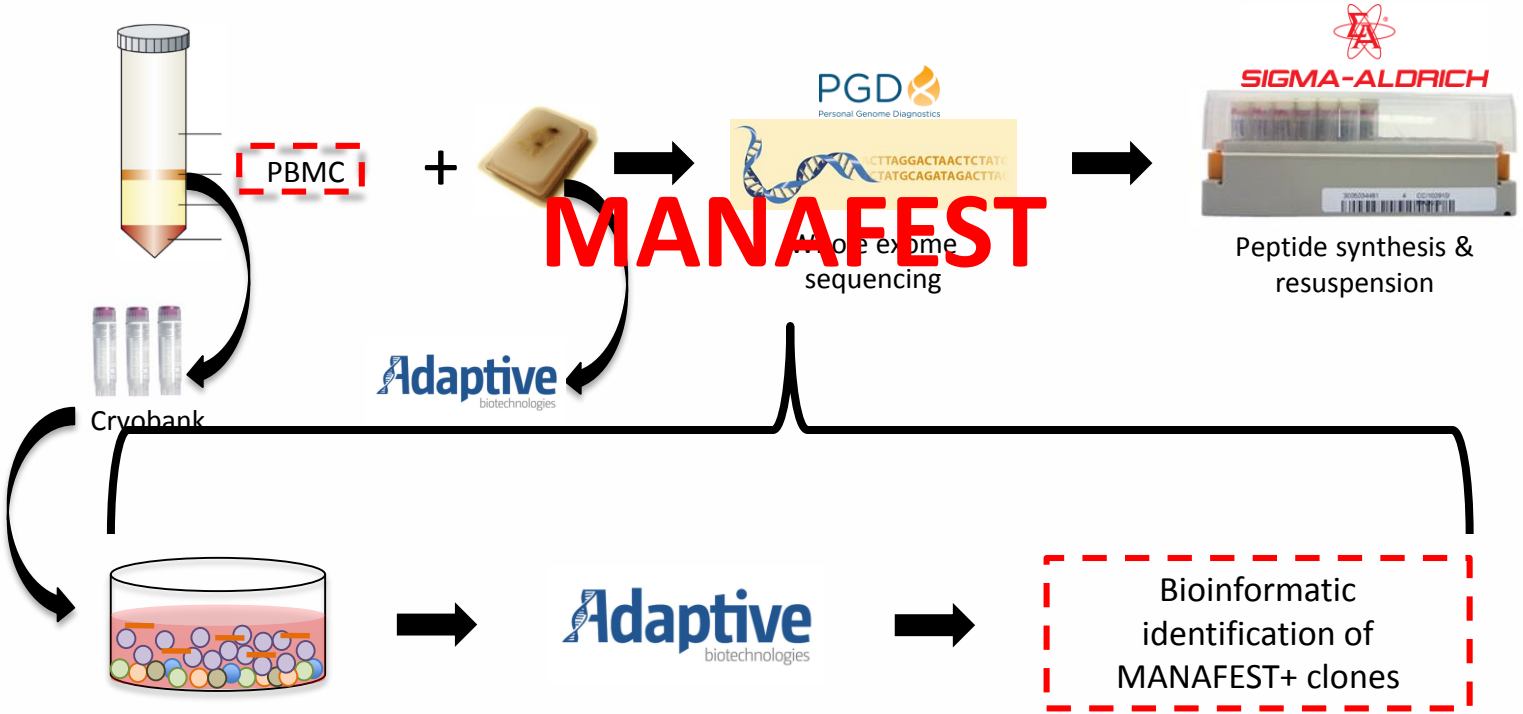


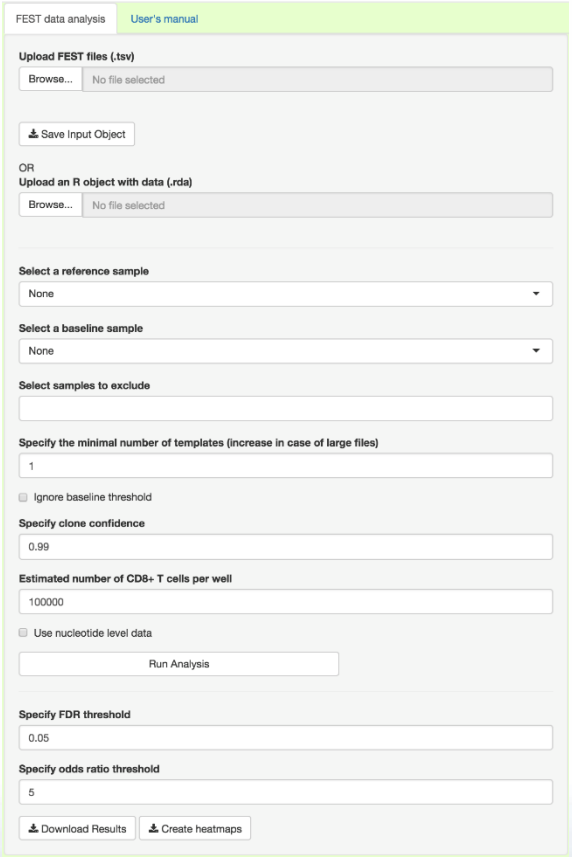
The Functional Expansion of Specific T Cells (FEST) Assays

- Combines experimental T cell culture with in silico identification of clones corresponding to Ag-specific T cells
  - ViraFEST
  - TAAFEST
  - BactiFEST
  - OncoFEST
  - AutoFEST
  - MANAFEST

Basic Workflow of the MANAFEST Assay



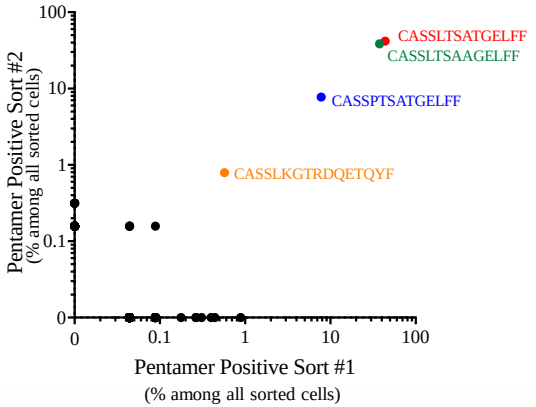
Data Analysis & Bioinformatic Pipeline



- What is considered “MANAFEST+”?
