SITC Advances In Cancer Immunotherapy Series: *Immunotherapy on the Horizon*

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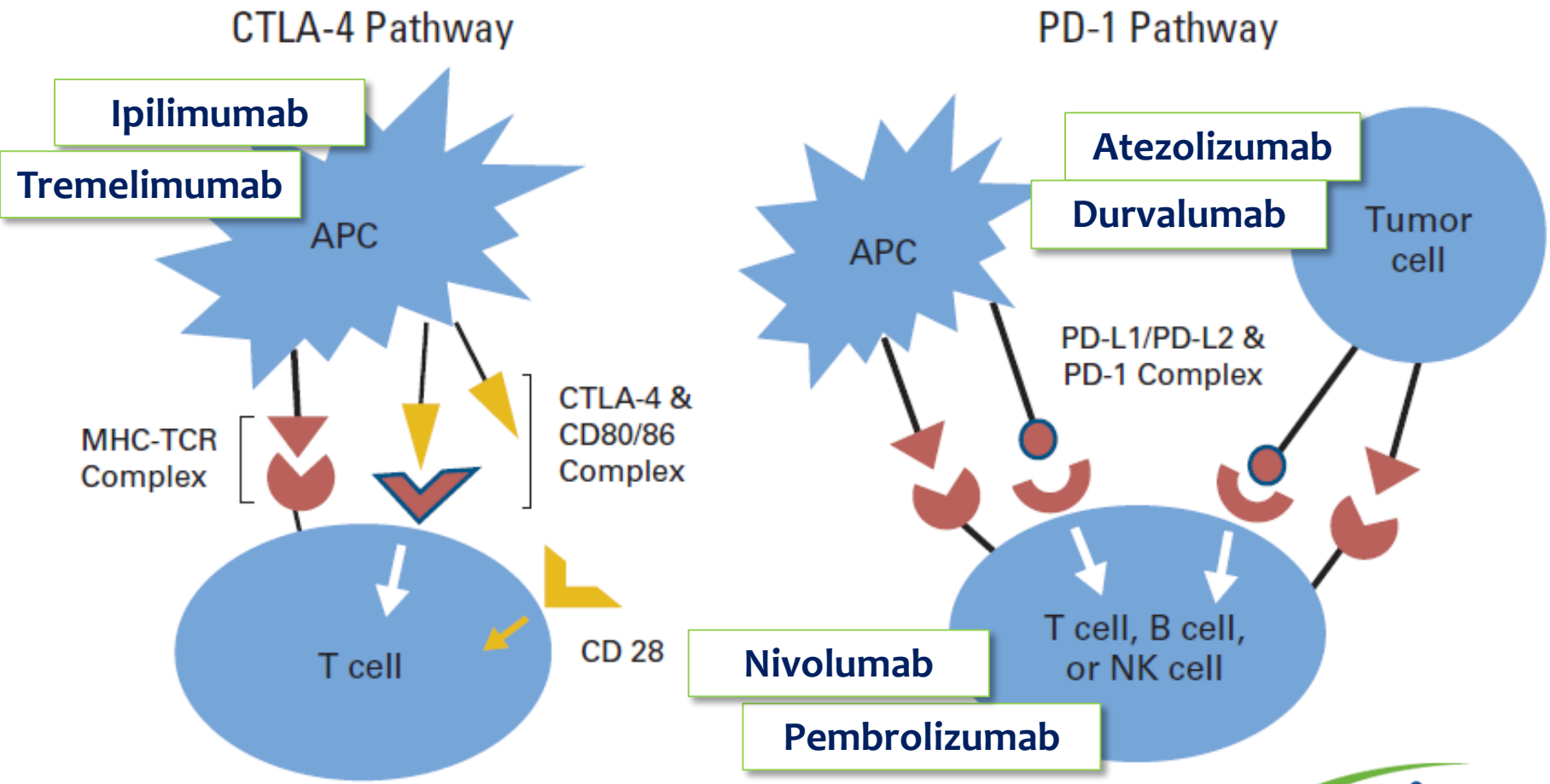
The Angeles Clinic & Research Institute

Disclosures

* Speaker's bureau for Bristol Myers Squibb
* ASCO Government Relations Committee Member
* Non-FDA approved therapies will be discussed

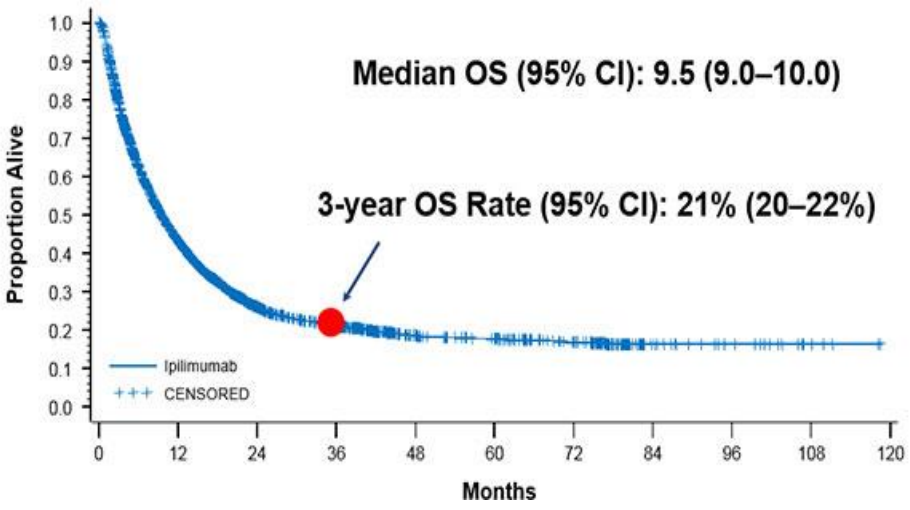
Immunotherapy's Great Promise



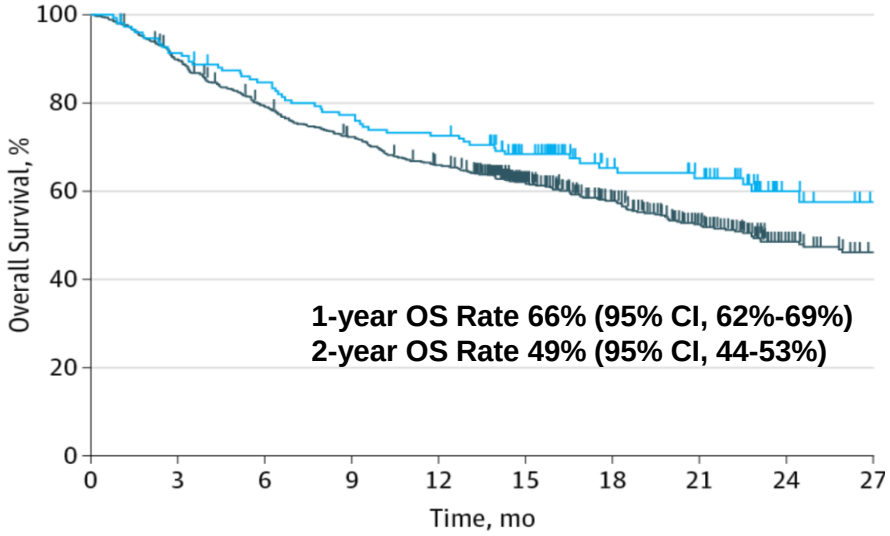
Brahmer JR. J Clin Oncol. 2013; 31:1021-28.

Improving Long-Term OS

Ipilimumab



Pembrolizumab



Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. JCO 2015
Association of Pembrolizumab With Tumor Response and Survival Among Patients With Advanced Melanoma. JAMA. 2016;315(15):1600-1609.

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Improving Toxicity*

Toxicity	Nivolumab		Ipilimumab		*Nivolumab and Ipilimumab	
	All grade	Grade 3 and 4	All grade	Grade 3 and 4	All grade	Grade 3 and 4
Diarrhea	19.2	2.2	33.1	6.1	44.1	9.3
Fatigue	34.2	1.3	28	1	35	4.2
Rash	25.9	0.6	32.8	1.9	40.3	4.8
Increased ALT	3.8	1.3	3.9	1.6	17.6	8.3
Increased AST	3.8	1.0	3.5	0.6	15.3	6.1
Hypothyroidism	8.6	0	4.2	0	15	0.3
Colitis	1.3	0.6	11.6	8.7	11.8	7.7
Arthralgia	7.7	0	6.1	0	10.5	0.3
Dyspnea	4.5	0.3	4.2	0	10.2	0.6

