Biomarkers: How to Select the Correct Patient for Which Therapy

Gregory A Daniels MD PhD

UC San Diego Moores Cancer Center

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Disclosures

- None

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Goals

- Appreciate the value and limitations of biomarkers in selecting therapy.
- Understand the difference between prognostic and predictive biomarkers.
- Have a general overview of biomarkers in cancer therapy.
- Discuss possible biomarkers in immune therapy.

Key Concepts

- Biomarkers should be practical-cost, timing, reproducible
- Biomarkers ideally reflect therapeutic mechanisms of action and select the subset of patients most likely to respond
- Ideally, biomarkers provide a clear yes/no stratification and a basis for combination therapy

How to Select Therapies?

- Genomics
  - Targeted agents based upon somatic mutations
  - Tumor types—ie Vismodigen in BCC
- Clinical
  - Pace of disease, tumor burden and performance status
  - Tumor types—ie IL2 for melanoma, RCC

Types of Biomarkers

Now

- Peripheral blood
  - Cytokines
  - Cell subtypes
  - Clonal frequency
- Tissue
  - IHC
  - Expression profiles
  - Genomics

Developing

- Single cell networking profiling
- Multispectral imaging on tissue sections Single cell networking profiling
- Non-invasive immune phenotyping

Longo J Immunol 2012

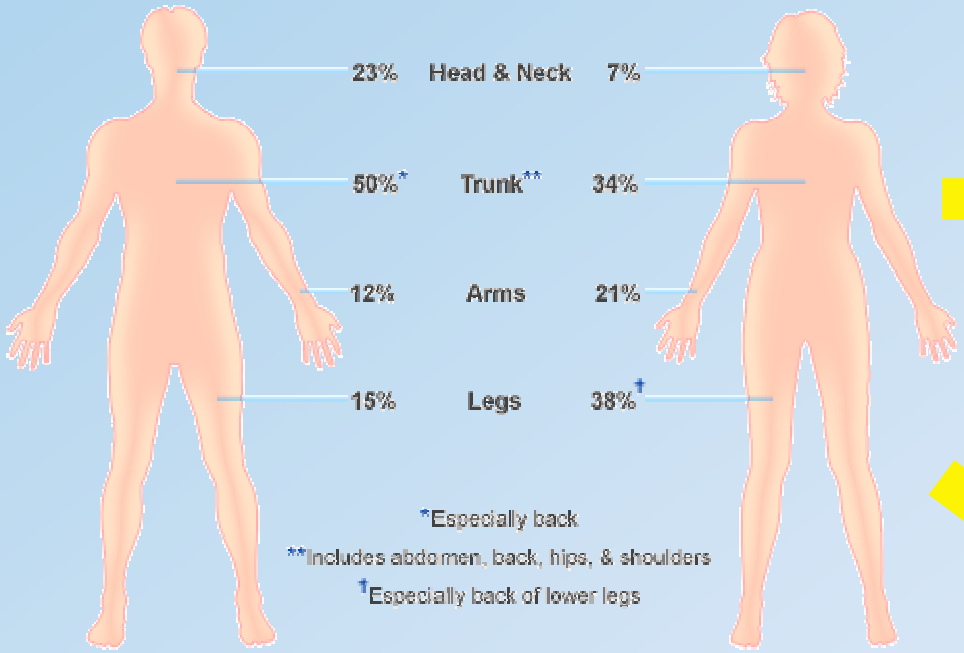
Patter of Somatic Mutations Reflects Causal Origins

GNAQ 45%
GNA11 32 %

uveal

scalp/face

Chronic sun
BRAF 28%
NRAS 18%



Trunk

Intermittent sun
BRAF 57%
NRAS 18%

mucosal

c-Kit 39%
NRAS 10%
BRAF 5%

NRAS 10%
BRAF 20%
c-Kit 36%

acral

Davies Nature 2002, Wan Cell 2004,
Flaherty, Hodi and Bastian Current Opinion in Oncology 2010

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Prognostic?

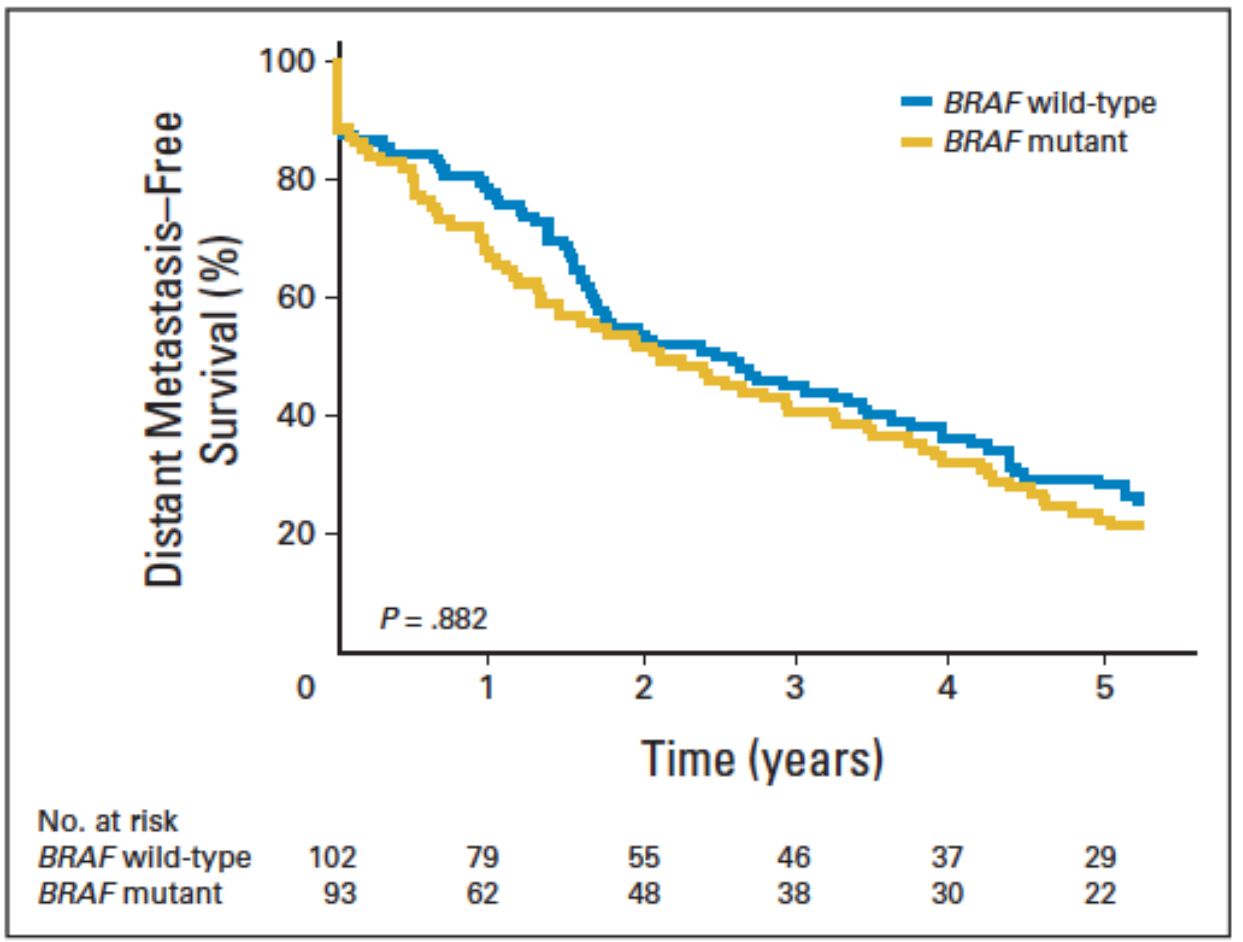

Fig 1. Disease-free interval from diagnosis of first-ever melanoma to first distant recurrence.

Long JCO 2011

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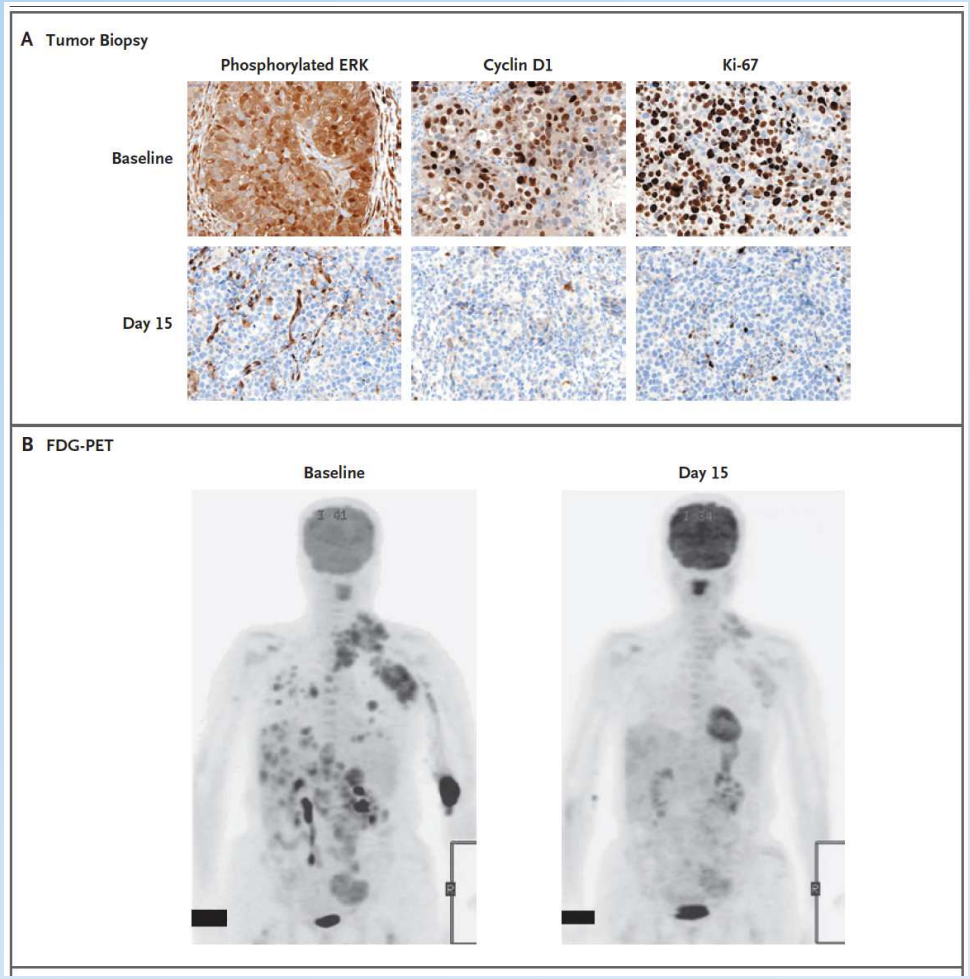
BRAF Mutations Predictive of Response

- Somatic mutation
  - Early event that drives phosphorylation of the ERK pathway
  - Testing done on fresh or fixed tumor samples
- Inhibits BRAF V600E or V600K mutated proteins
- Stops cell proliferation