  - Significant expansion compared to “no peptide”
  - Significant expansion compared to every other peptide
  - Odds ratio >5
  - No significant expansion in response to any other peptide
    - ~46 peptides for optimum specificity
- Match w/ clones found in tumor
- Evaluate peripheral dynamics of MANAFEST+ clones

Sensitivity & Specificity

EBV EBNA 4NP A*11:01



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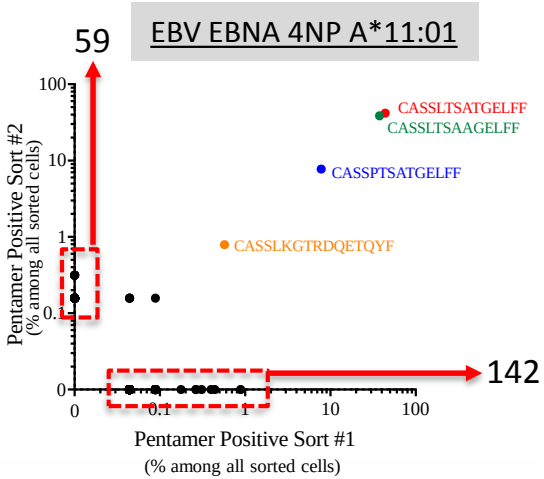