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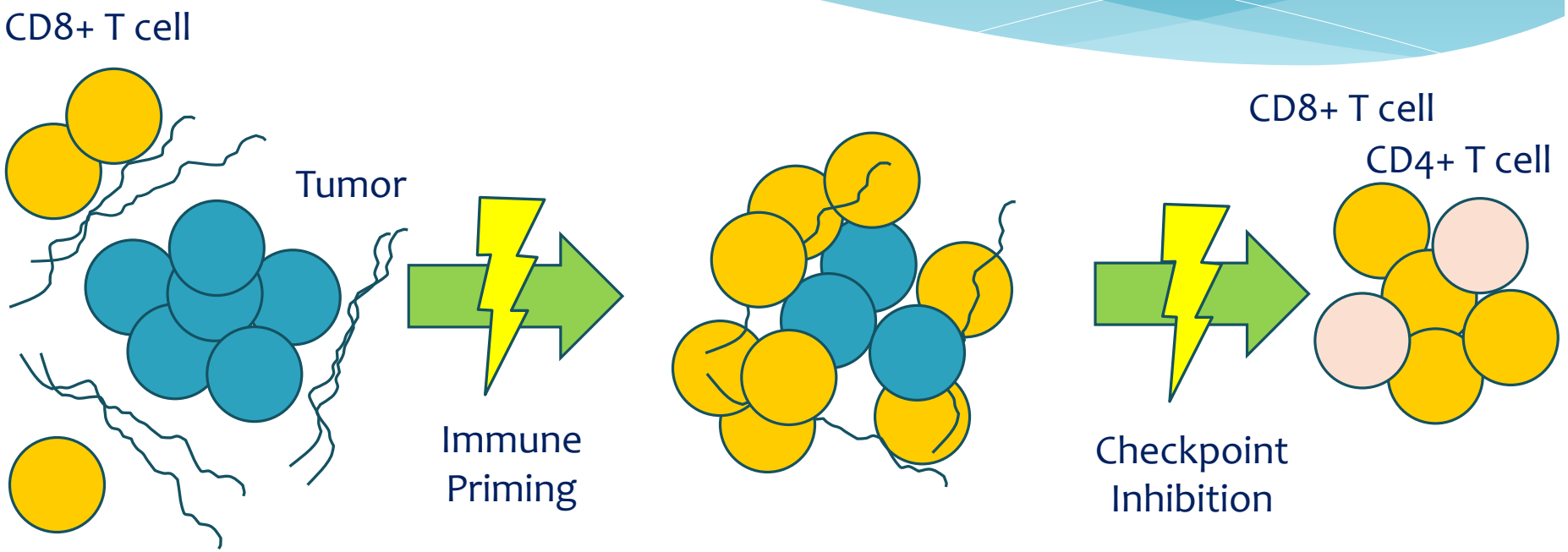
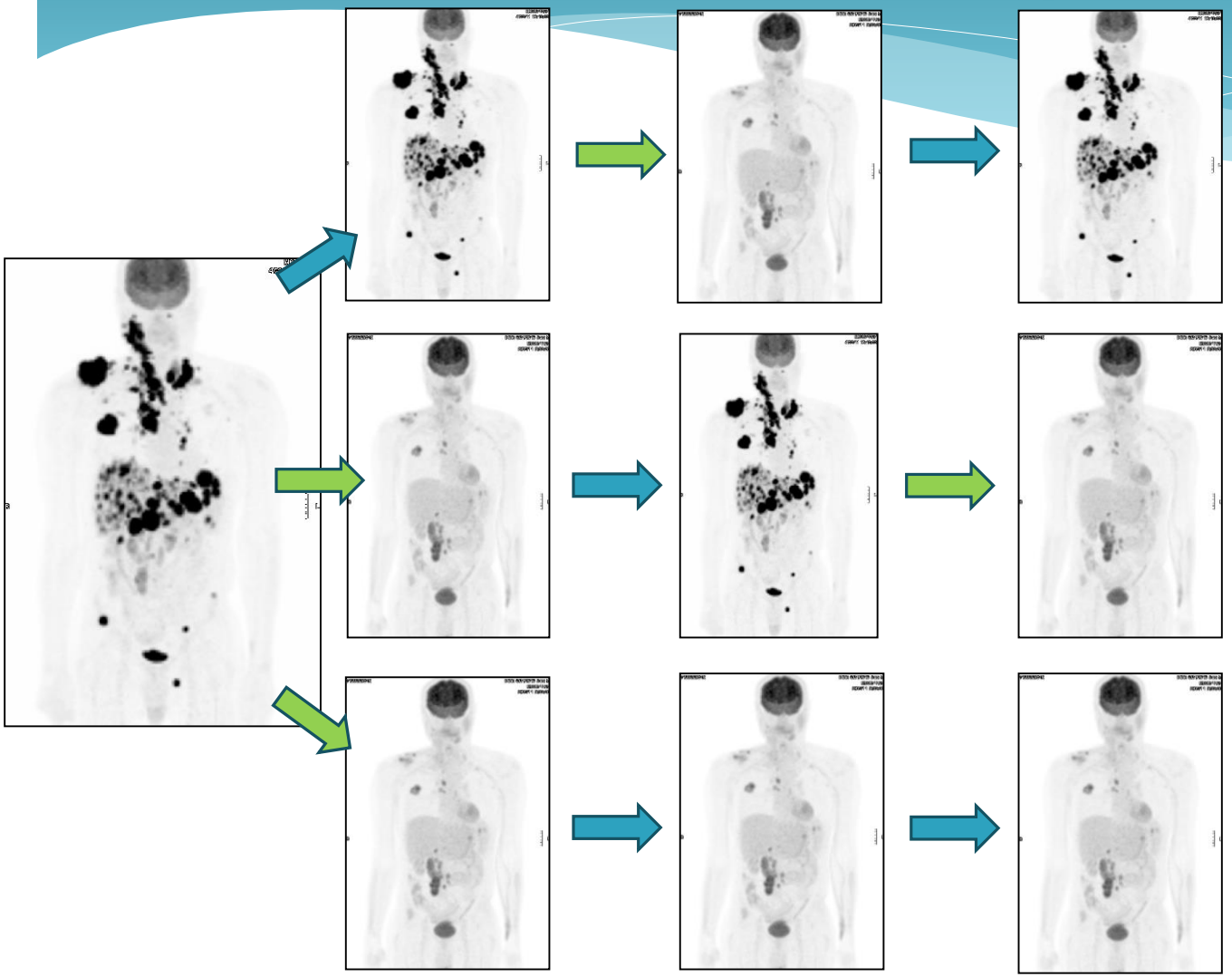
Larkin J et al. N Eng J Med. 2015; 373:23-34.

Simple… Right?



Prediction & Prognosis
Critical "Checkpoints"



Host, Tumor Characteristics Have a Major Role in Outcomes

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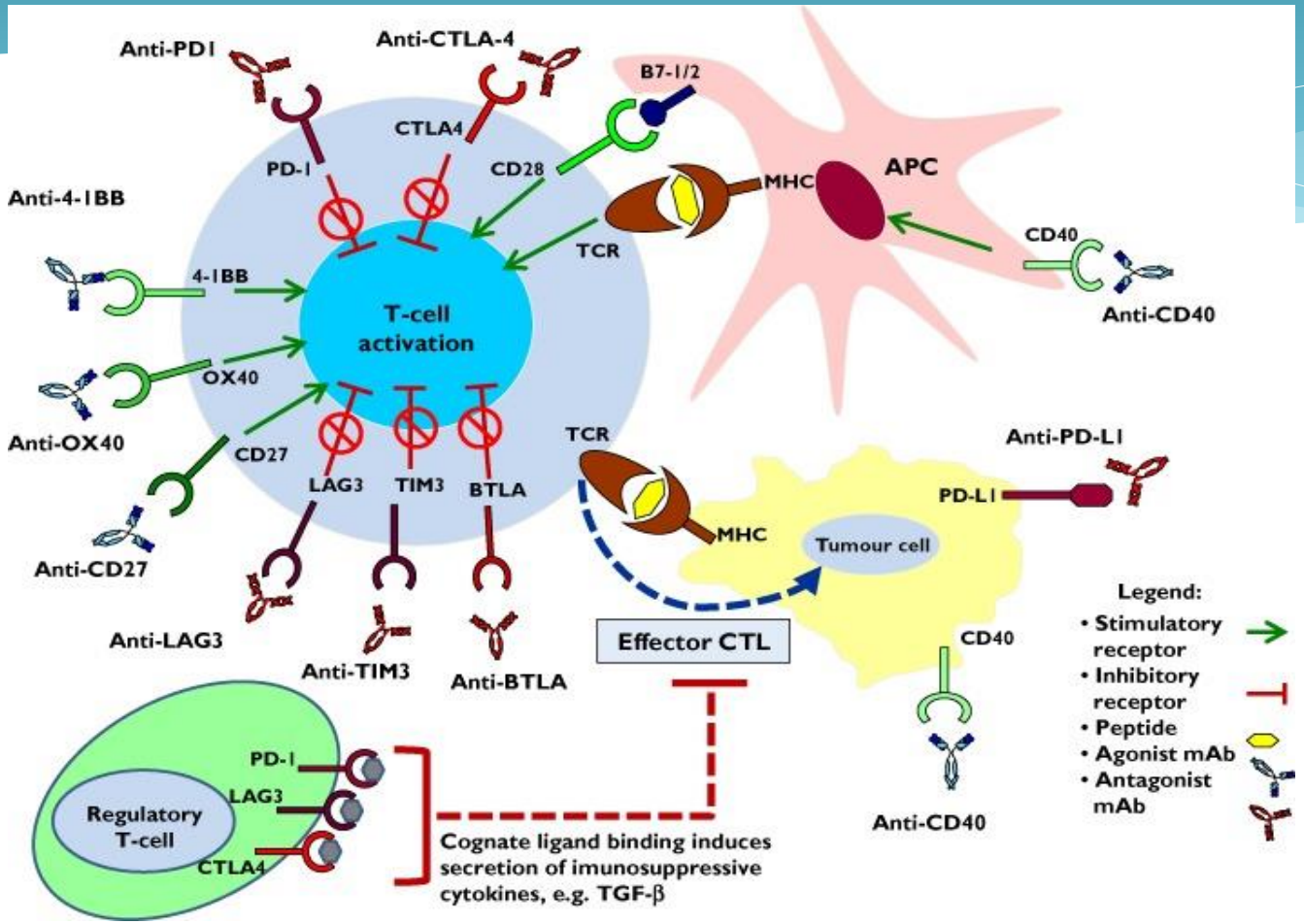
A Theatre of Mighty Designs

Eight Bells, Winslow Homer, 1886

A Theatre of Mighty Designs



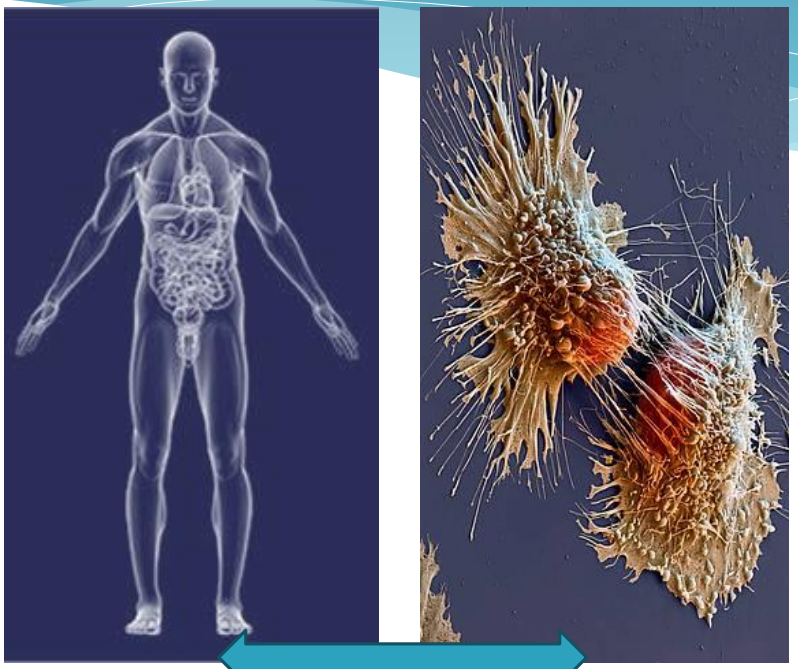
Novel antibodies targeting immune regulatory checkpoints for cancer therapy
Bri J Clin Pharm 76(2) May 2013

Host and Tumor Profiling

T Cell Priming (TILs, PDL1, microenvironment)

Multivariate Analysis (Immunoscore)

Immune Competence (CyTOF)



Mutation Burden & Neoantigens

Multispectral Imaging (microenvironment)

Tumor IHC , CTC DNA (serology)

Match Patient to Therapy

Immunomonitoring
(Predictive Biomarkers, Host Response)

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Learning Objectives

* Describe current state of biomarker analysis
  * Patient (tissue & liquid biopsies)
  * Tumor (tissue & liquid biopsies)
* Discuss technologies for identifying signatures of immune function
  * Pre- and post- therapeutic intervention
* Applying the science to everyday practice