Flaherty NEJM August 2010

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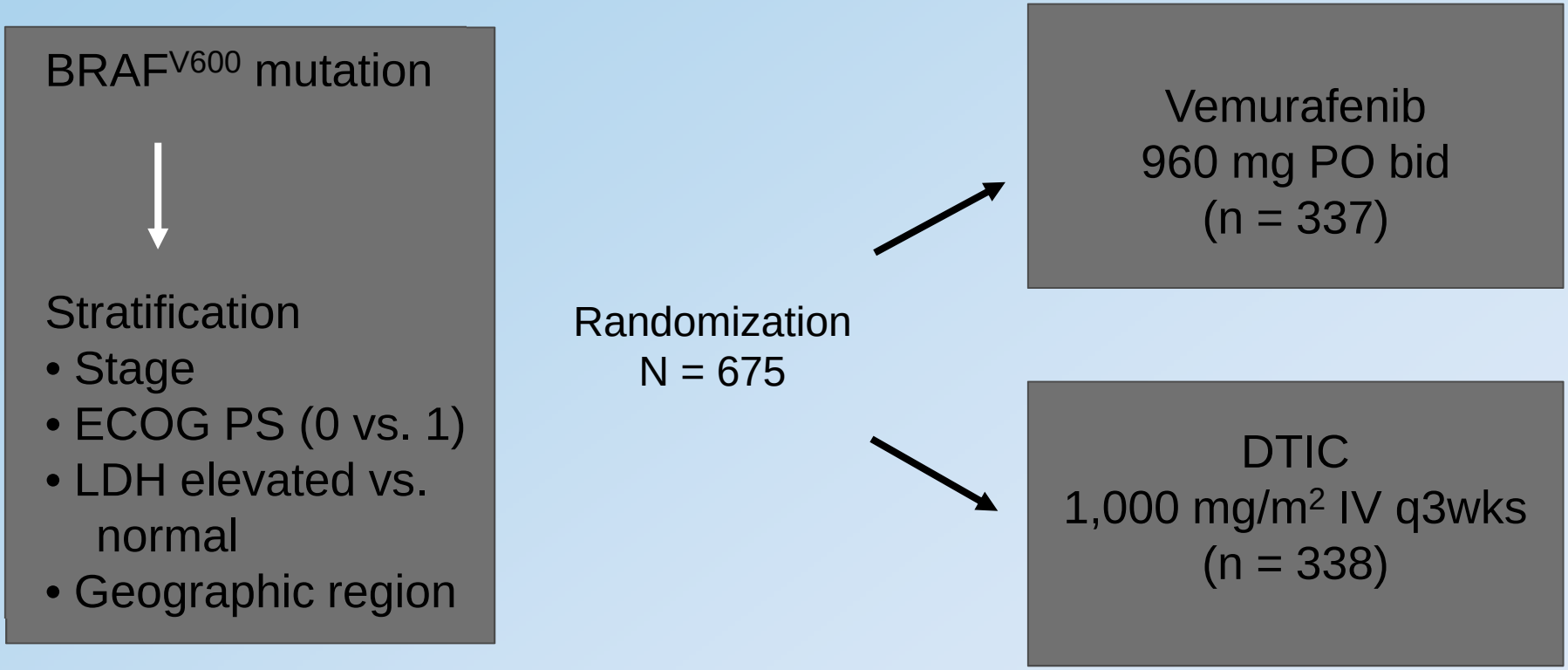
Phase 3 Trial Comparing Vemurafenib to DTIC in Patients With V600 Mutated BRAF Melanoma



Chapman NEJM 2011;364:2507

DTIC = dacarbazine; IV = intravenous.

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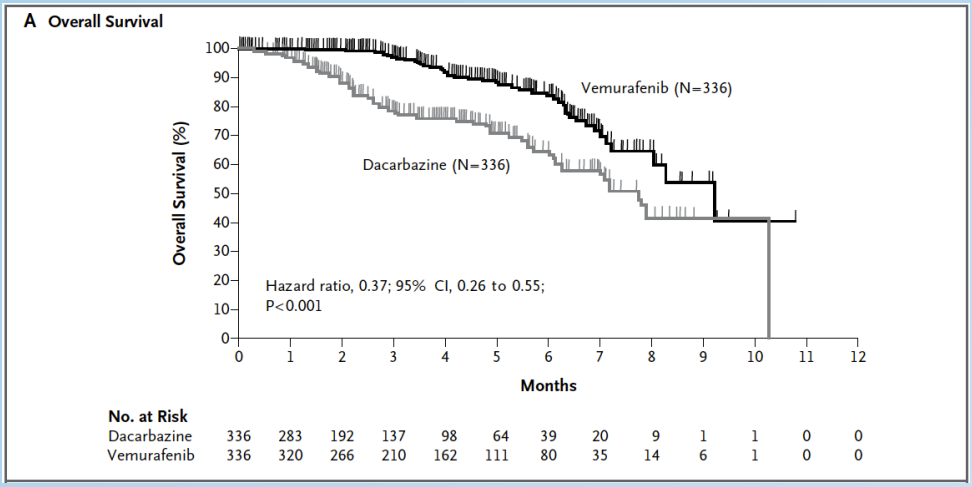
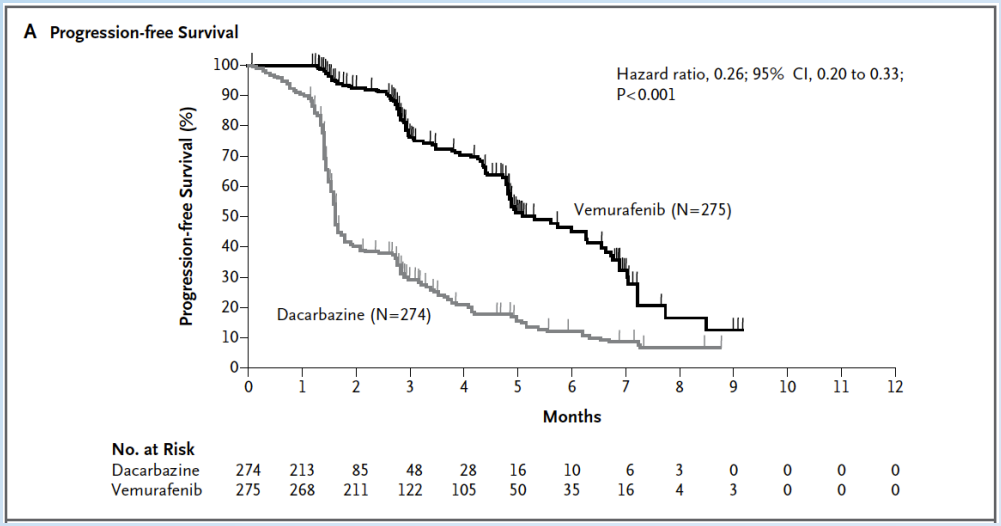
Improved Progression Free and Overall Survival





Chapman NEJM 2011;364:2507

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Immune Biomarkers
"Issues"

- **Systemic**-can one measure peripheral changes that guide therapy? Cytokines, VEGF, T cell markers, etc
- **Tumor microenvironment**
  - Primary tumor
  - Metastasis
  - Dynamic, response to therapy, heterogeneity, location
  - Measurements related to mechanism of action

Systemic Changes with Cancer Progression

Table 1. Study patient population distributed by clinical category, age, sex, and assayed immune variables

Clinical category	Total patients	Age, mean ± SD (range)	% Female	Assayed immune variables				
				Cell subset	Plasma cytokines	Tetramer	T-cell function assay	RNA array
Benign nevi	34	51 ± 12 (21-71)	68	26	34	7	2	0
Atypical/dysplastic	25	52 ± 16 (25-84)	44	22	16	11	1	0
In situ melanoma	36	61 ± 16 (26-84)	36	30	35	16	3	0
Stage I	45	54 ± 17 (21-82)	44	36	44	16	4	0
Stage II	16	55 ± 17 (22-81)	44	11	12	9	0	0
Stage III	16	53 ± 19 (23-83)	44	14	16	6	1	0
Stage IV	37	56 ± 14 (28-85)	43	32	30	27	16	24

Nevala CCR 2009

Systemic Changes in Melanoma Patients

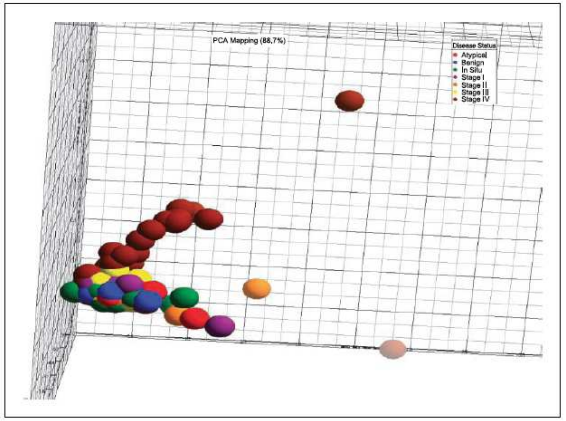

Fig. 1. Principal component analysis of cytokine levels across all stages of disease. Plasma collected from patients diagnosed with atypical nevi (*red*), benign nevi (*blue*), melanoma *in situ* (*green*), stage I melanoma (*purple*), stage II melanoma (*orange*), stage III melanoma (*yellow*), and stage IV melanoma (*brown*) was tested in the 27-plex cytokine array to determine plasma levels of 27 cytokines. Using principal component analysis, no difference in cytokine levels was noted among the early stages of disease (non-tumor-bearing) patients; however, cytokine levels were shown to be different in stage IV tumor-bearing patients, which is represented as separation of the brown spheres from all the others.

Higher levels of IL4, IL5, IL10 and IL13 in Stage IV melanoma patients

Higher circulating Treg cells and PD1 positive T cells
Decrease in functional recall antigens

General “Th2” bias with stage progression

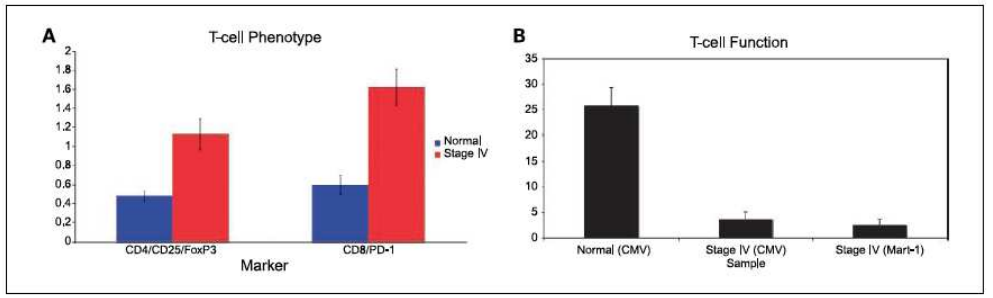

Fig. 2. Assessment of T-cell phenotype and function in healthy donors and stage IV melanoma patients. The number of T cells exhibiting the FoxP3 (regulatory T cells) or PD-1 phenotype in peripheral blood was determined in healthy donors and stage IV melanoma patients (*A*). Frequency of FoxP3-positive cells was measured by three-color flow cytometry: CD4-PC5, CD25-PE, and FoxP3-Alexa Flour 488. Mean ± SD percentage of FoxP3-positive cells was determined from the CD4 and CD25 double-positive population. Mean ± SD frequency of PD-1$^+$ cells was measured from the CD8$^+$ population. Mean ± SD frequency of tetramer-positive (CMV or MART-1) CD8$^+$ T cells was compared among normal volunteers and patients with stage IV melanoma (*B*).

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General Shift in Immune Regulation as Tumors Advance

- Melanoma patients have a bias for “Th2” state
- Higher VEGF levels may mark and contribute to this state
- Peripheral measures also suggest a general dysfunction
  - Higher PD1 positive cells
  - Reduction in functional recall antigens
- Multiple examples of systemic marker changes as tumors progress

Tumor Microenvironment

- Primary tumors
- Metastatic lesions

Immune Score
Prognostic in Primary CRC

- Leukocyte infiltration is a characteristic of almost all malignant tumors and these infiltrates include tumor associated macrophages, neutrophils, mast cells, NK cells, lymphocytes.
- Tumor progression depends upon the host immune response
- Immune ‘contexture’=nature, functional orientation, density and location influence risk of relapse in primary tumors.