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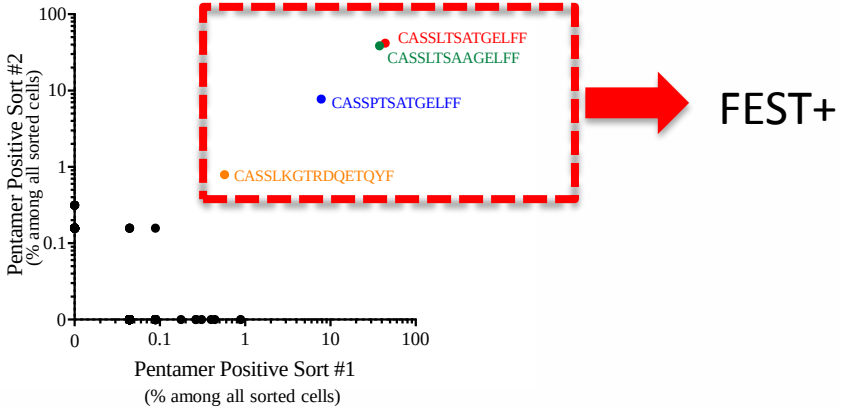

FEST+

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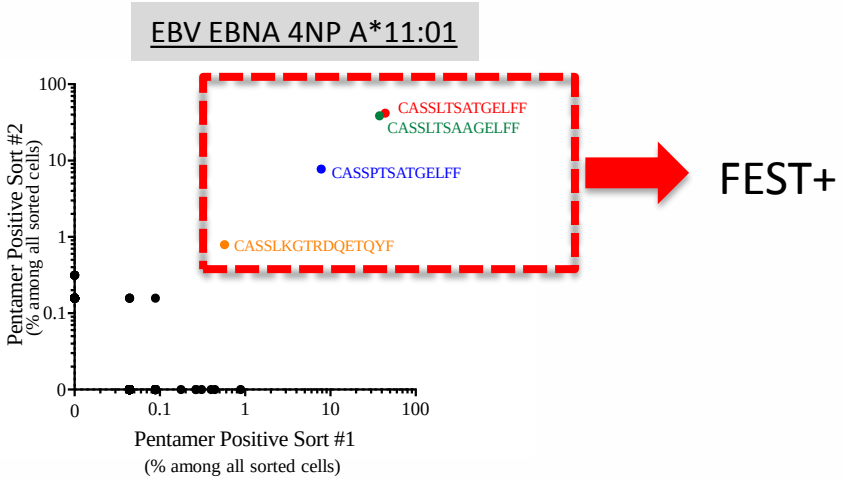
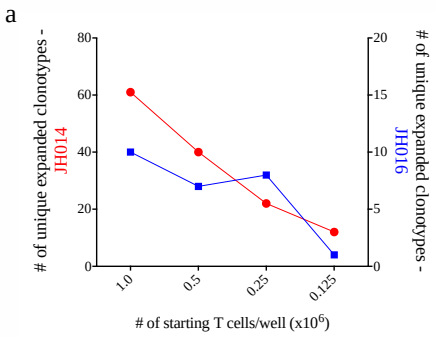
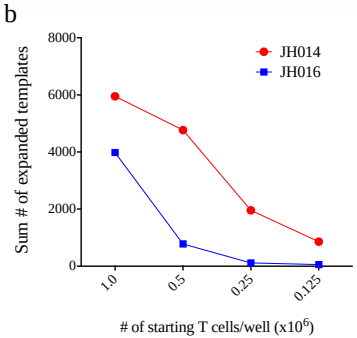
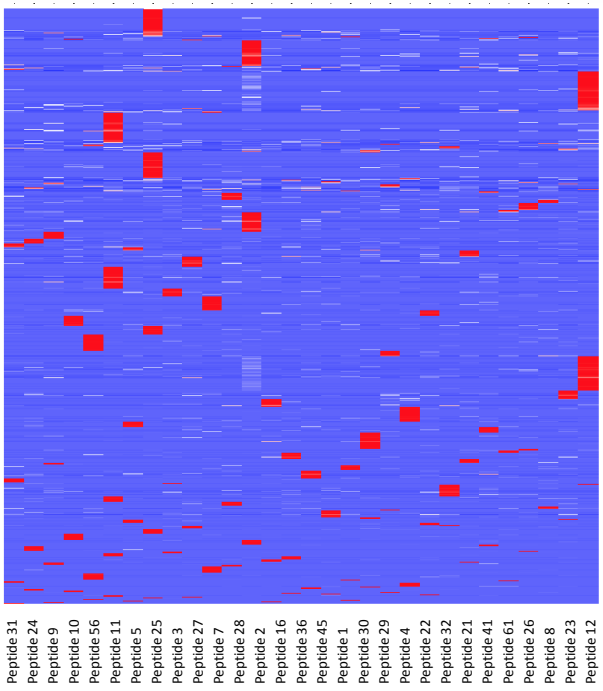