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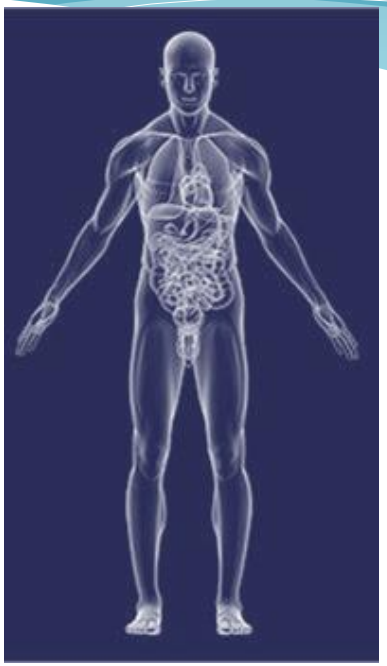

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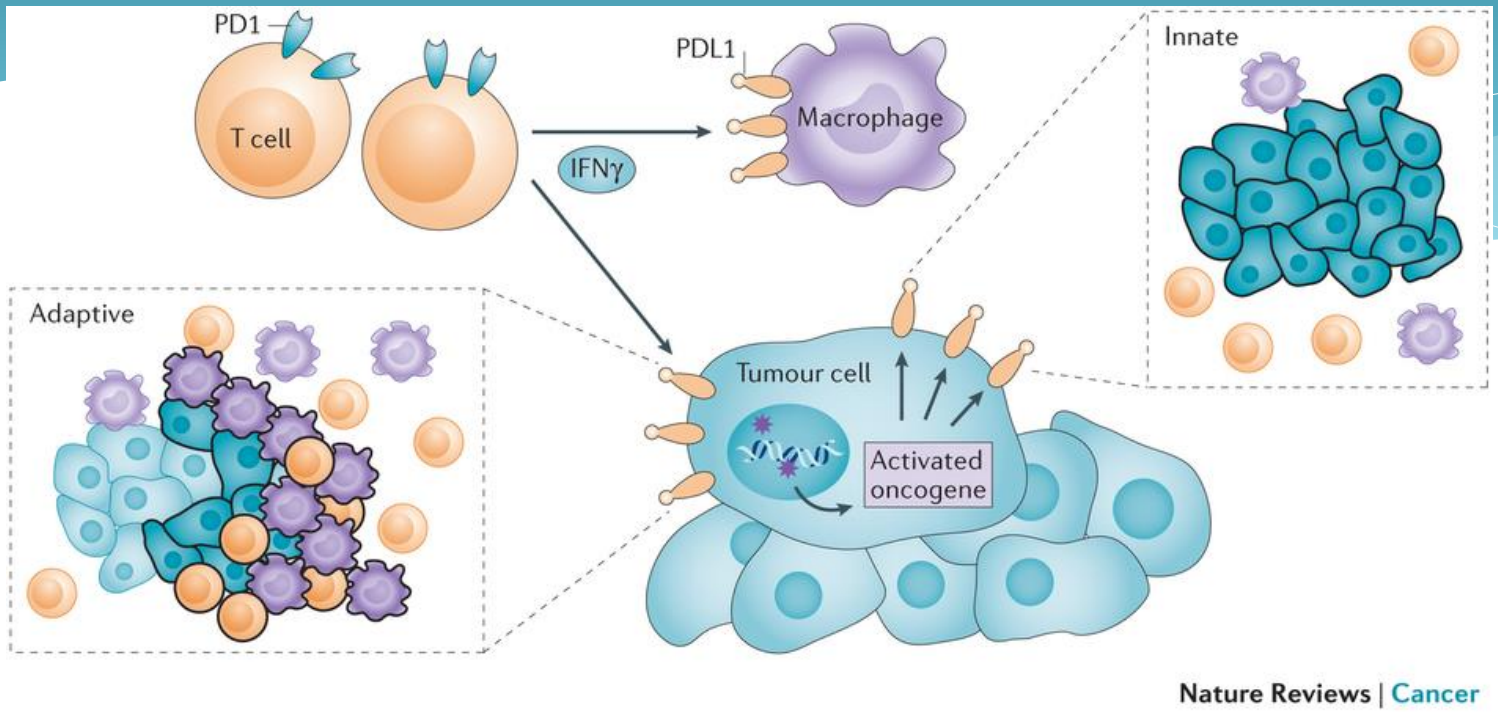
Setting The "Stage"



- Intratumoral lymphoid infiltrates, PDL1 expression have both been shown to impact outcomes
- Becoming as critical as TNM for staging in cancer

Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nature Reviews Cancer 16, 275–287 (2016)

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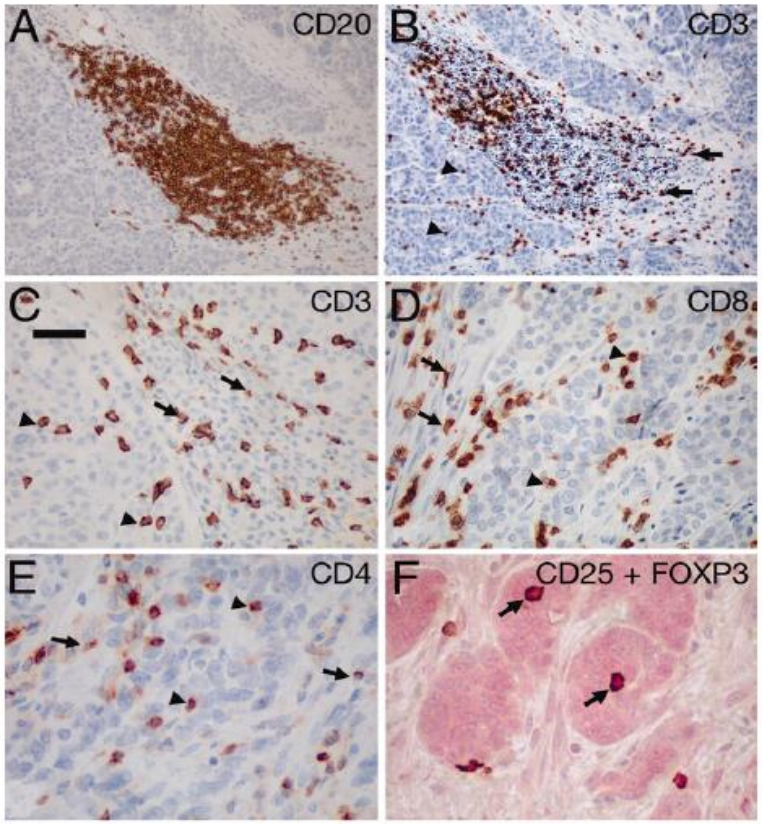
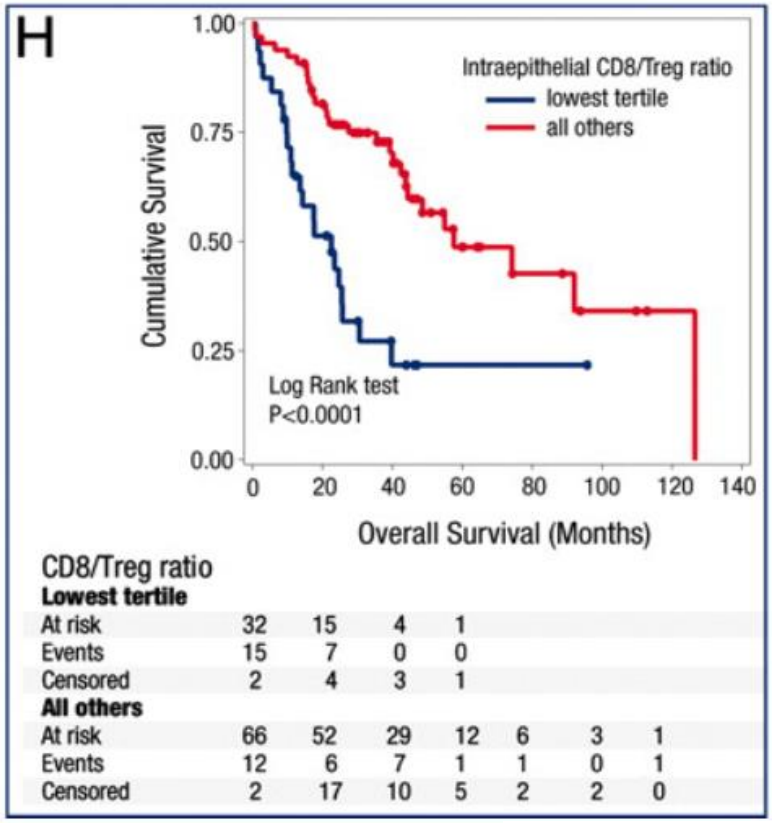
Intraepithelial CD8⁺ TILs and a high CD8⁺/Treg ratio are associated with favorable prognosis in ovarian cancer

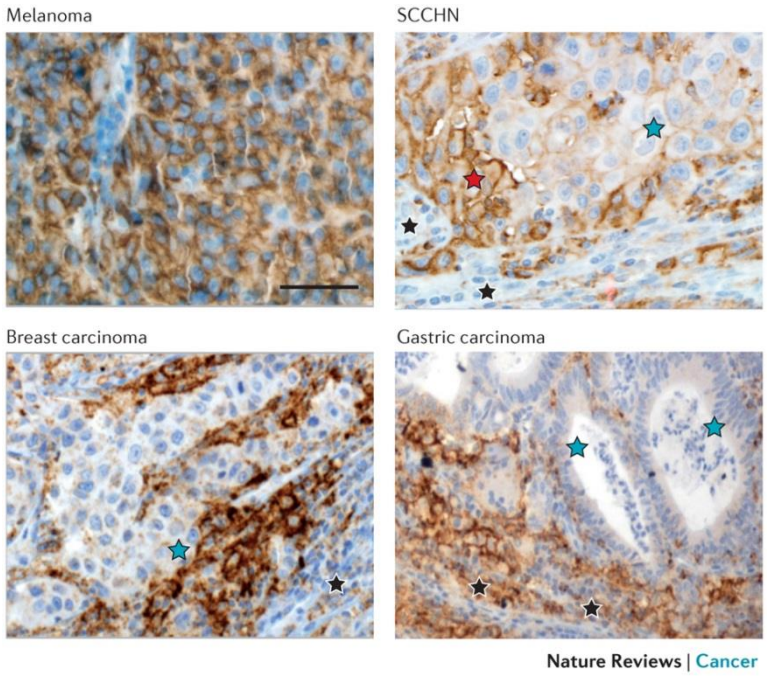




CD8/Treg ratio							
Lowest tertile							
At risk	32	15	4	1			
Events	15	7	0	0			
Censored	2	4	3	1			
All others							
At risk	66	52	29	12	6	3	1
Events	12	6	7	1	1	0	1
Censored	2	17	10	5	2	2	0

Proc Natl Acad Sci U S A. 2005 Dec 20;102(51):18538-43.