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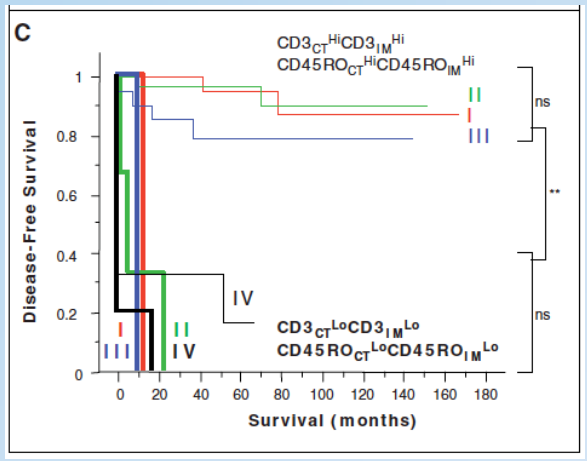
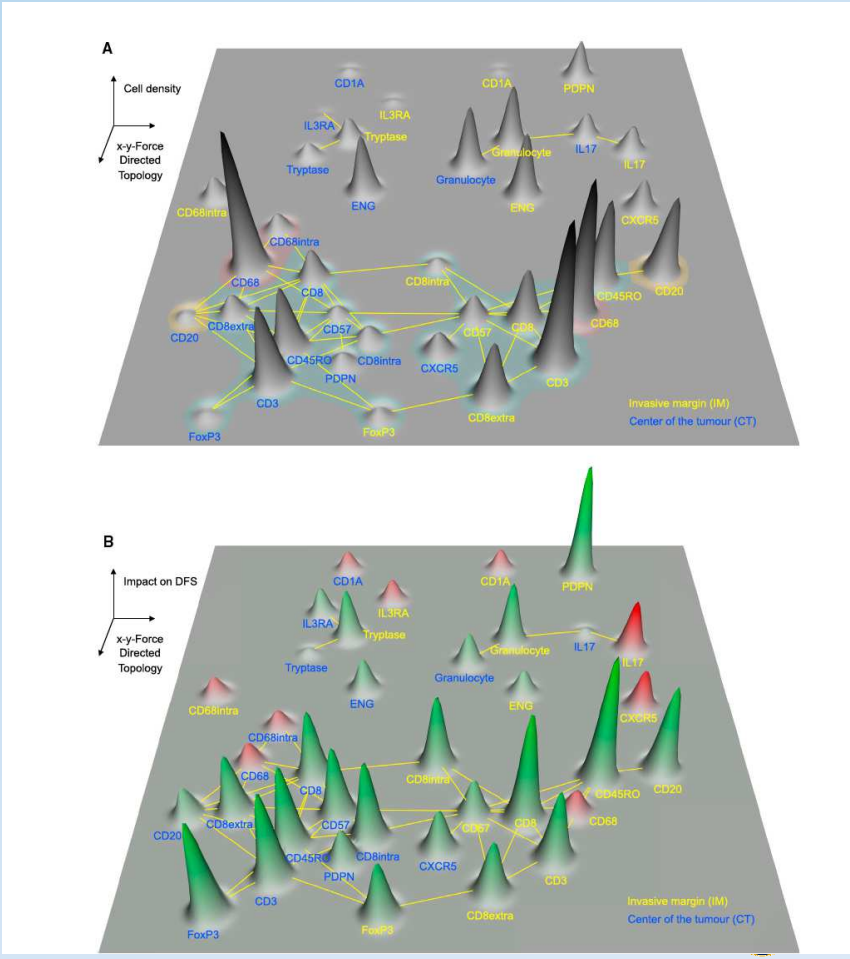
Immune Response Prognostic

- Type, density and location of immune infiltration predicts response in patients with colorectal CA patients





Galon Science 2006

Bindea Cell Immunity 2013

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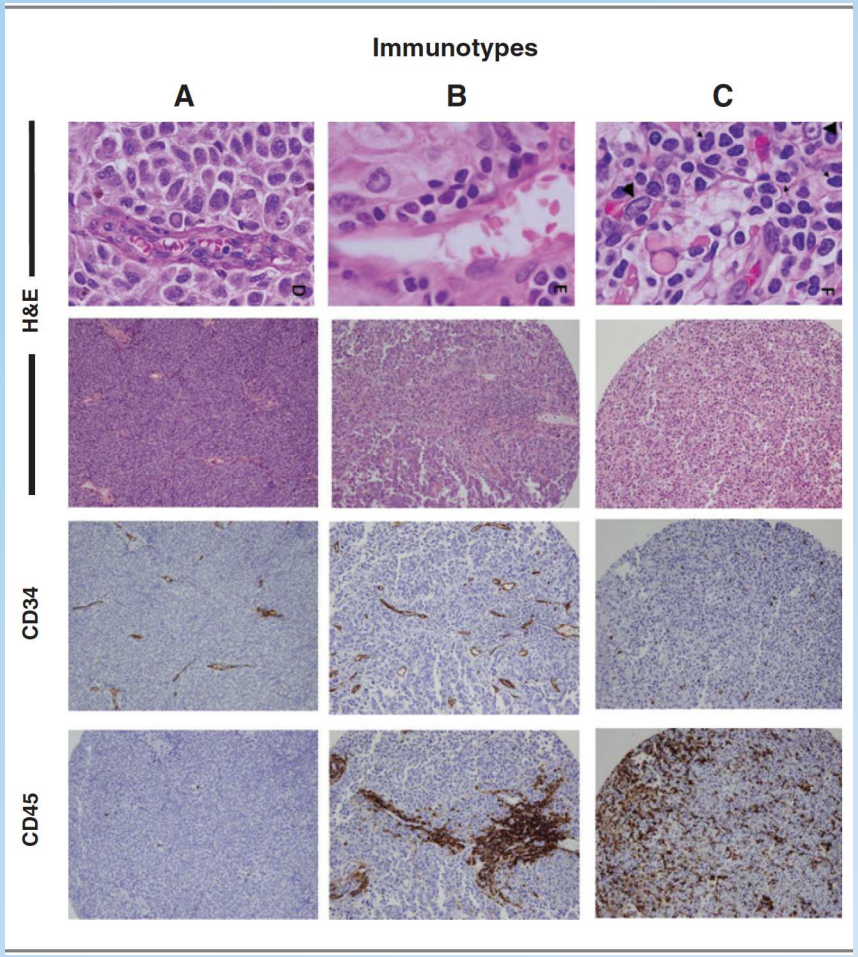
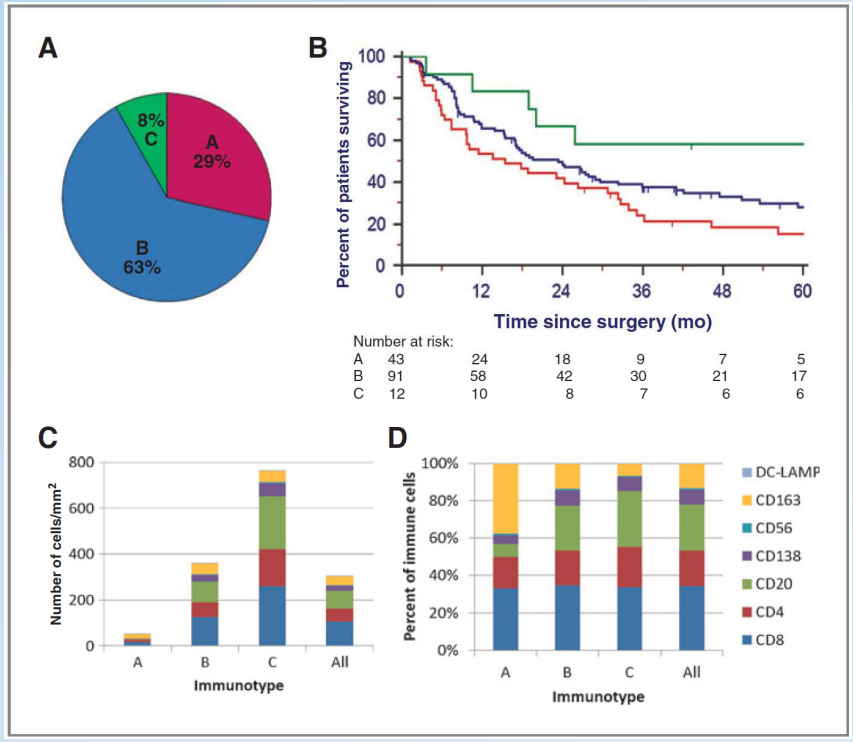
Metastatic Melanoma





Erdag Cancer Research 2012

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GIST

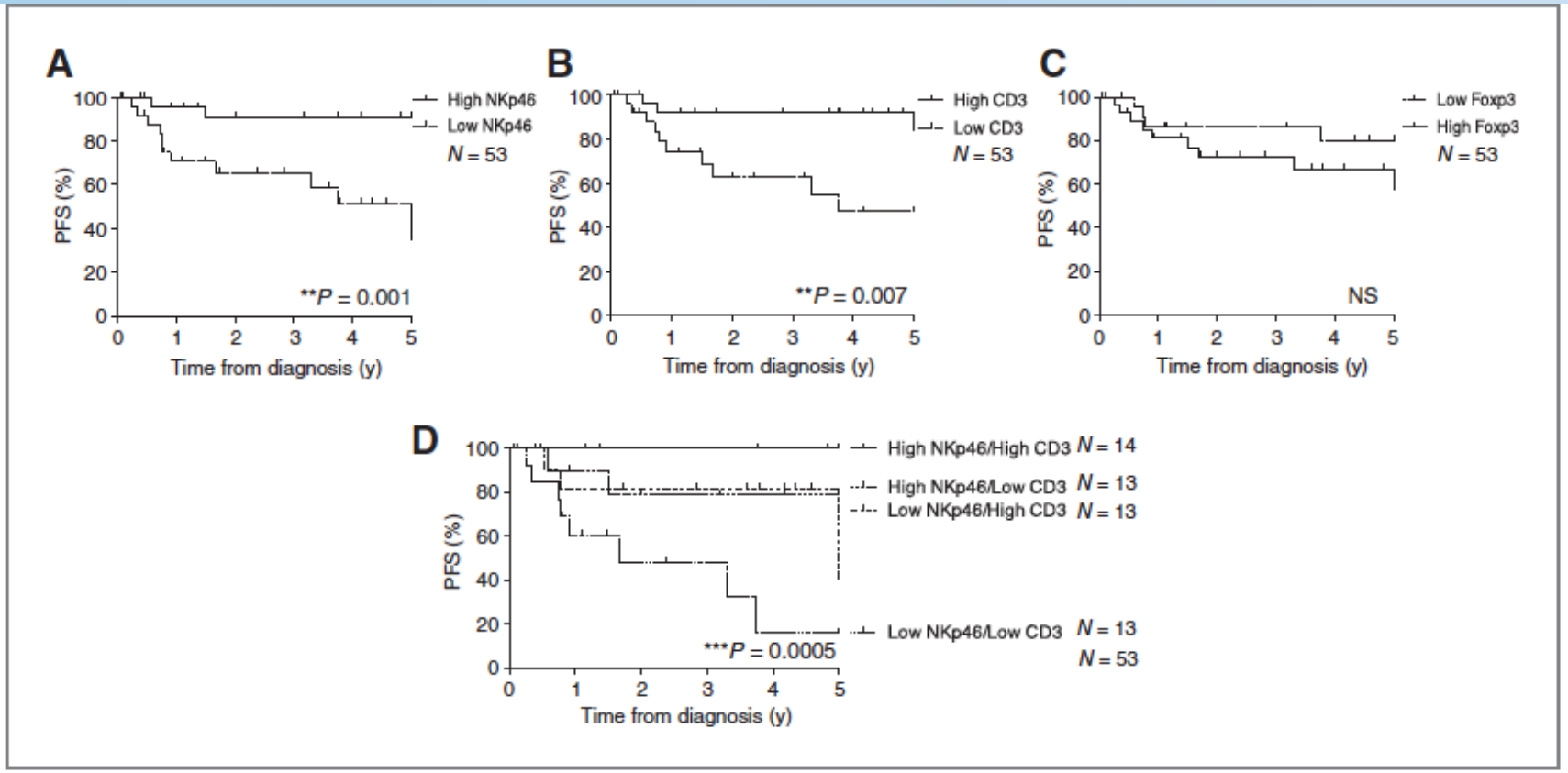

Figure 5. Prognostic value of T and NK cell infiltrates in localized GIST. A–C, PFS of 53 patients with localized GIST according to the median values of NKp46, CD3, or Foxp3$^+$ cells infiltrating the tumor at diagnosis (left, middle, and right). D, Kaplan–Meier curves of PFS obtained by stratifying the entire cohort of primary GIST into 4 groups according to the median of NKp46$^+$ and CD3$^+$ cells. Multivariate analyses are presented in Table 1.