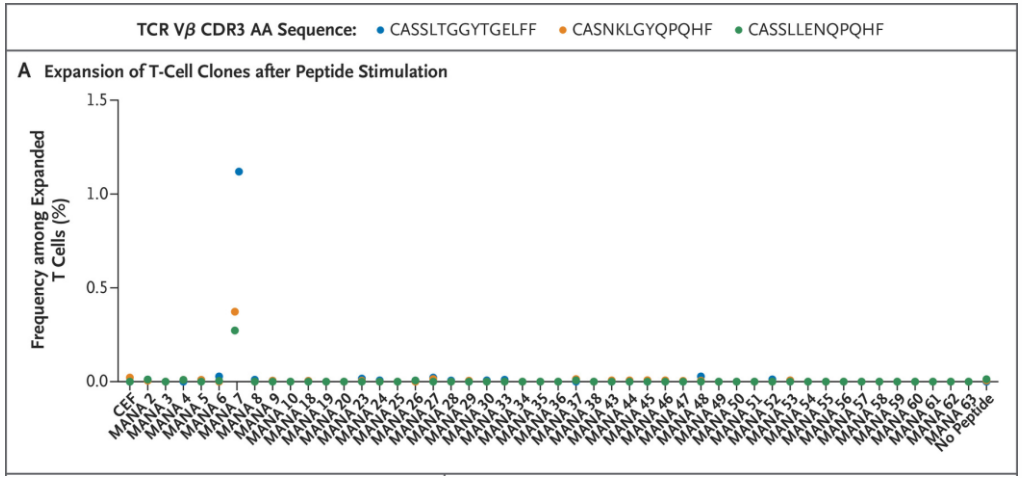
FEST+





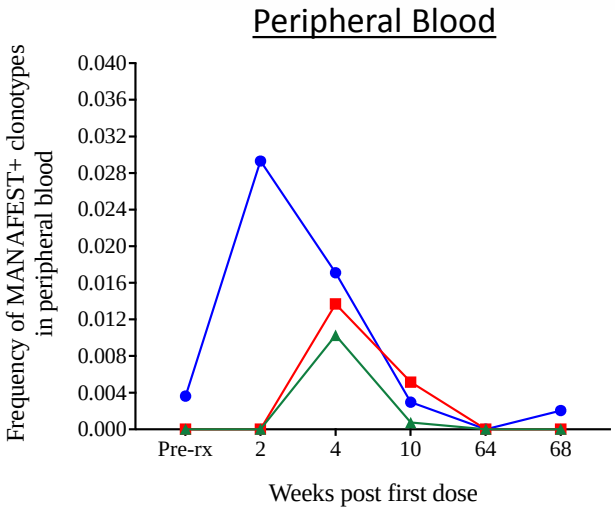
Example MANAFEST Data Visualization

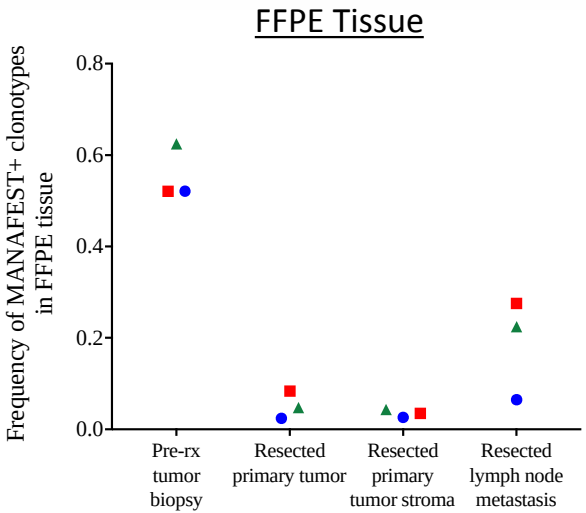


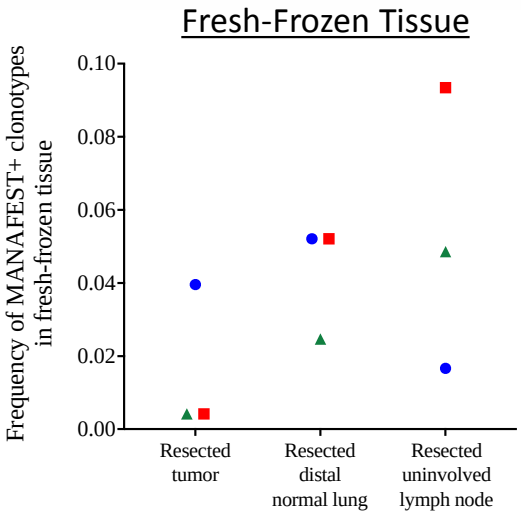