PDL1: To Be or Not To Be



- May be missed in small Bx specimens
- Can vary over time and by anatomical site
- XRT, chemo, kinase inhibitors may alter expression
- Variability in specimen handling & processing
  - Antibodies used for detection have different affinities and specificities
- Membranous vs cytoplasmic
  - Only membranous PDL1 is functionally relevant
- Expressed by multiple cell types within TME, posing challenges for scoring & interpretation

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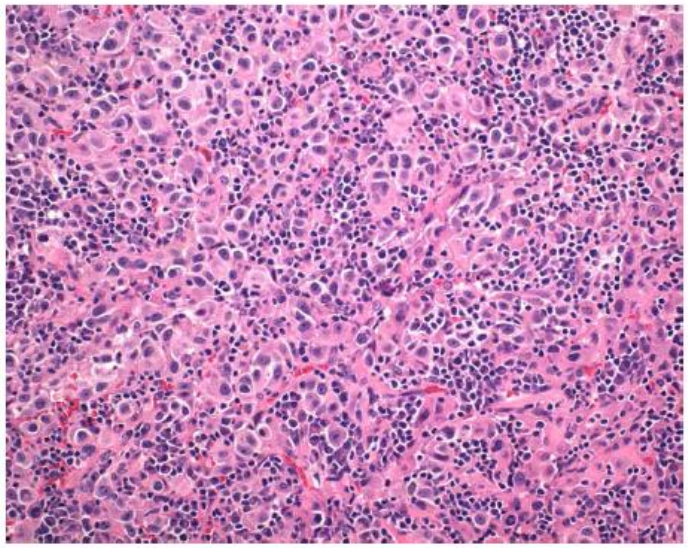
Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nature Reviews Cancer 16, 275–287 (2016)

TME: When T Cells Are Not Enough

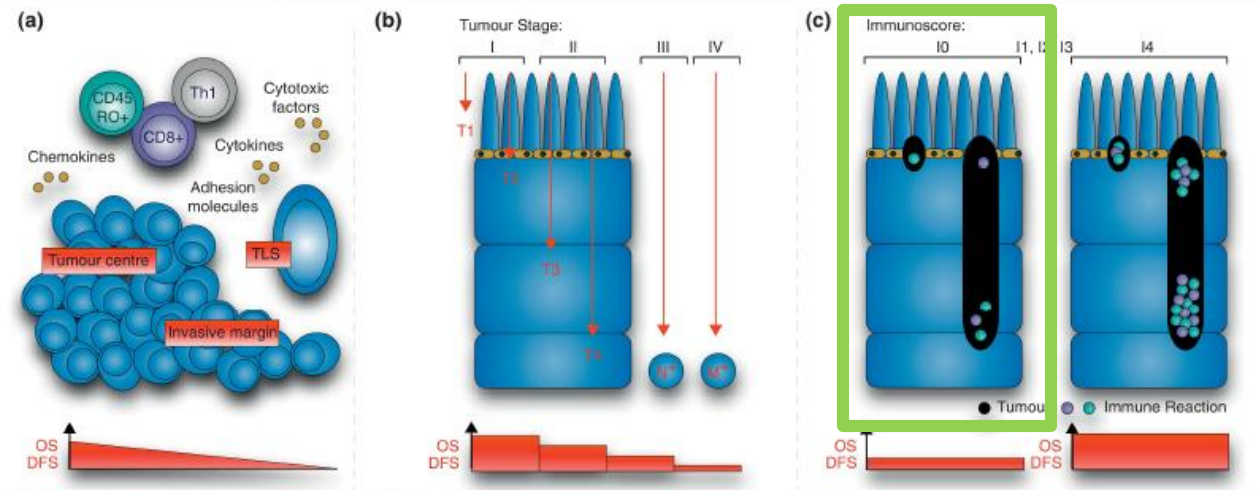
Inflamed melanomas containing CD8⁺ T cells have highest expression of immune inhibitory pathways



- **IDO** (indoleamine-2,3-dioxygenase)
  - Tryptophan depletion
- **PD-L1**
  - Engages PD-1 on T cells
- $CD4^+CD25^+FoxP3^+$**Tregs**
  - Extrinsic suppression
- T cell **anergy** (B7-poor)
  - T cell intrinsic TCR signaling defect

Gajewski T. Immunotherapeutic barriers at the level of the tumor microenvironment. SITC Nov 2012

Immunoscore: Type, density, and location of immune cells within human colorectal tumors predict clinical outcome

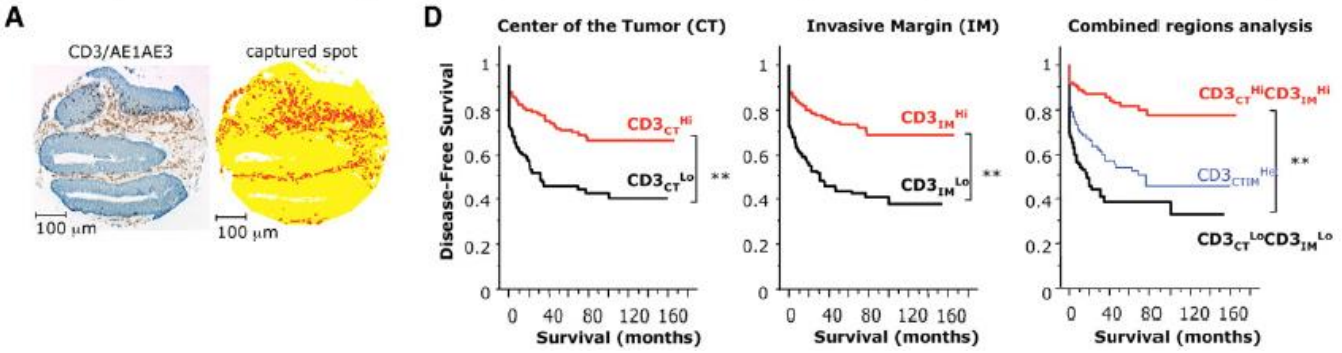


Immune Contexture	Key Parameters
Type	CTLs (CD3+CD8+) Memory T cells (CD3+CD45RO+)
Location	Tumour centre (CT) Invasive margin (IM) Presence and quality of TLS
Density	Continuous
Functional orientation	Th1 cell-associated factors Cytotoxic factors Chemokines, cytokines Adhesion molecules

TNM Staging	Key Parameters		
Tumour (T)	Longitudinal extent of tumour burden		
Lymph node (N)	Presence of cancer cells in draining and regional lymph nodes		
Metastases (M)	Evidence of distant site metastases		
Cox analysis (ref. 23)	DFS HR/P-value	OS HR/P-value	DSS HR/P-value
	1.38/0.09 ns	1.18/0.29 ns	1.43/0.10 ns

Immuno-score	Key Parameters		
Type	CTLs (CD3+CD8+) Memory T cells (CD45RO+)		
Location	Tumour centre (CT) Invasive margin (IM)		
Density	Predefined cut points		
Cox analysis (ref. 23)	DFS HR/P-value	OS HR/P-value	DSS HR/P-value
	0.64/<0.0001	0.71/<0.0001	0.63/<0.0001

Adapted from Angell et al.
Curr Opin Immunol. 2013;25:261



Adapted from Galon et al.
Science 2006;313:1960-4

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Gnjatic S. Immune Monitoring of T and B Cell Responses. SITC Primer Nov 2013

Liquid Biopsy: CyTOF

* Mass cytometry (CyTOF) enables simultaneous analysis of up to 40 PBMC markers in a single staining panel
  * B-cell subset analysis in pts with allergy vs non-allergy showed distinct B cell population in the allergy group, which correlated with IgE levels
  * Demonstrated heterogeneity in B cell malignancies (DLCBCL, HL)
  * Correlated with immune expansion in pts treated with XRT and PD-1 inhibition

Using mass cytometry to identify novel B cell subsets in red meat allergy. Journal of Immunology 196.1 Supplement (2016): 191-25.
Mass Cytometry Based Classification of Inter-and Intra-Tumoral Heterogeneity in Diffuse Large B-Cell Lymphoma. Blood 126.23 (2015): 3908-3908.
Local tumor irradiation combined with α-PDL-1 immune checkpoint inhibition results in local and systemic anti-tumor responses: Successful translation of a mouse model to a human case series. Cancer Research 74.19 Supplement (2014): 2941-2941.

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Peripheral Blood Immunoscore

* Metastatic breast CA pts randomized to chemo + vaccine
  * Predefined analyses of immune subtypes (CD4, CD8, NK, Tregs, MDSC) showed no difference in TTP in chemo groups
  * **Did show statistically different TTP in pts getting vaccine**
  * ? surrogate biomarker for vaccine/immunotherapy
* Baseline characteristics of melanoma pts prior to ipilimumab
  * **Low** absolute monocyte counts, **MDSC frequencies** and **high** absolute eosinophil counts + CD4/CD25/FoxP3 **Treg frequencies** were significantly associated with better survival

Combination of docetaxel and recombinant vaccine enhances T-cell responses and antitumor activity: effects of docetaxel on immune enhancement. Clinical Cancer Research 14.11 (2008): 3536-3544.
Baseline peripheral blood biomarkers associated with clinical outcome of advanced melanoma patients treated with ipilimumab. Clinical Cancer Research (2016).