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Cell Populations Predict Outcome

- Increases tumor associate neutrophils is associated with worse outcomes
- Independent predictor of DFS and OS
- Seen in multiple tumor types

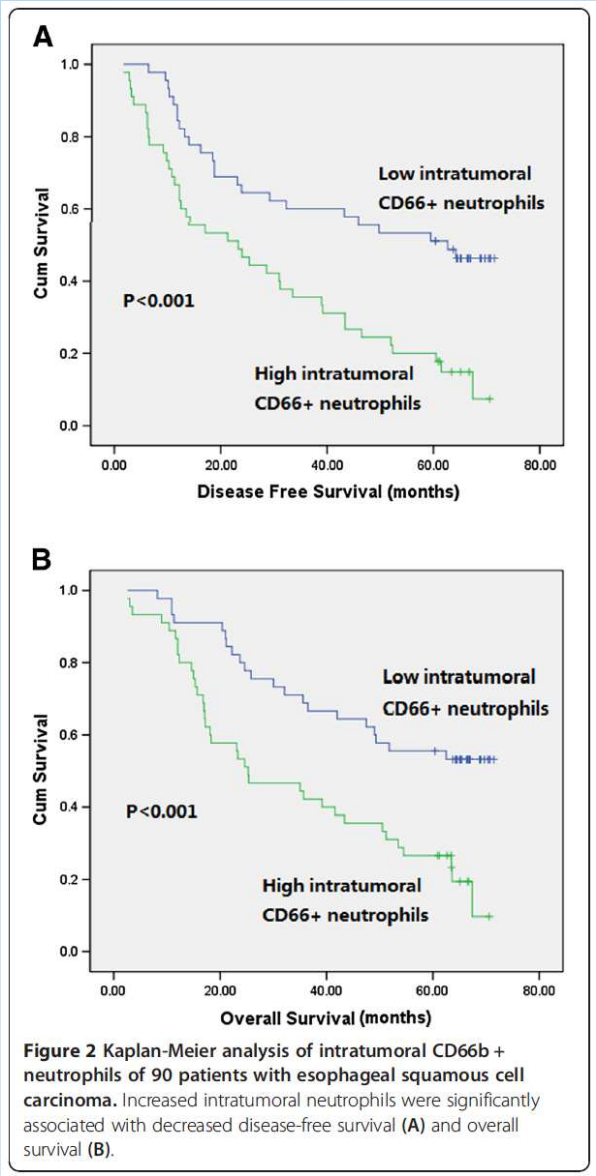

Figure 2 Kaplan-Meier analysis of intratumoral CD66b + neutrophils of 90 patients with esophageal squamous cell carcinoma. Increased intratumoral neutrophils were significantly associated with decreased disease-free survival **(A)** and overall survival **(B)**.

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Challenges with Characterizing TME

- Sampling tumors
  - Cell populations vary per location of the tumor
  - Invasive
- Quantitative assessments
  - Time consuming
    - Counting
    - Controls
  - Reproducibility
- Dynamic
- Multiple cell populations

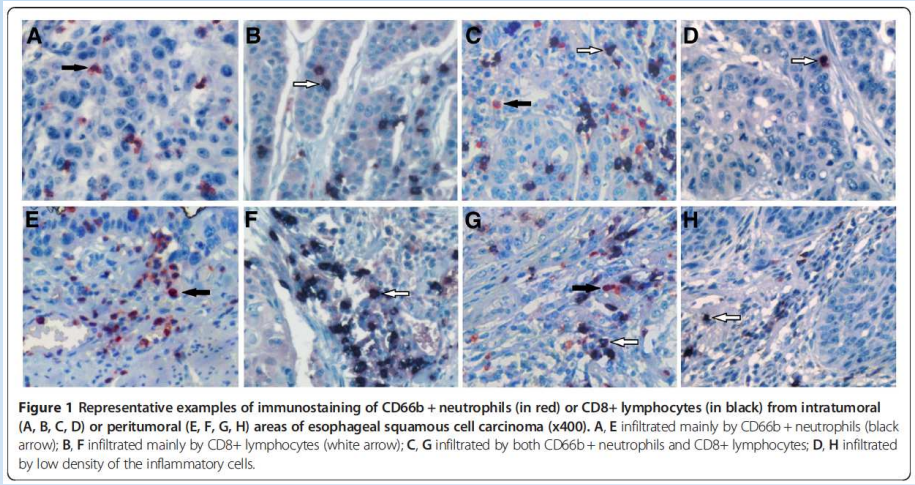

Figure 1 Representative examples of immunostaining of CD66b + neutrophils (in red) or CD8+ lymphocytes (in black) from intratumoral (A, B, C, D) or peritumoral (E, F, G, H) areas of esophageal squamous cell carcinoma (x400). **A**, **E** infiltrated mainly by CD66b + neutrophils (black arrow); **B**, **F** infiltrated mainly by CD8+ lymphocytes (white arrow); **C**, **G** infiltrated by both CD66b + neutrophils and CD8+ lymphocytes; **D**, **H** infiltrated by low density of the inflammatory cells.

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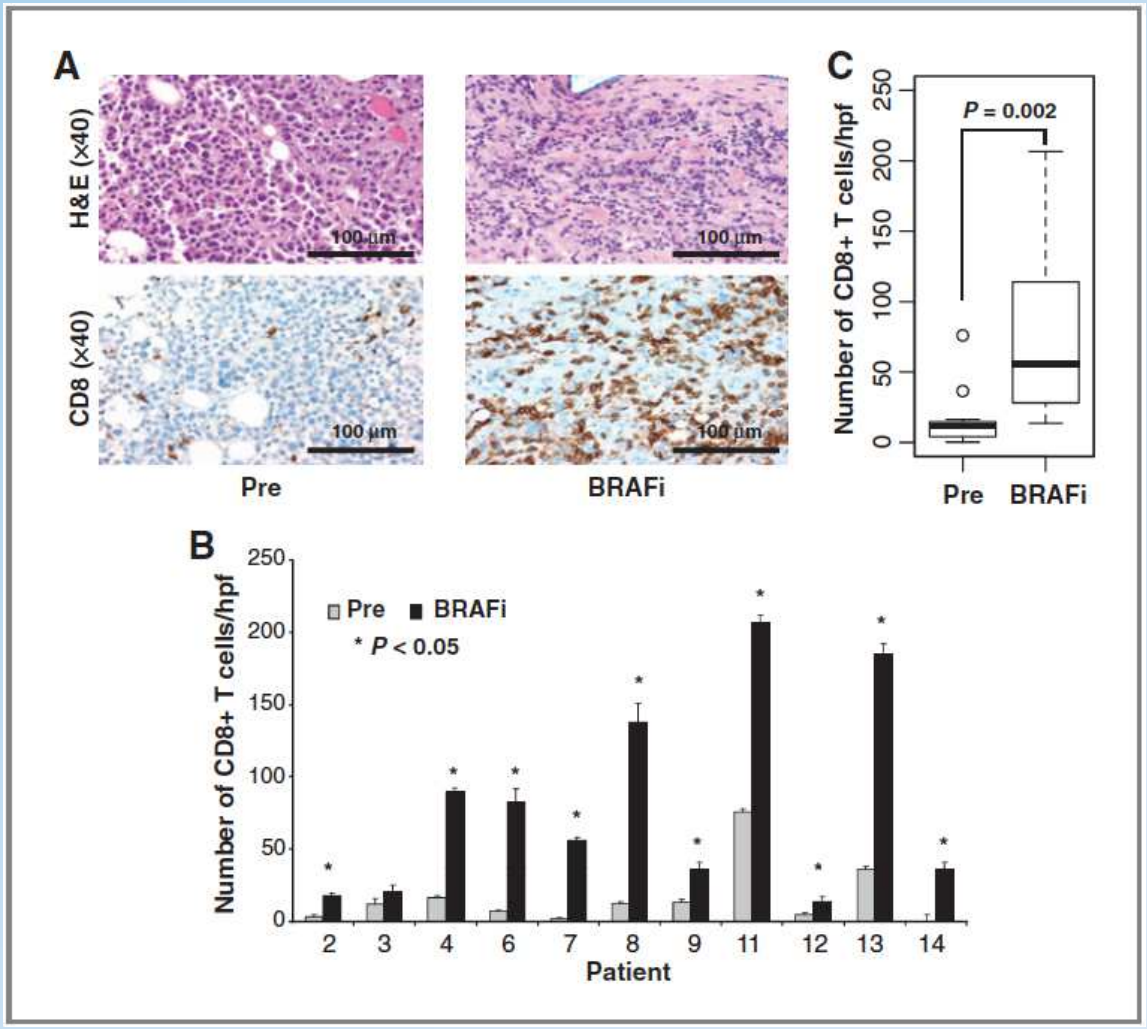
Tumor Microenvironment

- Changes associated with therapy

BRAF Inhibition is Associated with Increase in CD8 T-cell Infiltrate



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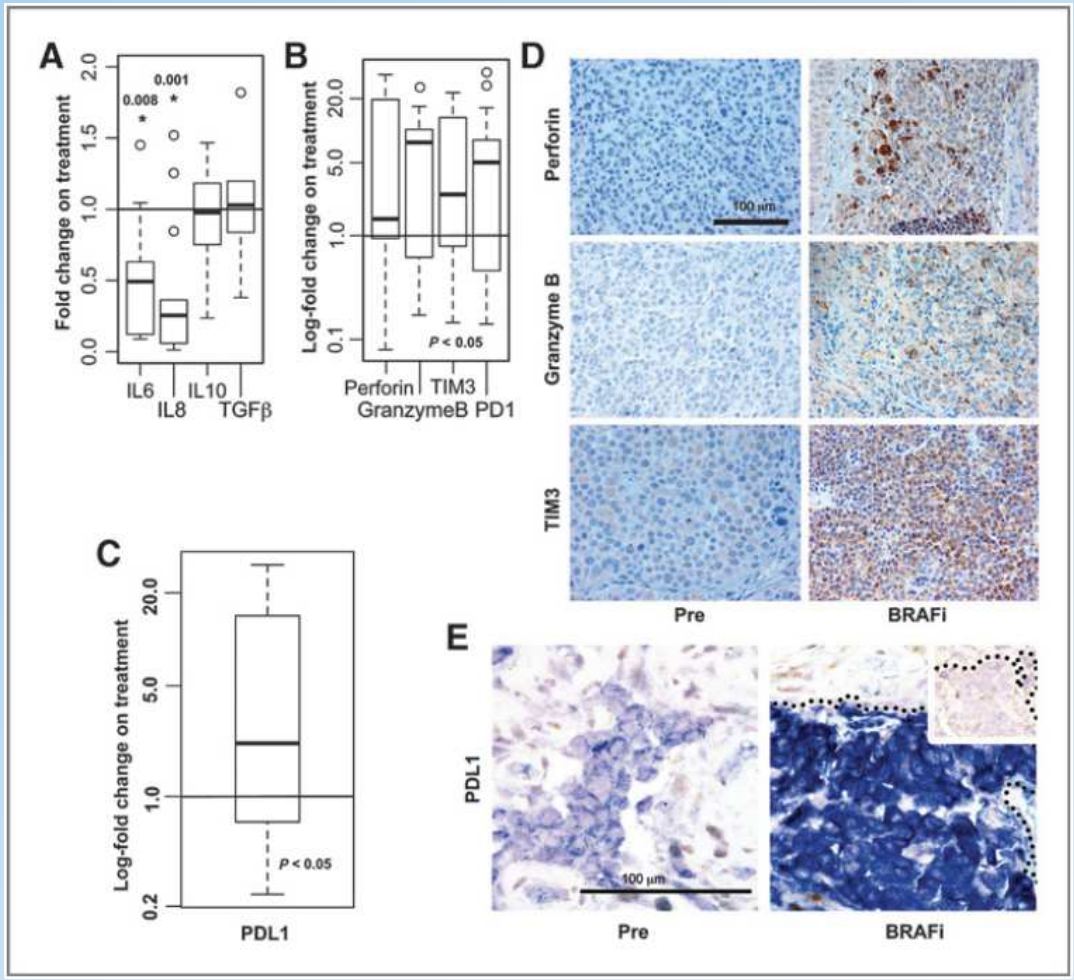
BRAF Inhibition is Associated with Expression of T Cell Exhaustion Markers