Forde, Chaft, and Smith et al, NEJM, 2018

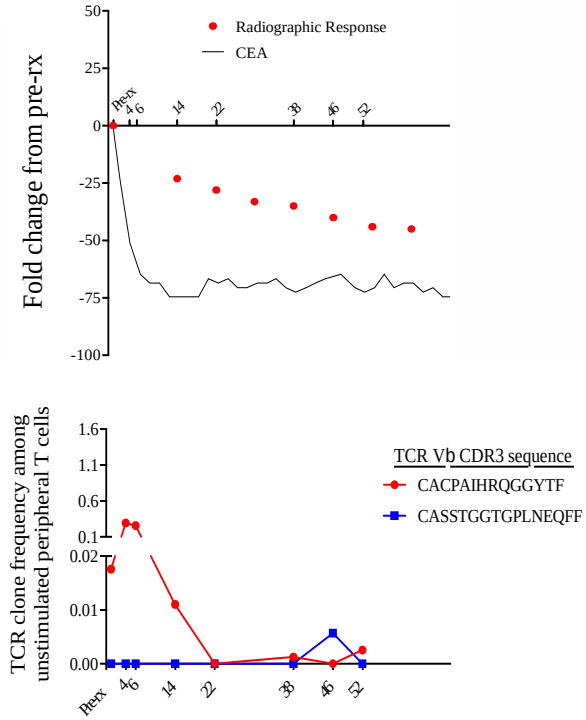
Possibilities: tracking antigen-specific T cells in every biological compartment & specimen type