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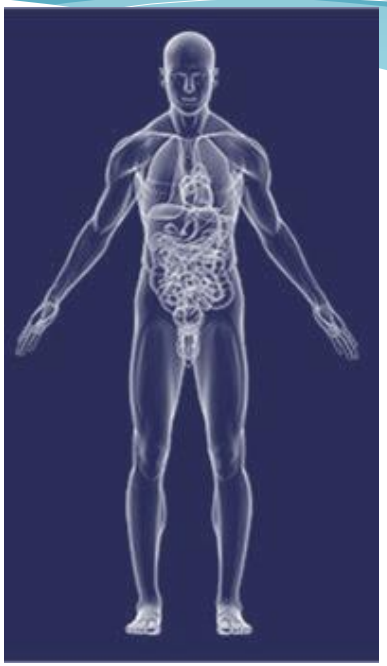
Tumor Profiling

T Cell Priming
(TILs, PDL1, microenvironment)

Multivariate Analysis
(Immunoscore)

Immune Competence
(CyTOF)



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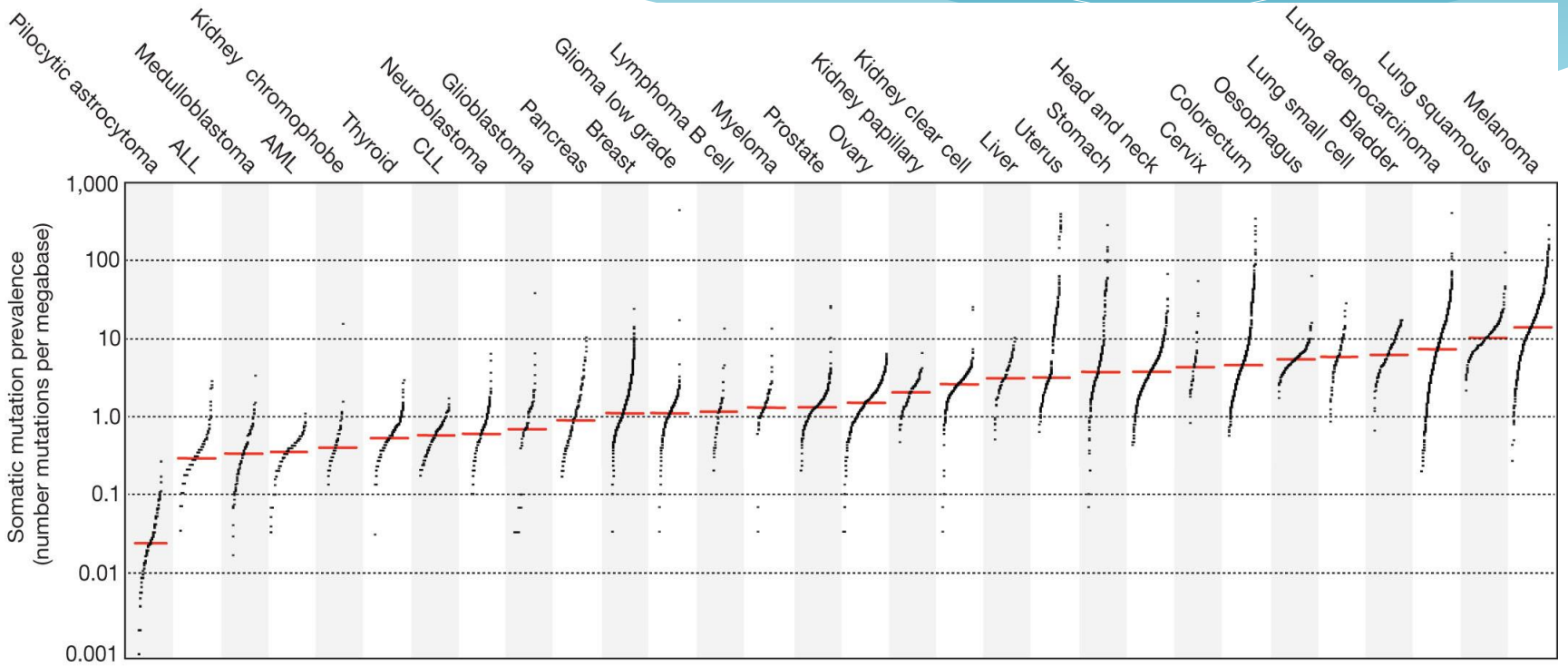
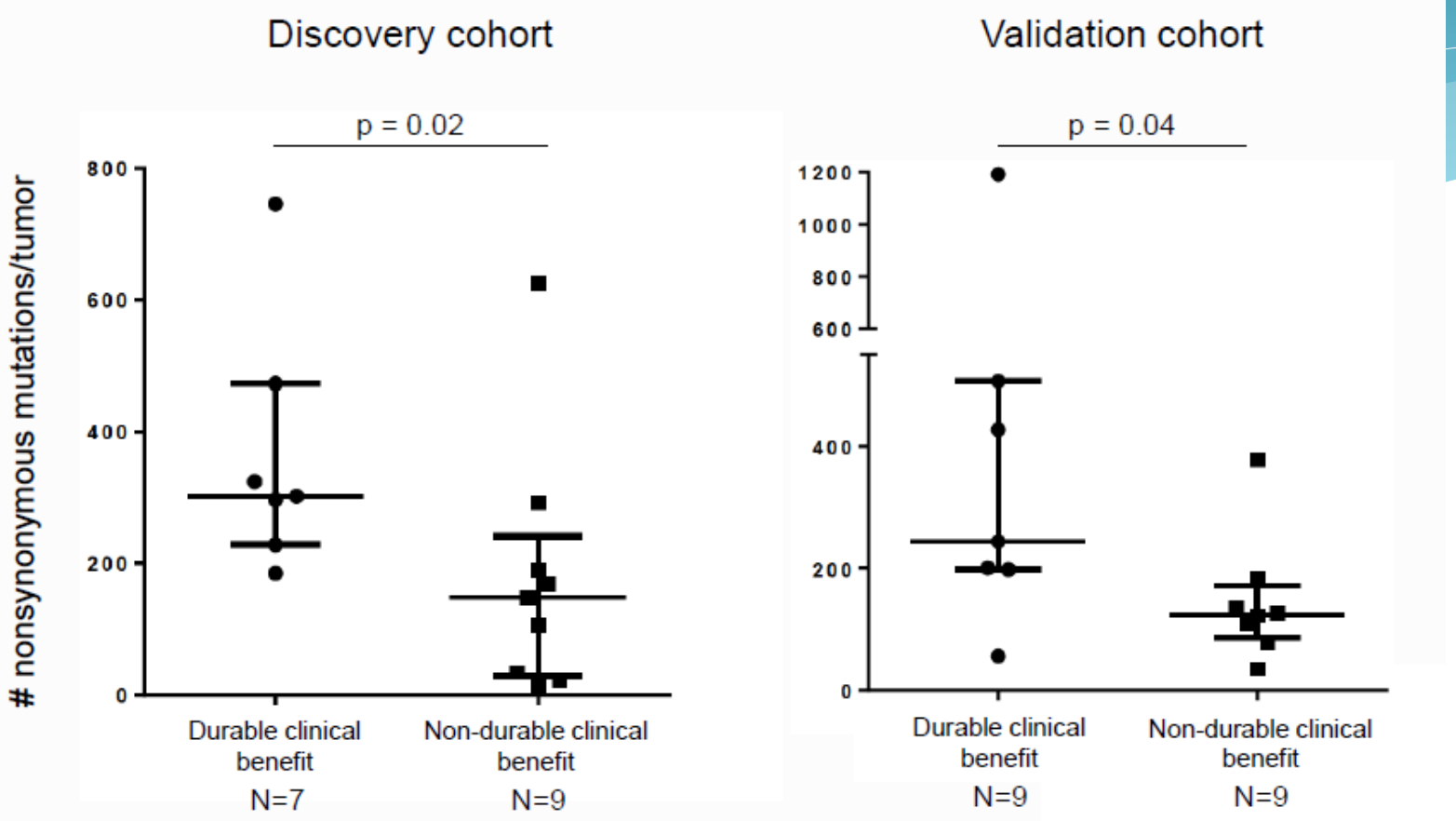


LB Alexandrov et al. Nature 000, 1-7 (2013) doi:10.1038/nature12477

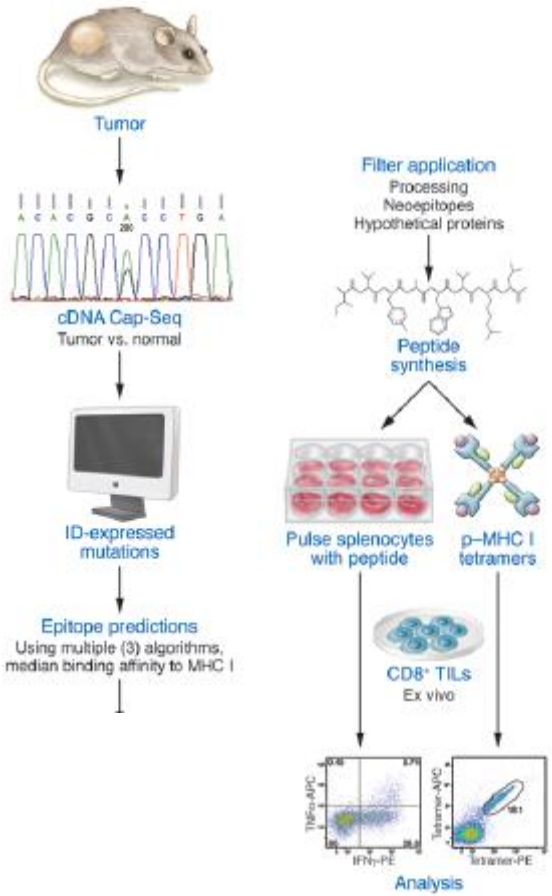
NSCLC: Mutational load and clinical outcome to antiPD-1 therapy (MSKCC)



Rizvi et al, *Science* 2015

Haanen J. Use of MHC multimers to identify tumor reactive T cells. SITC Nov 2014

Neoantigens: Personalizing Immune Tx



* **Inconsistencies in mutational burden and response to immunotherapy**
  * Mutated genes in *individual* tumors may be immunogenic
* Whole exome sequencing identifies neoantigen-specific CD8+
* Clinically applicable for TIL or personalized vaccine therapy + checkpoint inhibition

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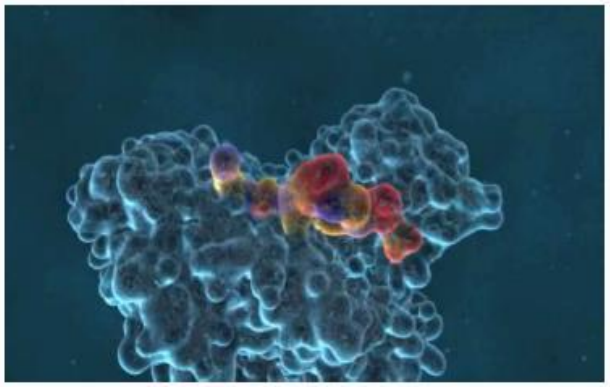
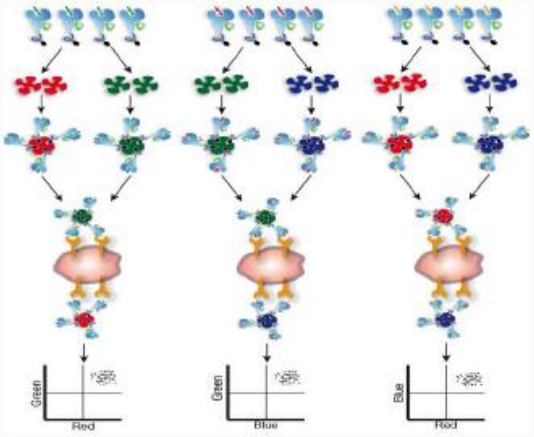
Tumor neoantigens: building a framework for personalized cancer immunotherapy. J Clin Inv 125.9 (2015): 3413-3421.