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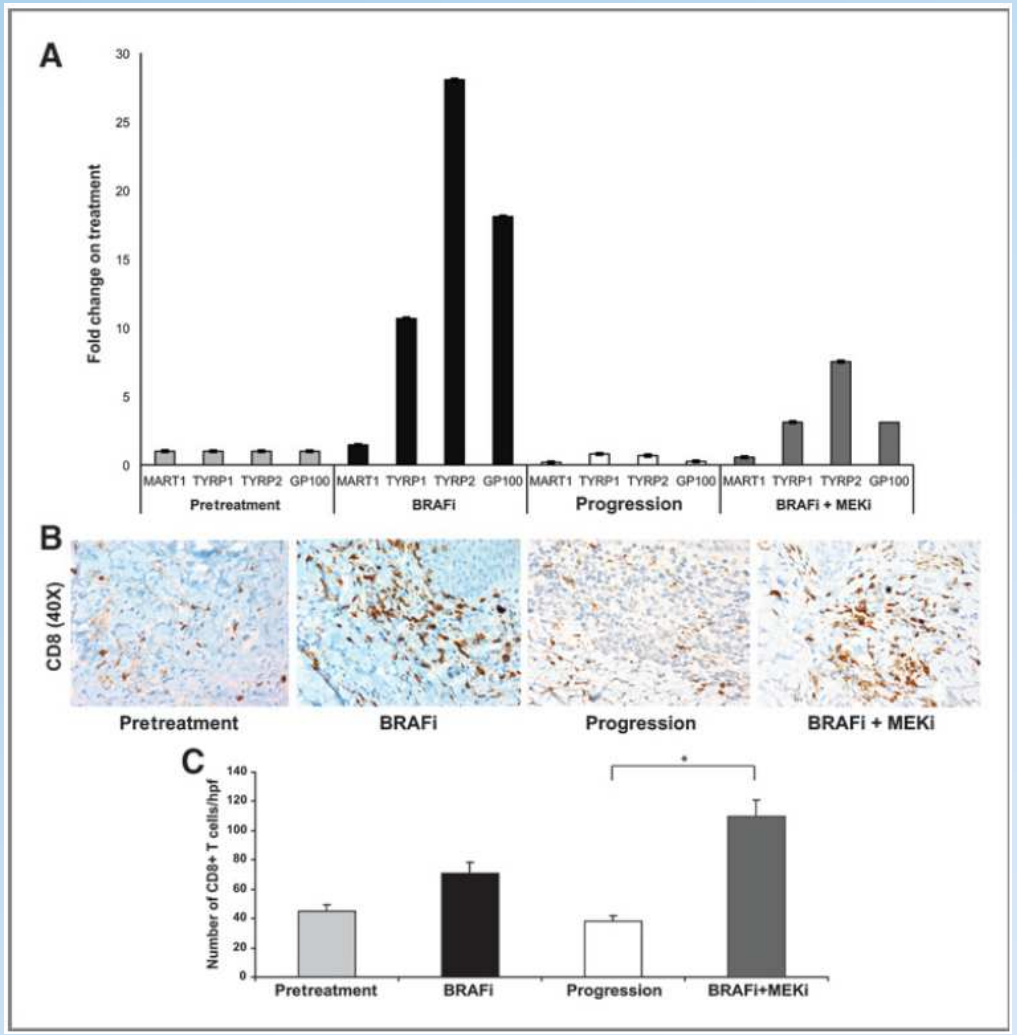
T cell Depletion Associated with Progression



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Tumor Micoroenvironment

- Biomarkers related to mechanism of action

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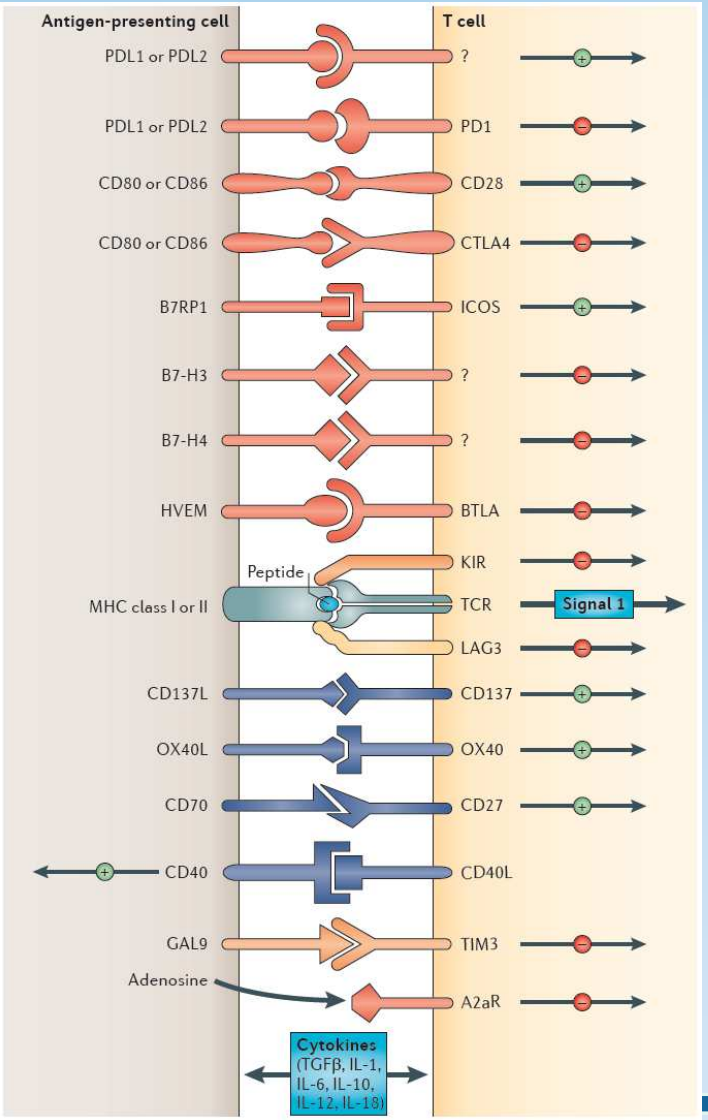
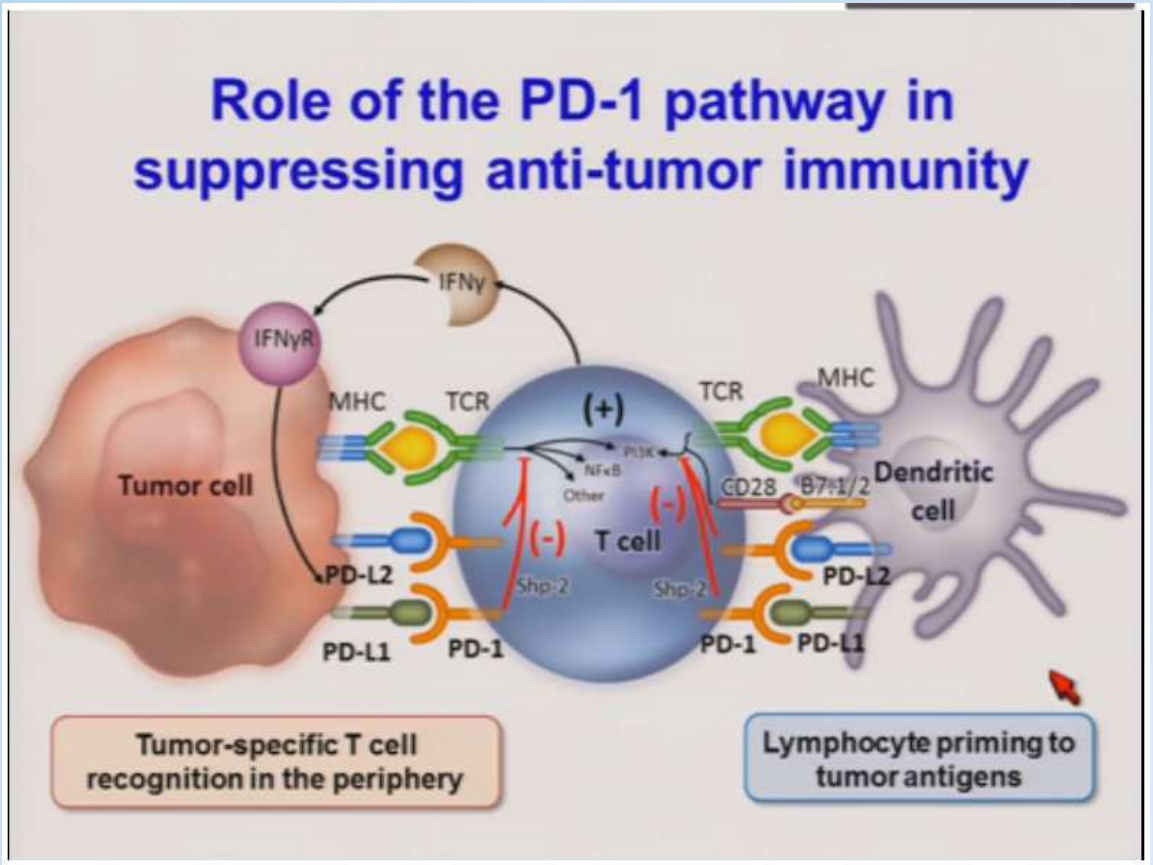
Immune Check Points





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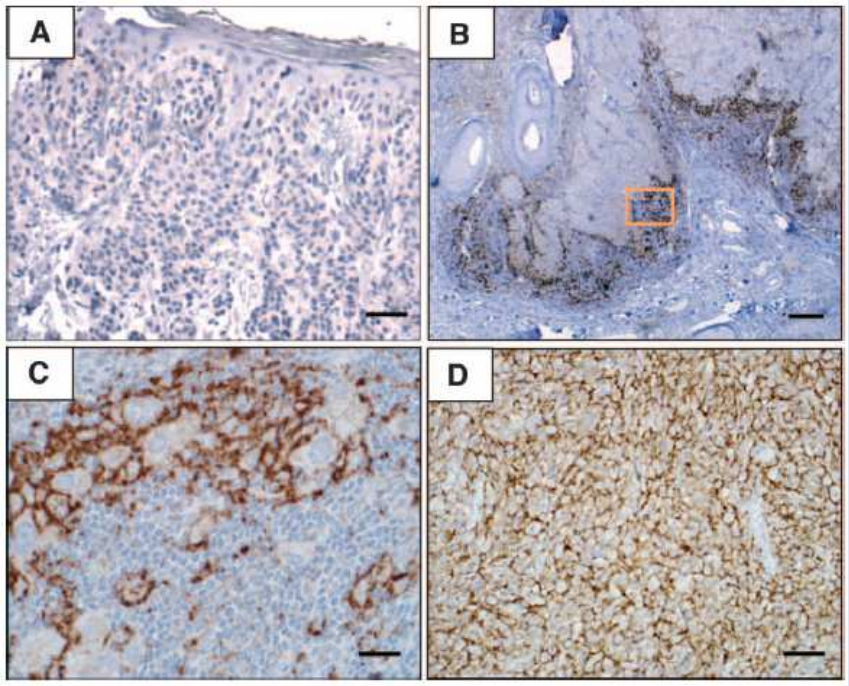
PDL1 Expression

- PDL1 (B7-H1) expressed by melanocytes adjacent to IFN secreting T cells
- Expression of PDL1 associated with improved survival
- ?PDL1 marks a successful immune response?



