Strategies for Incorporating Biomarkers in Clinical Trials for PD-1/PD-L1 Combinations

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Disclosures

(Not directly related to the presentation)

Simpatica, Scientific Advisory Board Member, Jan. 2017-present
Shire Advisory Board Meeting, Orlando, FL Feb. 25, 2017
StemImmune Scientific and Medical Advisory Board, April 6, 2017-present
SapVax Advisory Board meeting, Philadelphia, PA, Nov. 15, 2017
NextCure, Scientific Advisory Board Member, July 2018-present

Why don’t we have more useful Biomarkers?

Now that we have populations of clinical trial *clinical* responders:

1. We need the right specimens saved under standardized conditions. Variably banked specimens give noisy data. Many trials bank only non-viable tumor (often incomplete) and serum samples.

2. Immune assays can be costly; testing small numbers don’t give robust, reproducible signals; guessing at 1-2 assays may miss the true biomarker.

No sample left behind

…the reality is that most immune profiling efforts remain at a pilot scale. …require greater attention to how samples are acquired and analyzed and community agreement on how store, share and interpret data.

…samples are acquired for specific purposes, such as tumor biopsies for diagnosis or blood draws for determining tumor burden.

Once a sample has been used to answer a research question, often the remaining tissue or cell sample is lost. …

in industry-sponsored studies, samples often remain sequestered in company freezers….Drug companies have little incentive to fund unsupervised analyses of their patient cohorts.

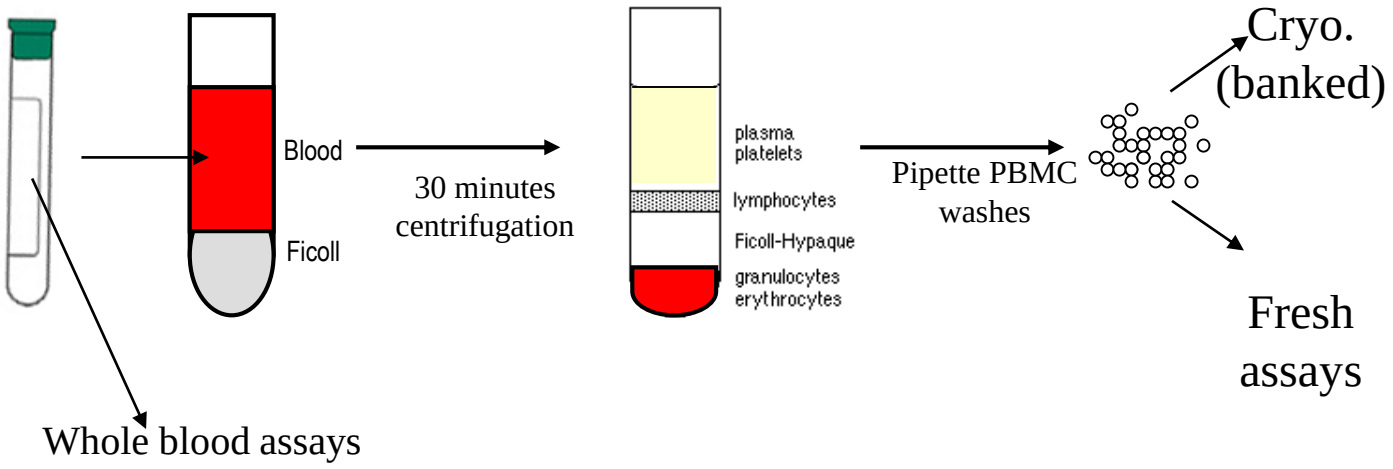
Grants focus on an investigator's one-dimensional analysis of samples and fail to provide funding for sample studies beyond that analysis.

…institutional support is often a hard-fought gain….

Nature Biotechnology 08 October 2015

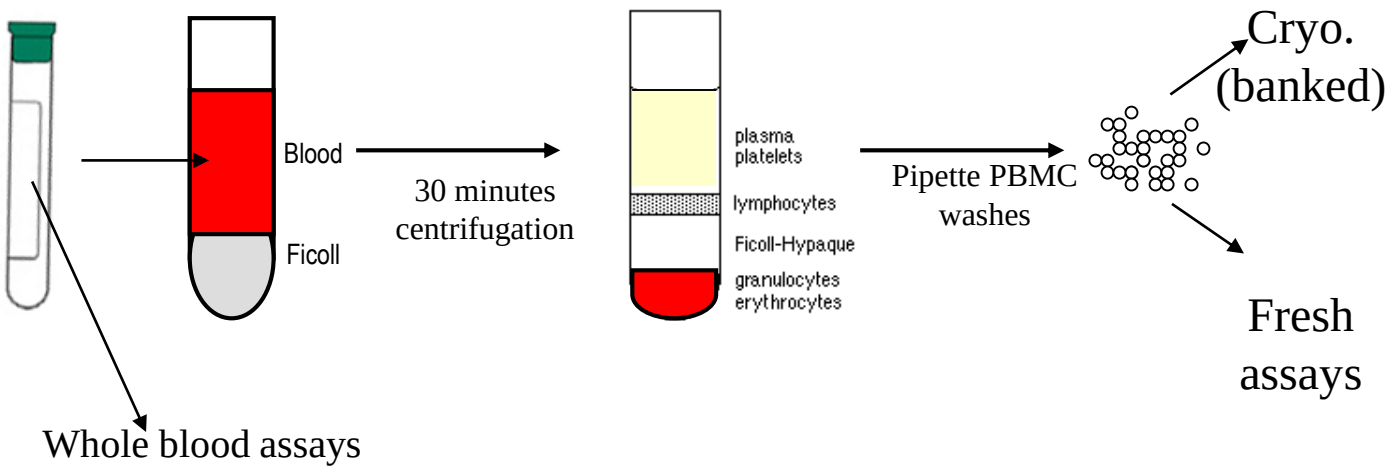
Peripheral Blood: easy to obtain at multiple time points

Separation of blood components on a Ficoll-Hypaque gradient.