Tools for high-throughput analysis of neo-antigen specific CD8 T cell responses



Toebes et al. *Nat. Med.* 2006
Bakker et al. *PNAS* 2008



Hadrup *Nat Methods* 2009
Kvistborg *Science Transl Med* 2014

Allows analysis of T cell responses against *100s-1000s of antigens*

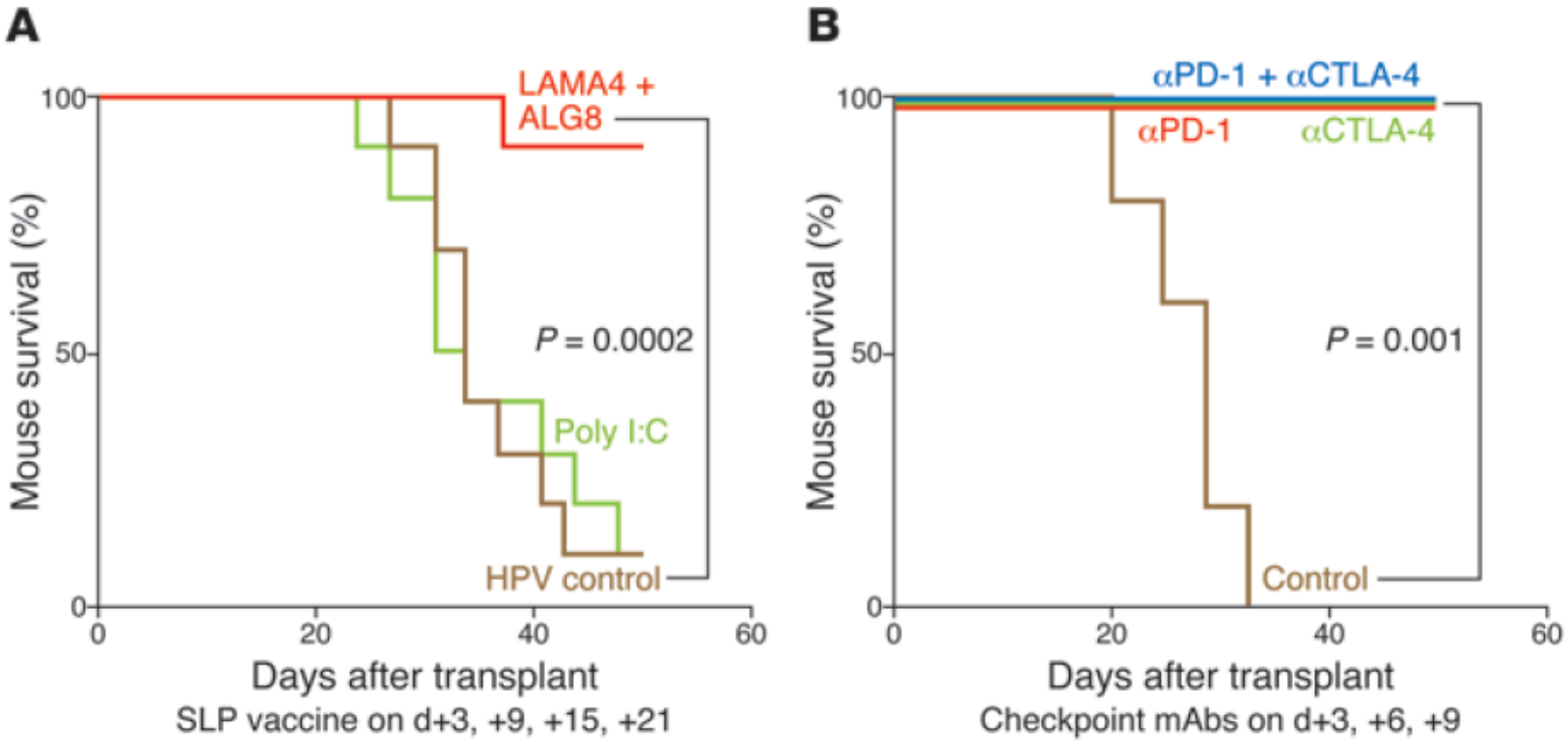
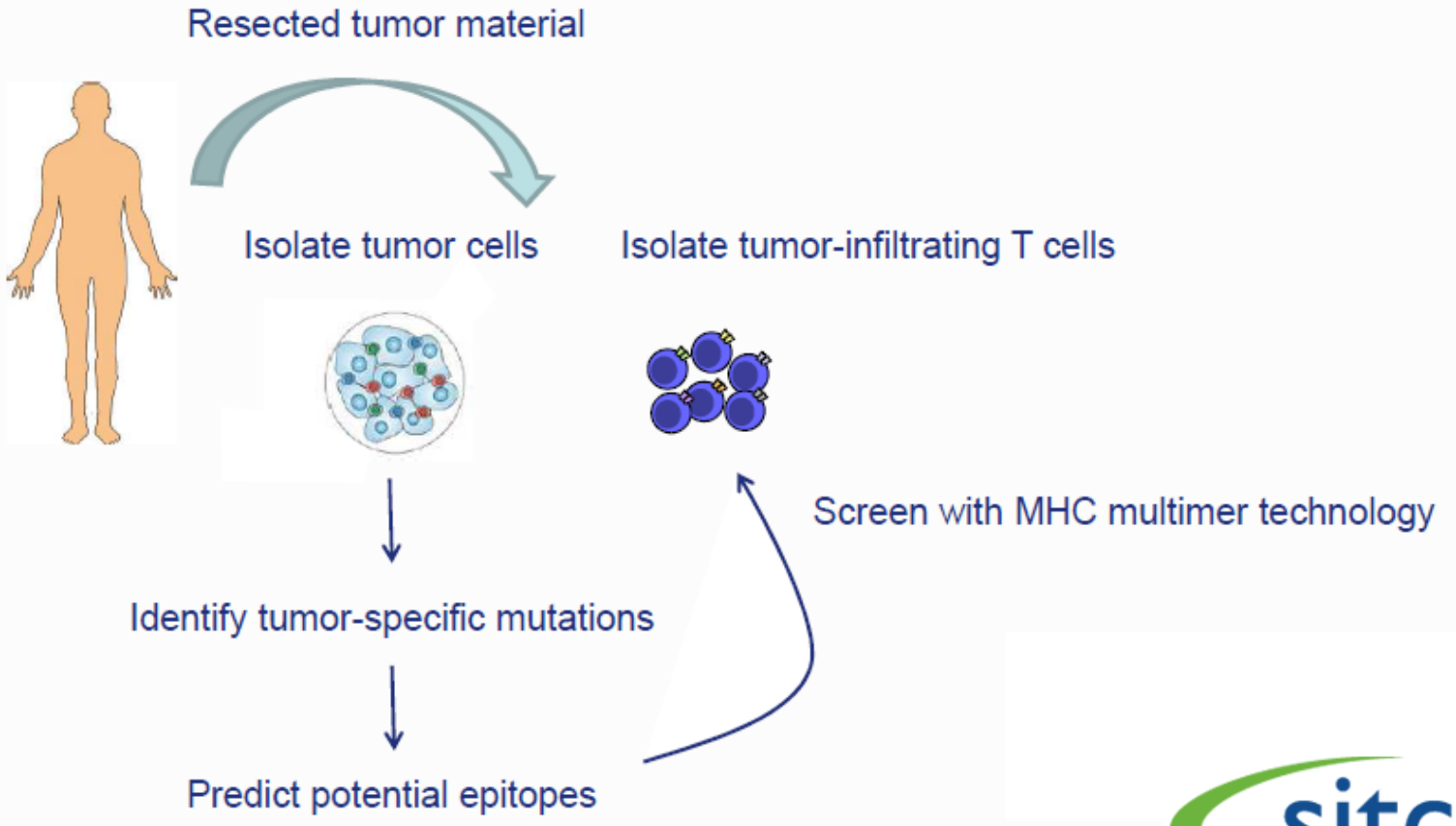


Figure 2. Mutant neoantigen–specific peptide vaccines induce therapeutic effects comparable to those of checkpoint blockade therapy. Kaplan-Meier survival curves of tumor-bearing mice therapeutically vaccinated with a vaccine comprising poly I:C plus either ALG8 plus LAMA4 SLP, control SLP (HPV peptide), or buffer (**A**) or therapeutically treated with mAbs to CTLA-4 and/or PD-1 immune checkpoints (**B**). Adapted with permission from *Nature* (ref. 77; Figure 1A and Figure 2, D and E)

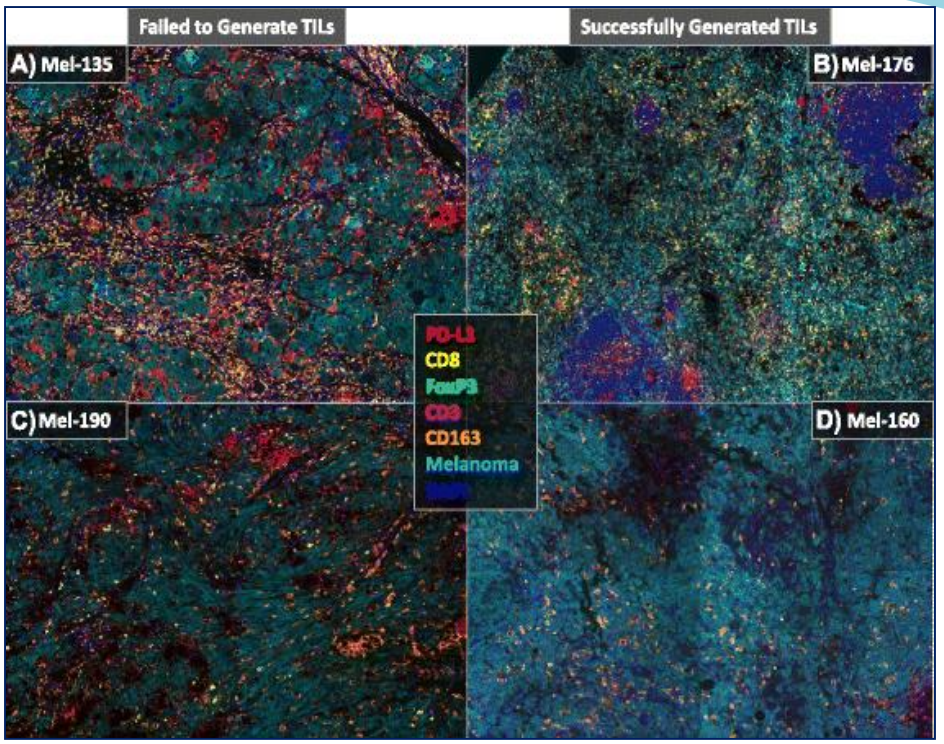
Tumor neoantigens: building a framework for personalized cancer immunotherapy. J Clin Inv 125.9 (2015): 3413-3421.

Analyzing the neo-antigen-specific T cell repertoire in human cancer?