Taube Science Translational Medicine 2012

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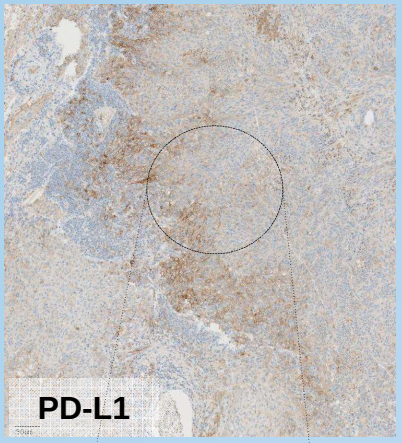
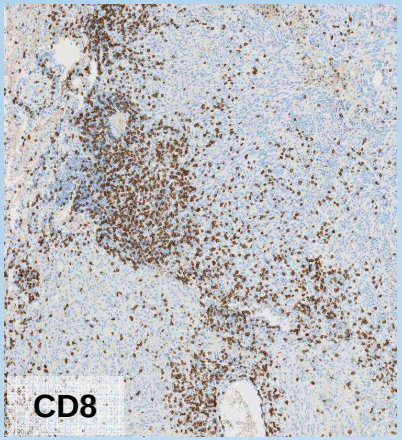
Adaptive Regulation of Tumor PD-L1 Expression May Be an Indicator of Local TILs Attacking Tumor

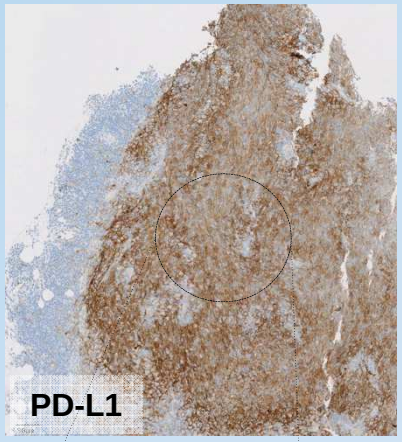
Baseline

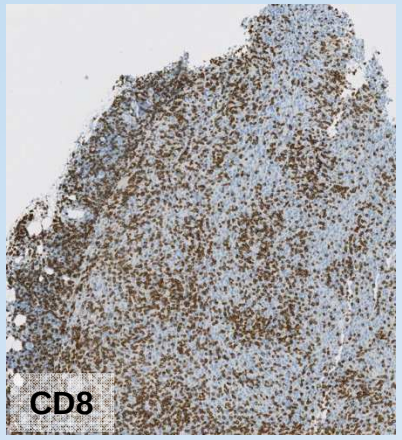


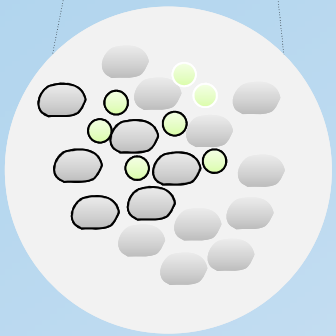


Anti-PD-L1

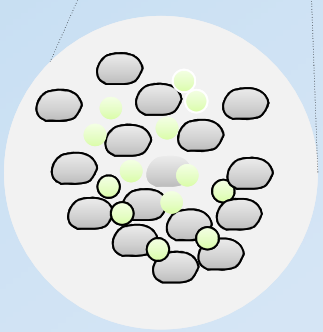
On-Tx







- T-cells and PD-L1+ tumor cells co-localize
- Focal PD-L1 expression may represent interface between cancer cells and immune cells (anti-tumor T-cell attack may be controlled by tumor PD-L1 or immune cell expression)



- T-cell mediated killing of tumor cells leads to T-cell proliferation and activation
- Activated T-cells release IFNγ and may induce PD-L1 expression in neighboring tumor cells

Tumor cells
PD-L1 expressing tumor cells
TIL/Immune cells

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Melanoma

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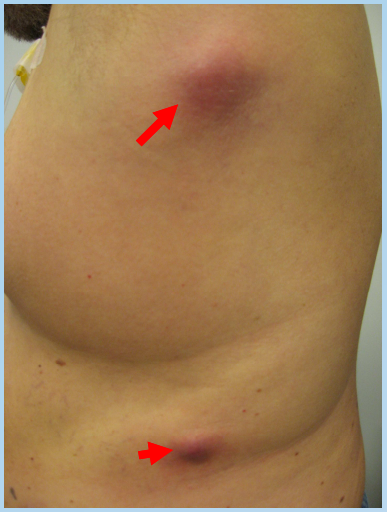
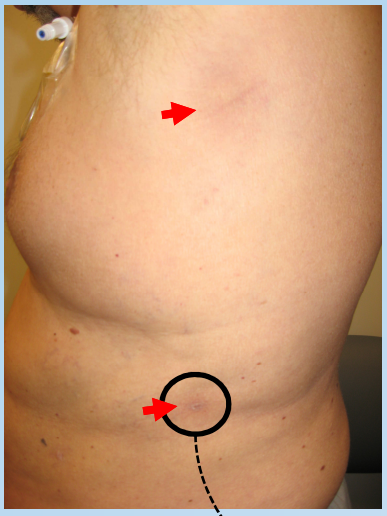
Serial Biopsy in a PD-L1+ RCC Patient with a Rapid Response to MPDL3280A (antiPDL1)

Baseline

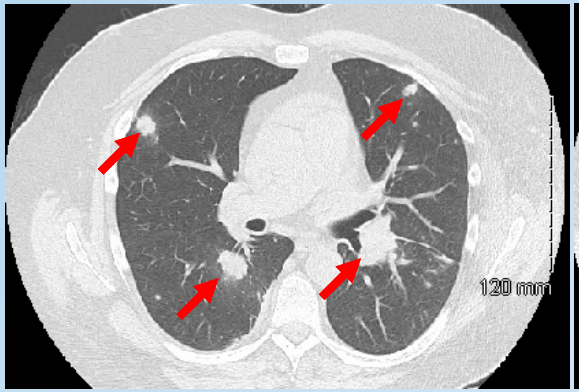


After 4 weeks

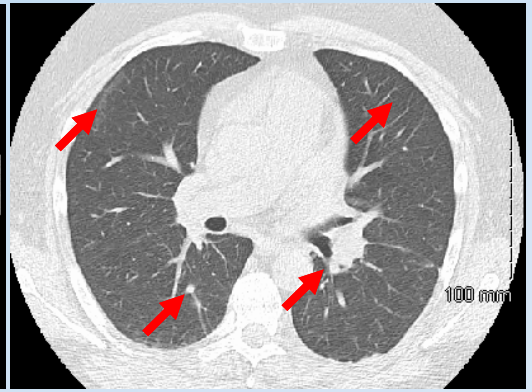


surgical resection of responding mass,
0.75 x 0.75cm at time of resection

Baseline



After 6 weeks



51 yo male with RCC s/p L nephrectomy, Sunitinib, XRT T9, Temsirolimus
Tumor PD-L1+

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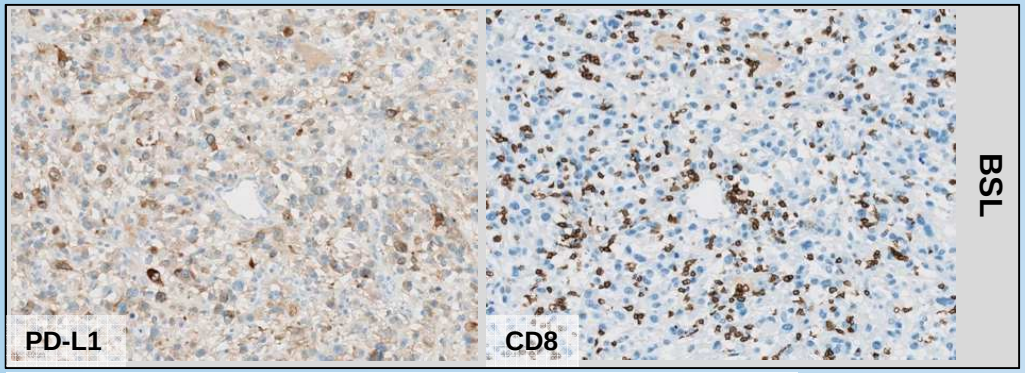
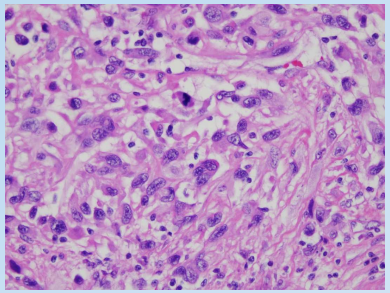
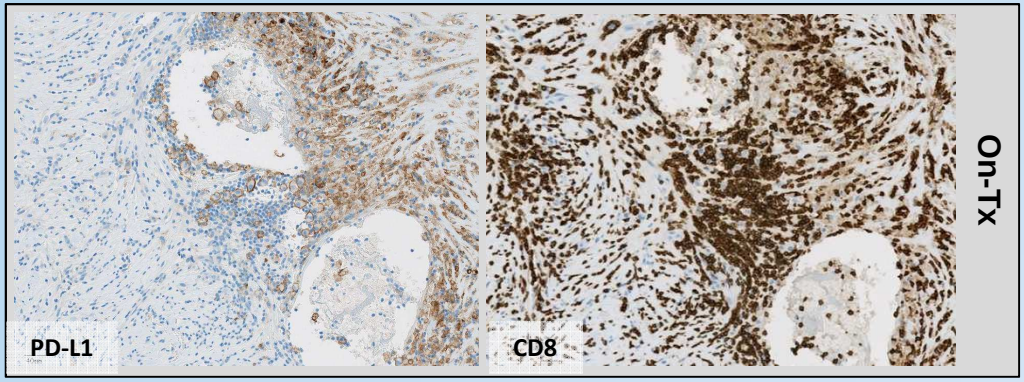
Serial Biopsy in a PD-L1+ RCC patient with a Rapid Response to MPDL3280A (antiPDL1)



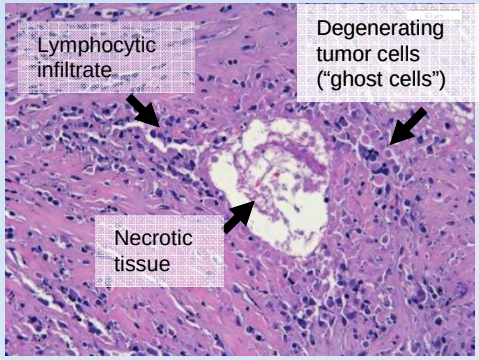
Biomarkers at baseline:
PD-L1+
Frequent CD8+ T-cells



Baseline H&E: RCC



Biomarkers at week 4:
PD-L1+
Dense CD8+ T-cell infiltrate



On-treatment H&E: dense lymphocytic infiltrate and *no viable* tumor cells seen

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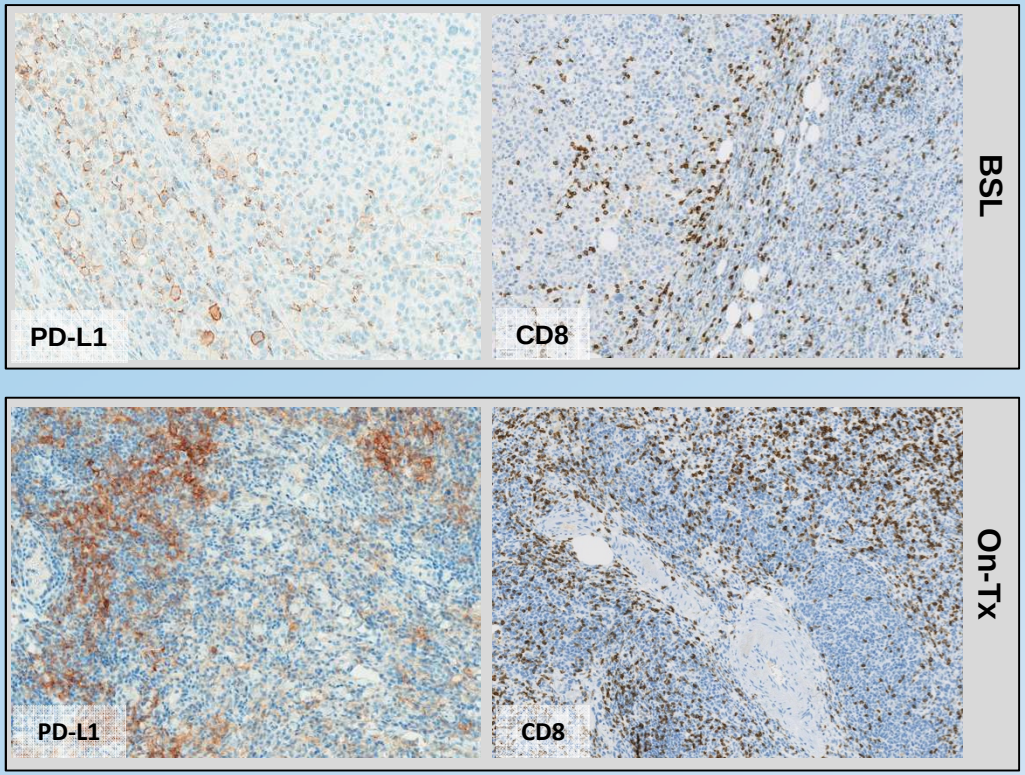
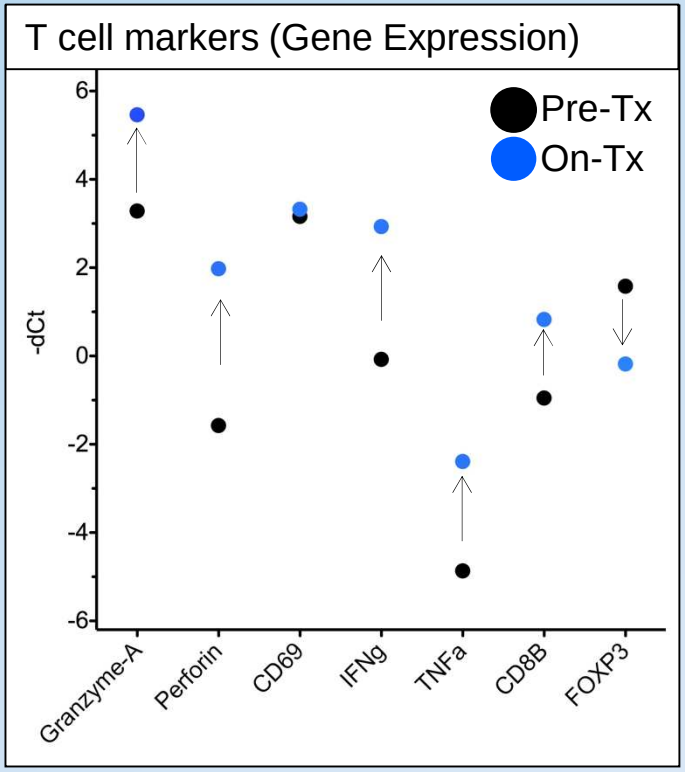
MPDL3280A Increased T-cell Activation in PD-L1+ Patient Responding to Treatment