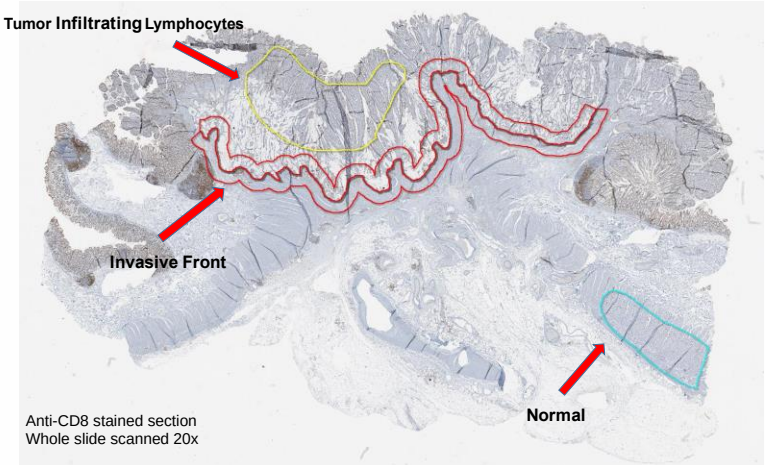


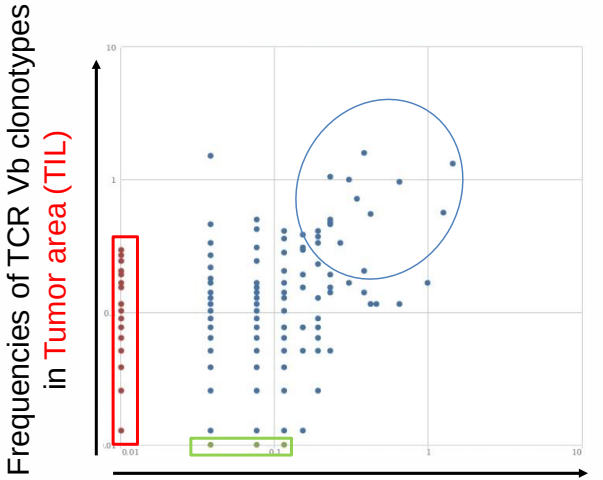
Possibilities: associating antigen-specific T cell dynamics with clinical parameters



Adapted from Le DT, Science, 2017

Possibilities:
tracking geographic distribution of antigen-specific T cell repertoire





Nicolas Llosa, MD
Franck Housseau, PhD

Quantitative outputs: a wealth of data

- # of positive peptides
- Frequency of each positive clone
- Combined frequency of positive clones
- Expansion compared to baseline
- Common motifs
- Peripheral and intratumoral dynamics

How does MANAFEST stack up?

	ELISpot/FLUOROSpot	MULTIMERS	F.E.S.T.
Assay	Enzymatic/fluorescence	Flow cytometry	NGS
T cell activation	In vitro	Ex vivo	In vitro
Signals	Colorimetric/fluorescence	Fluorescence	TCR sequences
Biospecimens	Fresh PBMC Antibodies Peptides	Fresh PBMC Antibodies Peptides HLA monomers	Fresh PBMC
Instrumentation	ELISPOT reader	Flow cytometer	None
Quantification	Frequency/intensity	Frequency	# clonotypes Frequency Fold change
Sensitivity	1/10,000-20,000	1/5,000-10,000	>1/100,000
Specificity	++ Background	++ Background Spectrum overlaps	+++
Resolution	>10-50 cells/well	>250 events/gate	>10 reads/clonotype
Throughput	Low	High	High
Functional readout	Cytokine / Granzyme	Multiparameter	N/A
Time course	1 ELISPOT/time point	1 staining/time point	1 test total
Archival specimens	No	No	FFPE/snap frozen
Geographic analysis	No	No	Yes, using FFPE

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Future Directions

- Opportunities for machine learning approaches
- Phenotyping neoantigen-specific clonotypes
- Association with clinical parameters in a variety of disease and treatment settings
- Assay development and optimization: cross-institutional validation with MSKCC

Acknowledgements

Cancer Immunology
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PATIENTS AND THEIR FAMILIES!

JOHNS HOPKINS MEDICINE | BLOOMBERG~KIMMEL INSTITUTE FOR CANCER IMMUNOTHERAPY