Haanen J. Use of MHC multimers to identify tumor reactive T cells. SITC Nov 2014

Multispectral Imaging



- Immune cell phenotypes visualized and quantified simultaneously
- Enables study of spatial distribution and proximity within TME
- Improves understanding of TME immune suppression, TIL harvest potential

Multispectral imaging of formalin-fixed tissue predicts ability to generate tumor-infiltrating lymphocytes from melanoma. Journal for immunotherapy of cancer 3.1 (2015): 1.
Multispectral imaging and objective assessment of immune-tumor interactions in non-small cell lung cancer. Journal for immunotherapy of cancer 3.2 (2015): 1.

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Multispectral Imaging

* Breast CA pts receiving neoadjuvant chemo
  * Pts with path CR showed no sig difference in stromal vs intratumoral CD8 (p=.11) or CD4 (p=.75)
  * Pts **without pCR** had **higher stromal** CD8 (p=.03) and CD4 (p=.05) vs intratumoral density

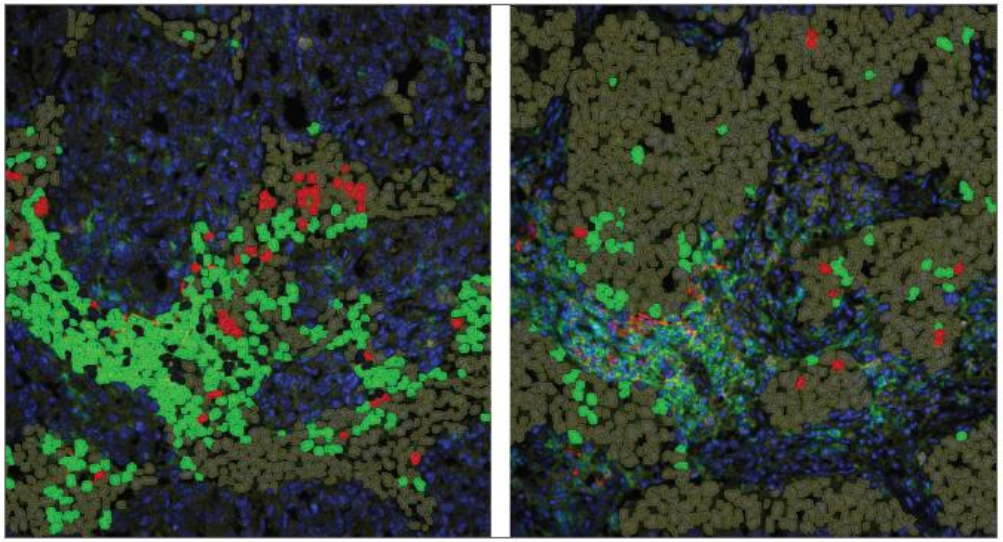
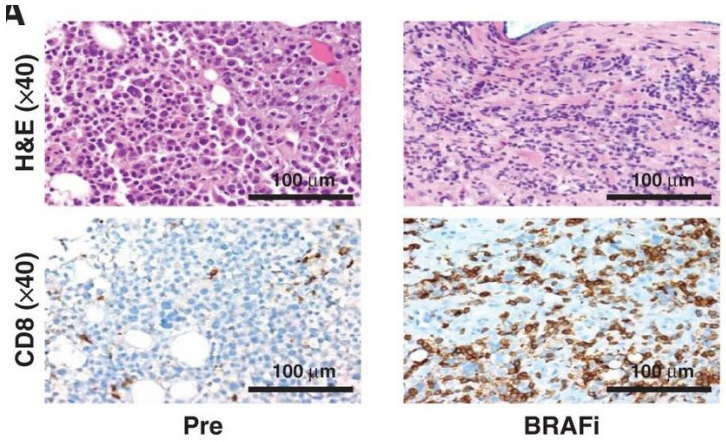


Figure 5. Helper T-cells and cytotoxic T-cells in breast cancer: CD4+ T-cells green, CD8+ T-cells in stroma (left). CD4+ T-cells green, CD8+ T-cells in tumor (right).

Abstract P5-04-07: Multispectral imaging allows visualization and quantification of multiple immunologic cell types in breast tumor tissues. Cancer Research 75.9 Supplement (2015): P5-04.

Tumor IHC & Liquid Biopsy



* Converting "cold" to "hot" tumors
  * Static vs dynamic sampling
* Detecting immune-resistant clones
  * Serum ULBP2 (NK ligand) associated with lower OS (independent predictor of prognosis in Stage I-III melanoma)
* CTCs emerging for baseline & ongoing treatment responses in lung, prostate CA

BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. Clin Cancer Res 2013; 19:1225–1231
Natural Killer Cell Recognition of Melanoma: New Clues for a More Effective Immunotherapy. Frontiers in immunology 6 (2015).
Abstract CT306: Radiolabeled anti-PSMA antibody J591 immunotherapy is associated with favorable circulating tumor cell (CTC) count control in men with castration-resistant prostate cancer." Cancer Research 75.15 Supplement (2015): CT306-CT306.

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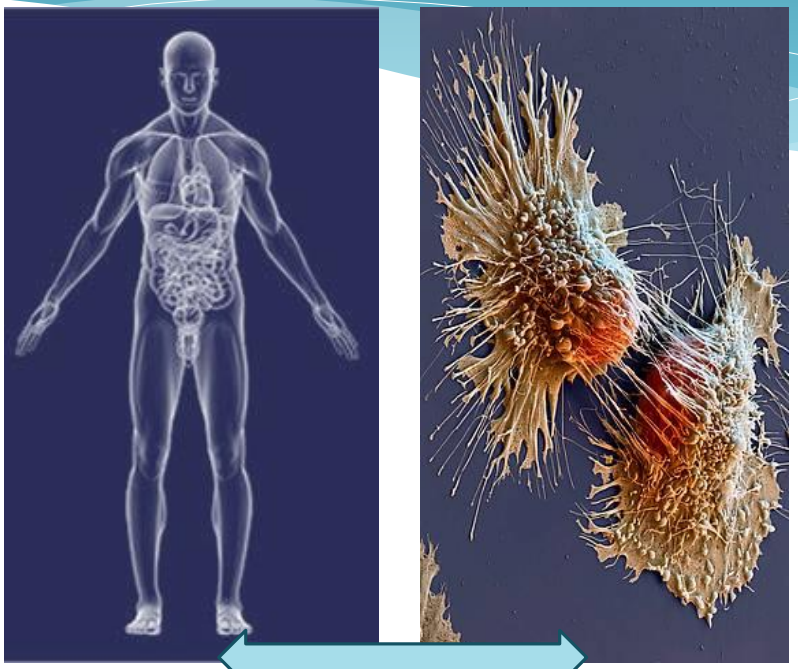

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(Predictive Biomarkers, Host Response)

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Challenges of Immune Monitoring

- **Is it a "cold" or a "hot" tumor?**
  - Should I use single, or combined agent therapy?
- **Is my immune therapy working?**
  - What should we check? Tissue, or peripheral blood?
  - When should we check, and how often?
- **Is the tumor adapting?**
  - Can we detect this before tumor grows on imaging?

TCR Sequencing

Adaptive biotechnologies

RESEARCH | DIAGNOSTICS | **PIPELINE** | INSIGHTS | PUBLICATIONS

QUANTIFYING TUMOR INFILTRATION LYMPHOCYTES IN SOLID TUMORS

Adaptive is validating a novel clinical diagnostic to quantify the presence and clonality of tumor infiltrating lymphocytes (TILs) that is a reliable measure of "immunocompetence" to predict and monitor response to immune-modulating cancer therapies.

Preliminary data in variety of tumor types including colorectal cancer, ovarian cancer and glioblastoma suggest that a higher level of clonally expanded TILs may correlate with better patient outcomes. Adaptive is invested in research and development to validate and incorporate into clinical practice this potentially new and meaningful immune molecular diagnostic for better prognostic staging of patients with solid tumors.

The TIL assay is also actively being validated as a potential predictive diagnostic to monitor response to immune-modulating cancer therapeutics. Several human and mouse studies are assessing response to a variety of cancer immunotherapies including anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and anti-programmed cell death-1 (PD-1). Preliminary results suggest that greater TIL clonal expansion and percent tumor infiltration in tumor tissue prior to treatment may be predictive biomarkers of response.

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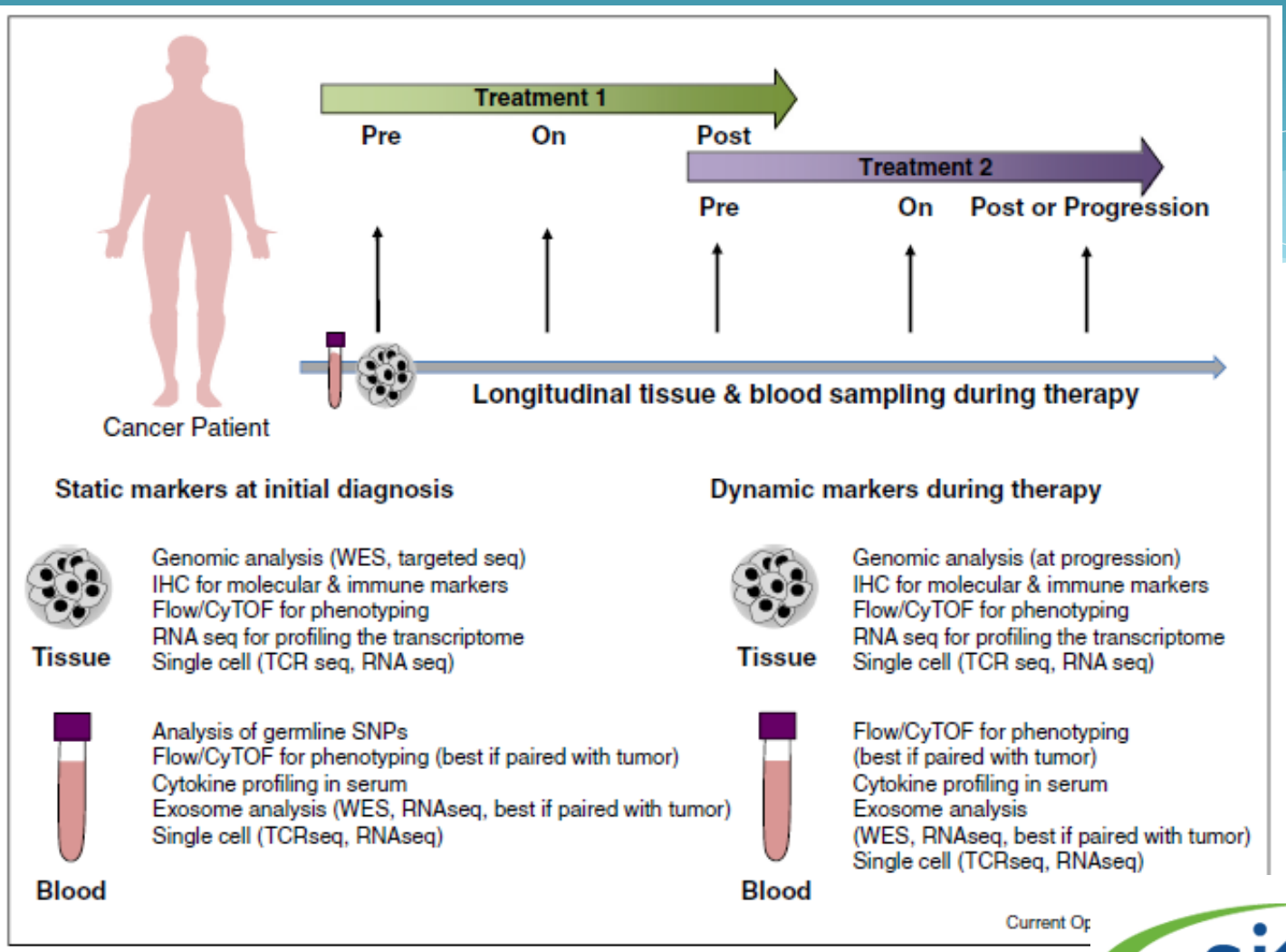
http://www.adaptivebiotech.com/immunoseq

Challenges of Immune Monitoring



Monitoring immune responses in the tumor microenvironment. Curr opn immunol 41 (2016): 23-31.