Possible MoA of response to MPDL3280A:

Melanoma

- Pre-existing intra-tumoral CD8+ T-cells
- Increase trafficking or proliferation of intra-tumoral CD8+ cells
- Increased T-cell activation and cytotoxicity (e.g. Granzymes and Perforin production)

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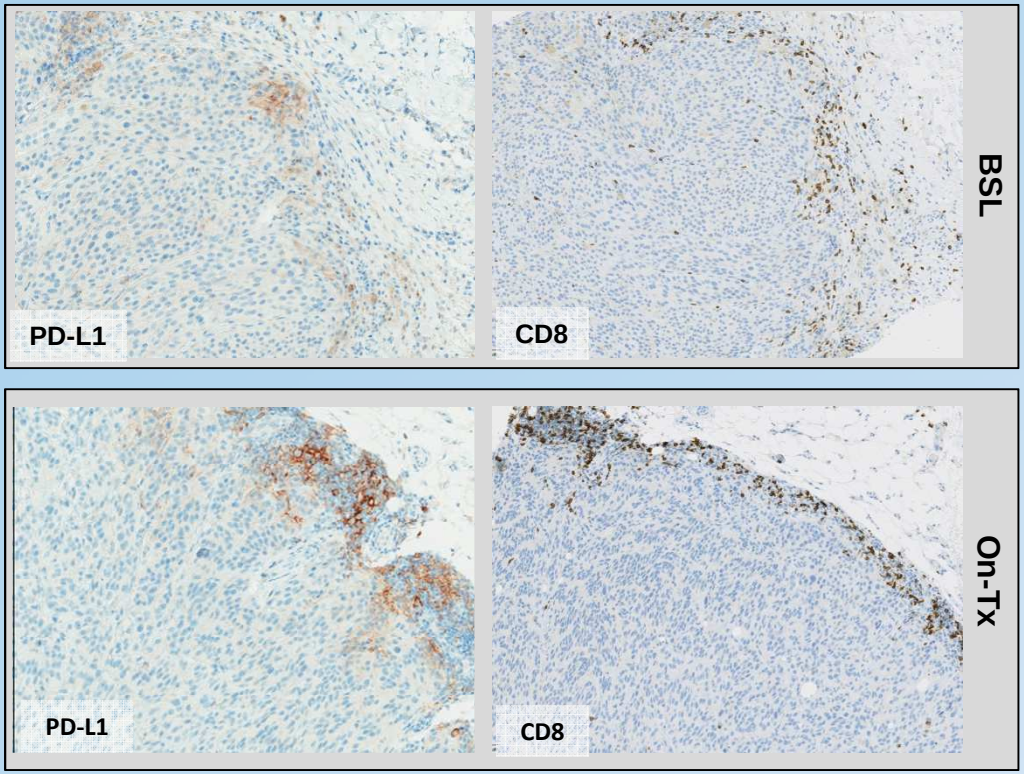
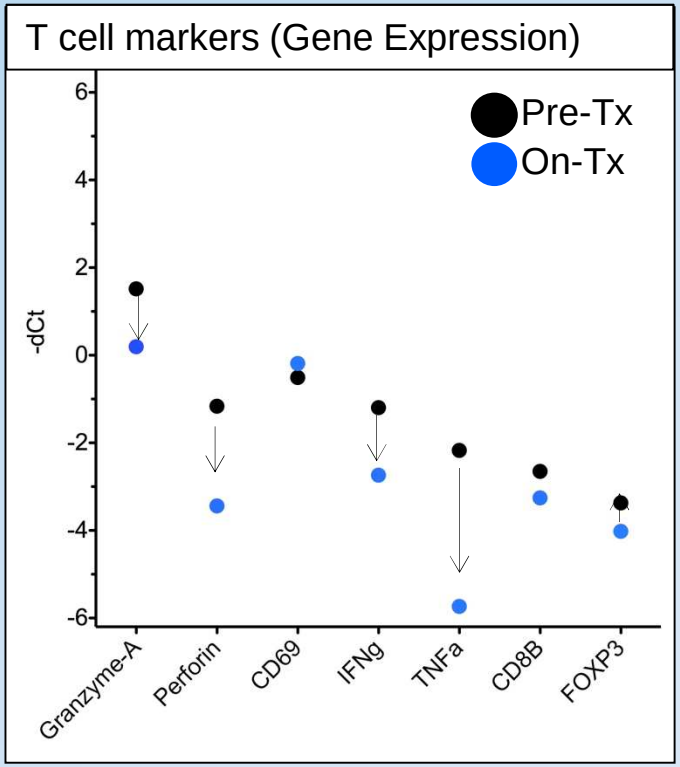
PD-L1- Patient Not Responding to MPDL3280A Exhibits Low Frequency of Intratumoral T-cells





Melanoma

Possible MoA of resistance:

- CD8+ T-cells remain at the edge of the tumor (possible impaired trafficking)
- No increase in T-cell cytotoxicity
- No T cell recognition of cancer antigens in this patient

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Adaptive Increase in PD-L1 Expression is Prominent in Patients Responding to MPDL3280A

Summary of responses to MPDL3280A in paired biopsies:

Max SLD Decrease†	Increase in tumor PD-L1*
>30% reduction	4/4 (100%)
0-30% reduction	2/6 (33%%)
0-20% increase	1/10 (10%)
>20% increase	0/4 (0%)
Unevaluable SLD (due to tumor excision**)	2/2 (100%)

- # of patients with increased PD-L1 expression following Tx with MPDL3280A, including sterilized tumor with PD-L1+ immune cells; Increase in tumor PD-L1
- †at any time point in study

** excision of responding tumor for purposes of biomarker analysis rendered the patient UE for max SLD change

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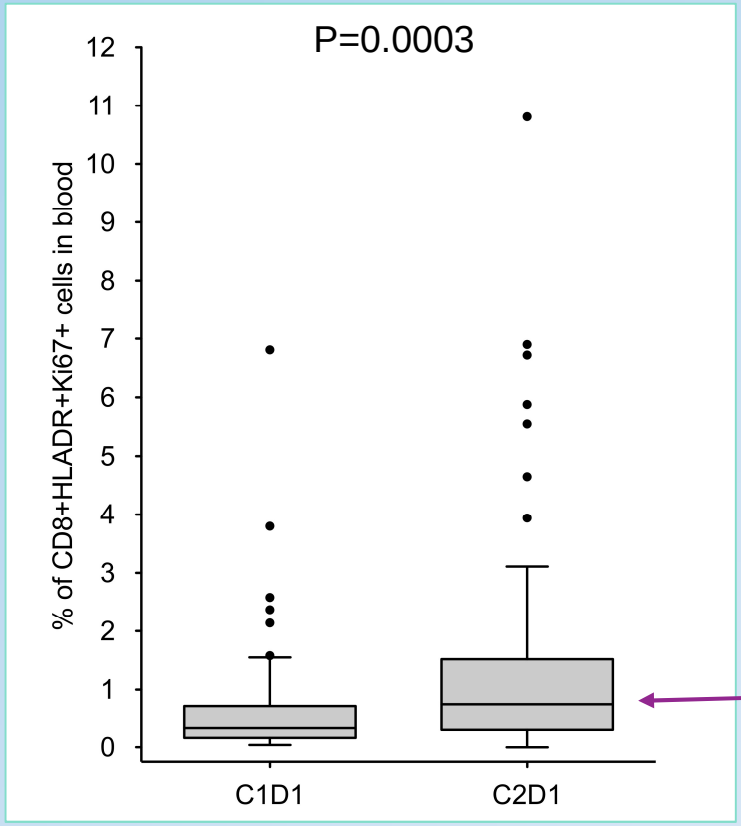
MPDL3280A Leads to Increased Frequency of Activated T-cells in Blood

Proliferating activated T-cells (CD8+/HLA-DR+/Ki67+) are more frequent at C2D1 in blood



- Activated T-cell proliferation in blood may serve as a pharmacodynamic biomarker of MPDL3280A treatment

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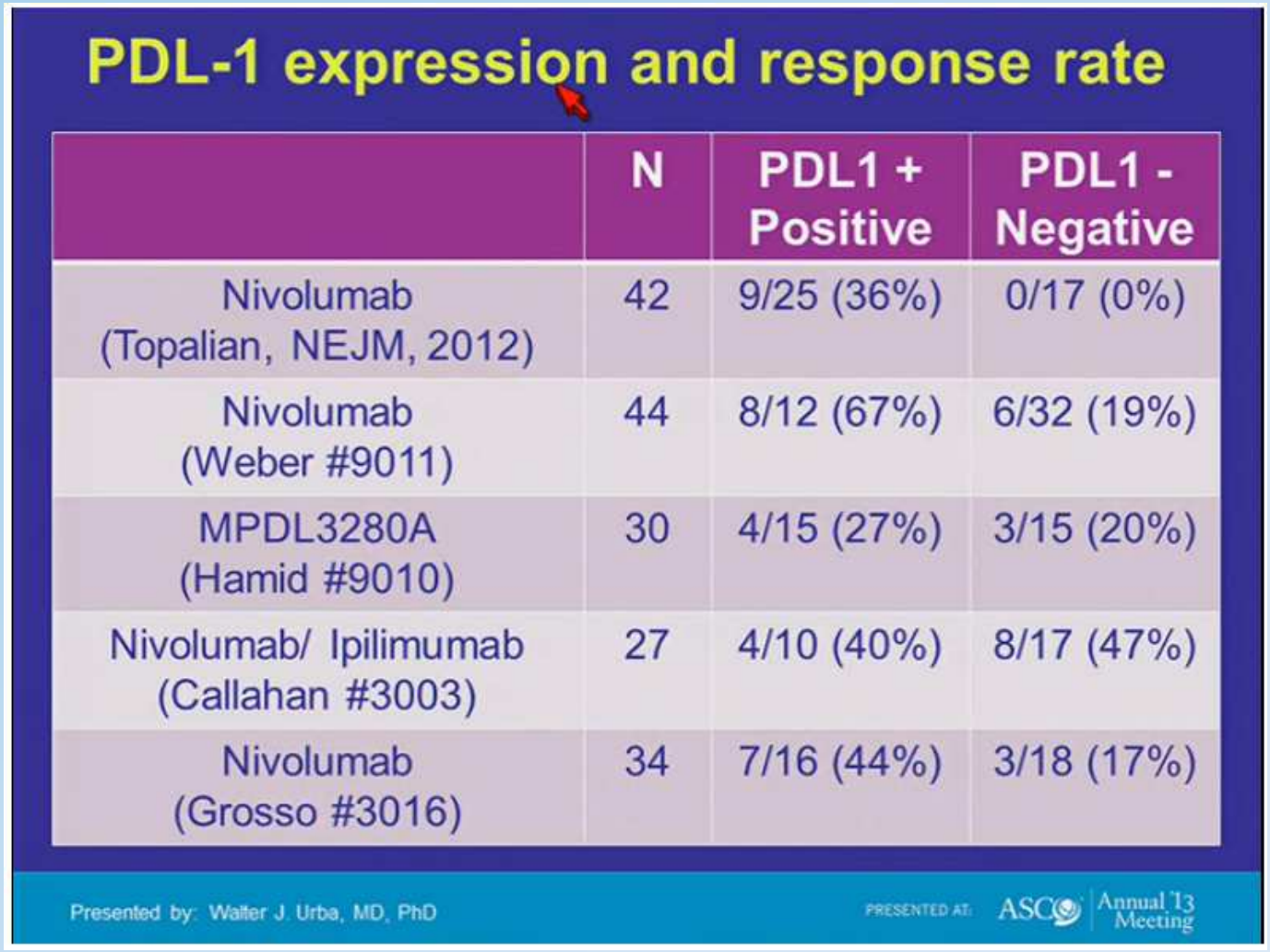
Why Does Initial PDL1 Expression Fail to Predict Response?