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

Patients with NY-ESO-1 antibodies 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

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

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Challenges of Immune Monitoring

ex vivo Immune Biomarker Monitoring in the Clinic

- The Challenge of Collecting active PBMCs in the Clinic
  - Multi-step processing, variable time intervals & temperature, preservation, transportation
  - Loss of signaling elements (granulocytes, platelets, RBCs)
  - Rejection/Acceptance Criteria?
- Whole Blood Cultures – Standardization and Incorporation into Clinical Trials
  - Novel technology; validation, reproducibility, and suitability for the clinic.
  - Cytokine, Chemokine and gene expression end-points.
  - Monitoring for Safety (Immunotoxicity) and Pharmacodynamics in cancer trials.

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Elsinger D. Standardization and validation of immune monitoring. SITC Nov 2014

ImmunoPET Imaging

* Thirteen pts with metastatic BC
* Immuno-PET/CT with radiolabeled CEA peptide injected 24-30h before, compared to CT TAP and PET/CT
* Immuno-PET sensitivity 93.8% (vs 74.6% and 84.7%)
  * 100% sensitivity for bone, liver, skin, and brain, 94% for LN
  * Brain lesions only detected by immuno-PET

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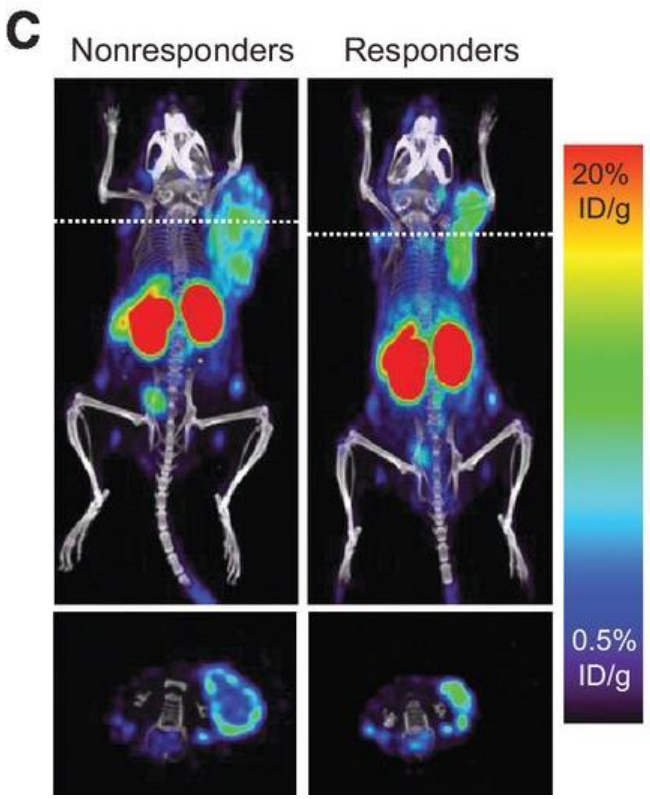
Improvement in imaging of metastatic breast cancer (BC) with a novel pretargeted immuno-PET targeting CEA: First clinical results. ASCO Annual Meeting Proceedings. Vol. 33. No. 15_suppl. 2015.

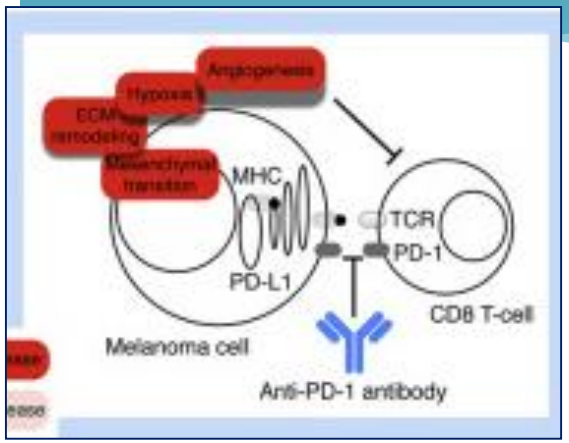
ImmunoPET Imaging

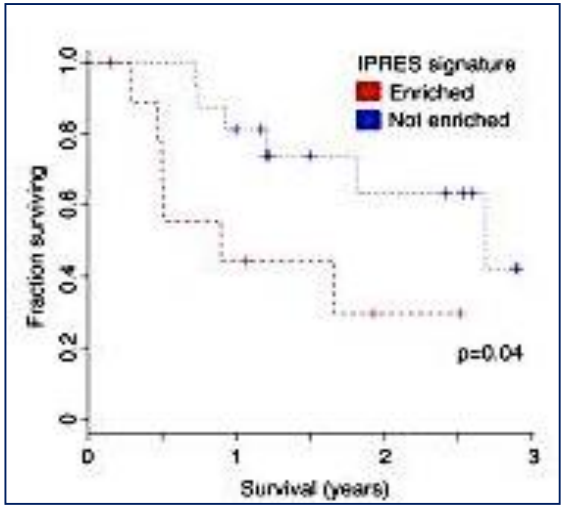


* Anti-CD8 immuno-PET sensitive for changes in systemic and tumor-infiltrating CD8+ in preclinical models using anti–PD-L1 therapy
* Demonstrated tx-induced alterations of a dynamic T-cell population
* May be new opportunity to evaluate antitumor immune responses

An effective immuno-PET imaging method to monitor CD8-dependent responses to immunotherapy. Cancer research 76.1 (2016): 73-82.

Transcriptomic Signatures of Resistance: IPRES





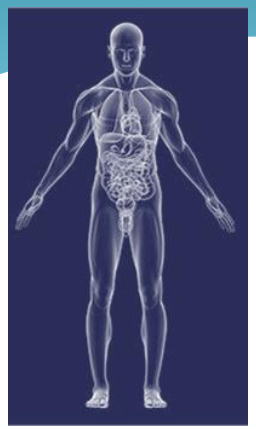
* **IPRES (innate PD-1 resistance)**
* Concurrent up-expression of genes involved in mesenchymal transition, cell adhesion, ECM remodeling, angiogenesis, & wound healing
* Identification of IPRES at time of progression may help guide next treatment selection
* IPRES signatures **can be induced by MAPKi,** which could further account for subsequent poor response to I/O therapy

Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. Cell 165.1 (2016): 35-44..

Learning Objectives

* Describe current state of biomarker analysis
  * Patient (tissue & liquid biopsies)
  * Tumor (tissue & liquid biopsies)
* Discuss technologies for identifying signatures of immune function
  * Pre- and post- therapeutic intervention
* Applying the science to everyday practice

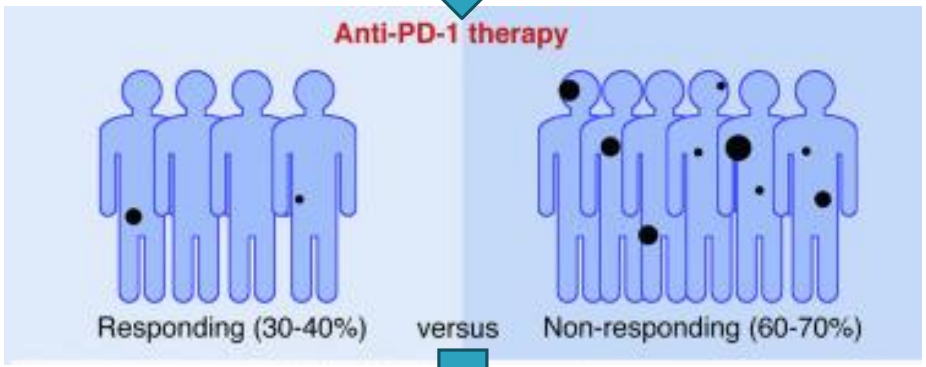
Applying the Science



T Cell Priming

Immune Competent

Clonal Selection & Persistence



Evasion

Immune Suppressive

Clonal Selection & Persistence

Can these questions be answered by RCTs?

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RCTs:
"Ideal" Biomarkers

* Feasible, reproducible
* Validated in prospective studies
* Minimal amount of clinical material (*inexpensive*)
* No *in vitro* manipulation (*rapid*)
* Quantitative, sensitive to a broad range of responses
* Able to be used with preserved/archived material (*convenient*)
* Relates to MOA (*interpretable*)

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Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy.
Nature Reviews Cancer 16, 275–287 (2016)

Regulatory Considerations: PD-L1

* IHC is currently only FDA-approved companion diagnostic
* Validation is needed; limitations described earlier
  * Performance, correlates, fresh vs archival tissue
* Validated CLIA test could be used to stratify patients for clinical trials
* Consensus remains: not a guide for treatment selection

Regulatory Considerations: Trial Design

* Immunomonitoring of pts should be considered an **essential component** of prospective clinical trials
  * Elucidate mechanism(s) of anti-tumor response
  * Monitor disease progression
  * Evaluate therapeutic effect
  * Identify novel targets for immunoTx
  * Predictive, prognostic markers for outcome

Regulatory Considerations: Data Sharing

* Multiple forms of data acquisition described here
* Danger of silo formation
* Expensive, dense data sets
* Integration becomes complex
* Fragmented data analysis may undermine “score”

Chasing The Horizon

The known is finite, the unknown infinite; intellectually we stand on an islet in the midst of an illimitable ocean of inexplicability.

Our business in every generation is to reclaim a little more land, to add something to the extent and the solidity of our possessions.

Thomas Henry Huxley

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Thank you to SITC and meeting organizers for the opportunity to collaborate & share knowledge!