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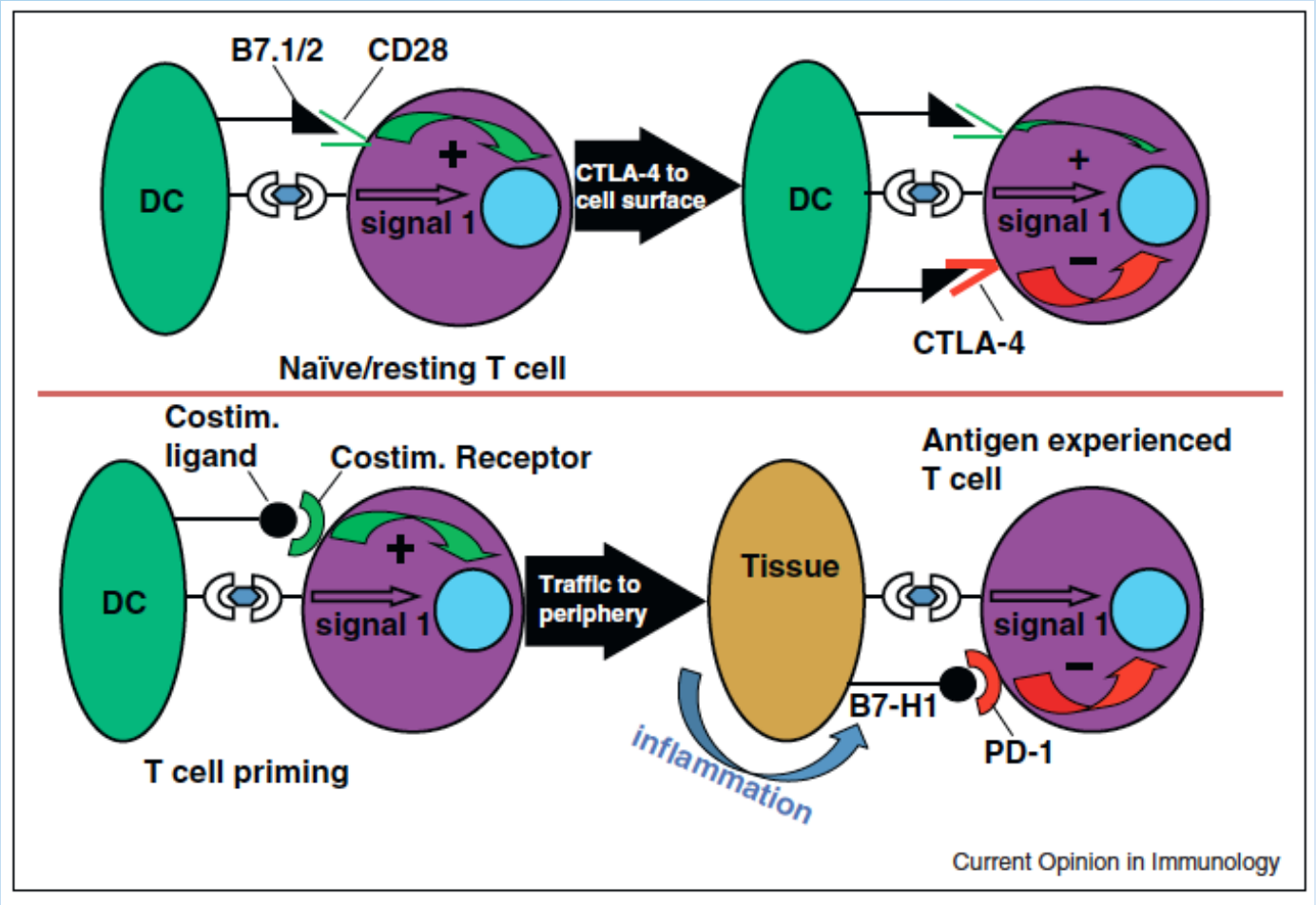
Immune Modulation



Topalian Current Opinion in Immunology 2012

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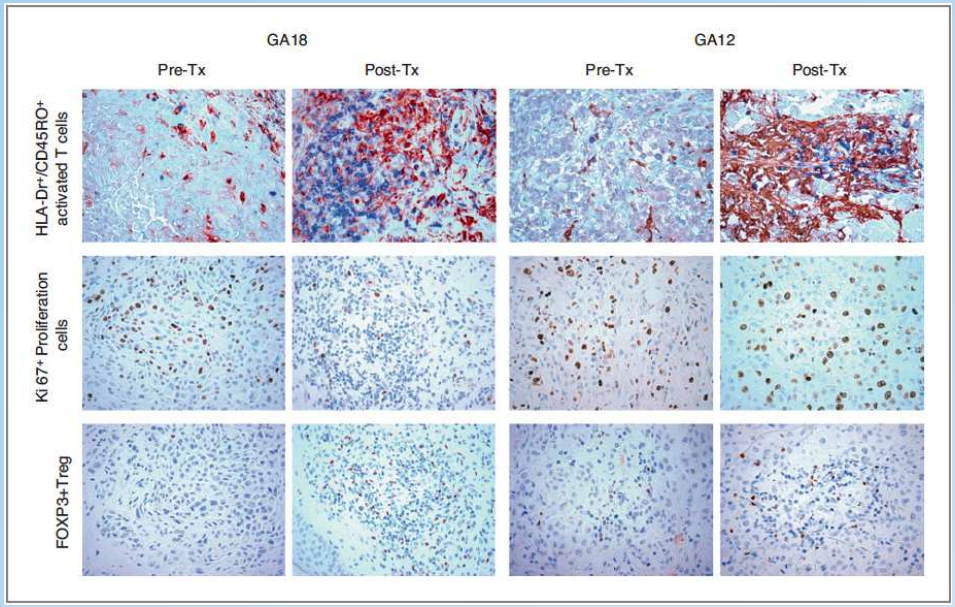
Immune Biomarker



Characterization of immune infiltrate 1 to 2 months after first doses of Tremilimumab

- T-cell infiltrates did not differentiate clinical response.
- CTLA4 blockade leads to frequent immune responses to CTLA4 blockade with infrequent clinical evidence of tumor regression.

Huang Clinical Cancer Research 2011

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Predictive Markers

- Dynamic
- Will vary with tumor type
- Require interrogation of the microenvironment
- Multiple immune modulators are becoming available that can be utilized in a rationale sequence and/or combination

Phenotyping Immune Cell Subsets

- Immune histochemistry limited to one stain per section
- FACS of solid tumors
  - Lack spatial definition
  - Need for nonfixed tissue
- Expression analysis
  - Loss of cellular data
- Ideally need to understand complex multimarker phenotypes

Technologies to Assess TME

- Imaging
  - Not invasive, dynamic
  - Resolution, number of markers
- Liquid biopsy
  - Minimally invasive, dynamic
  - Resolution
- Functional assays
  - Costly
  - Limited to measurable targets

Spatial Phenotyping

- Multiplex staining of a single section
- Multispectral imaging to allow quantitation and separation of chromogens or fluorophores
- Training based upon morphologic image analysis
- Automation

Mansfield http://www.mlo-online.com March 2014

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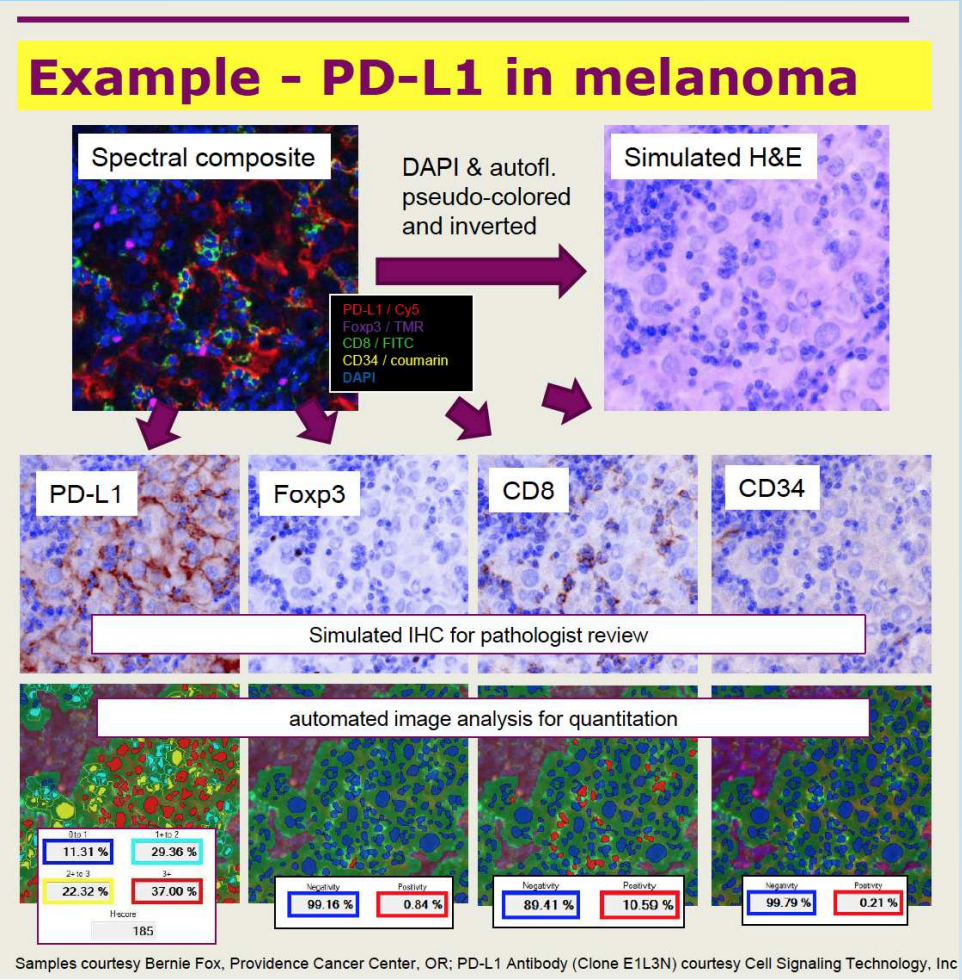
Quantitative IHC



- Quantitative
- Spatial information

- Dynamic microenvironment monitoring
  - Biopsy at clinical changes
  - Impact of therapy

Images courtesy of Cliff Hoyt, Kristin Lane and Chichung Wang, melanoma samples courtesy Bernie Fox, Providence Cancer Center, OR, PD-L1 Antibody (Clone E1L3N) courtesy Cell Signaling Technology, Inc.

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Take Away

- Given the multiple mechanisms underlying ineffective chronic inflammation, markers may vary between tumor types and even within individuals.
- Immune changes are dynamic and relational implying that predictive biomarkers may need to be assessed over time

Questions?

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