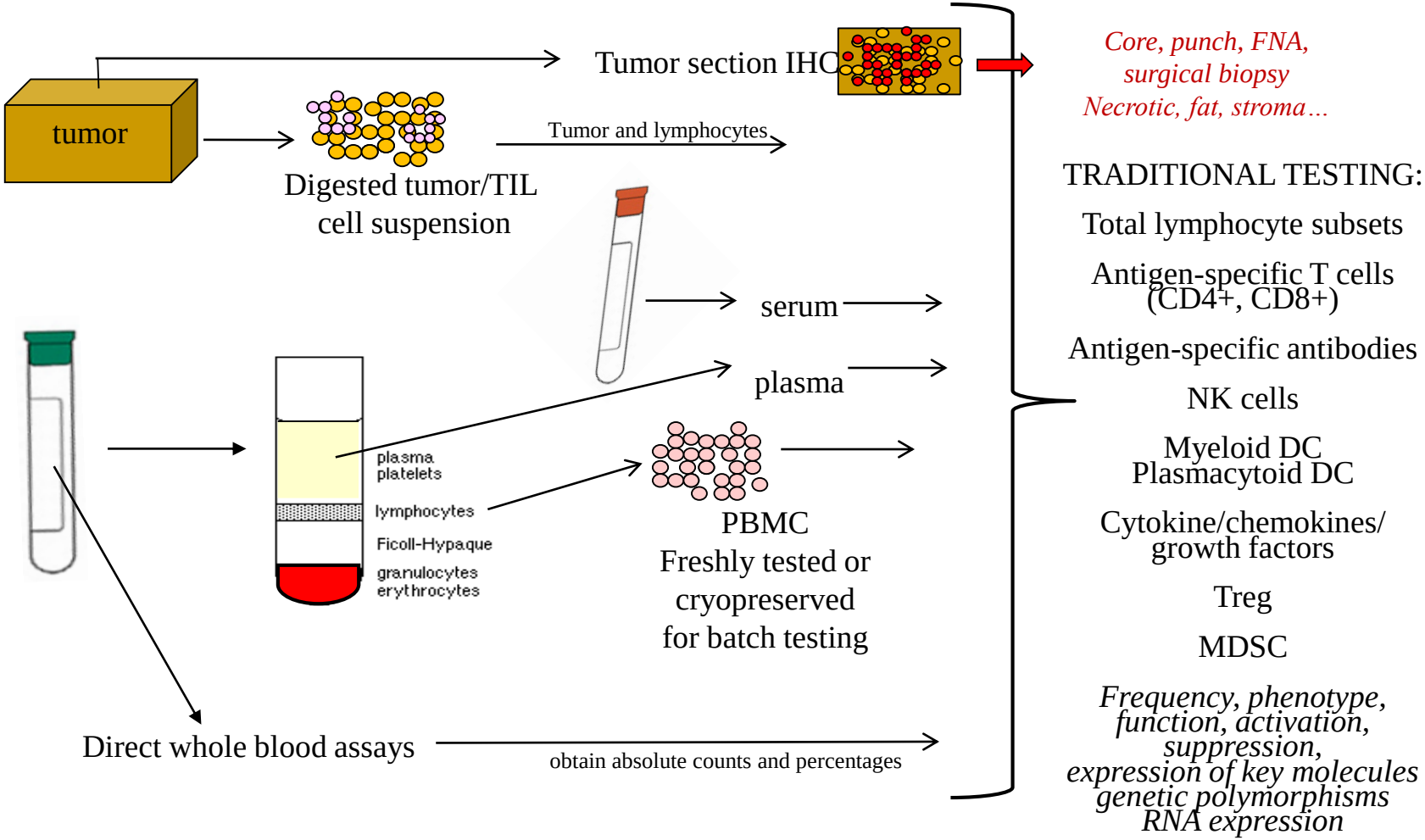


Limitations: for solid tumors, may not reflect what occurs at the tumor, and for leukemia/lymphoma, may be all tumor/blasts.

Peripheral Blood



Variability: *Hemolysed? Anti-coagulant in tube (heparin/EDTA)? Time/temperature since blood draw? Volume in tube?*



Patient-derived specimens used in immunologic monitoring

Measuring Immunity in Immunotherapy Clinical Trials:

- Was the cytokine induced (right time/place/level)?
- Did the vaccine activate tumor-specific T cells?
- Did the adoptively transferred effector cells survive/traffic to the tumor/kill the tumor?
- **Was immune suppression reversed?**
- **Were the target cells/molecules activated?**
- Did the target cells/molecules get to the tumor site and show activity?

- *Was the therapeutic intervention an improvement?*
- *Why or why not?*

Need: reliable, standardized measures of immune response.

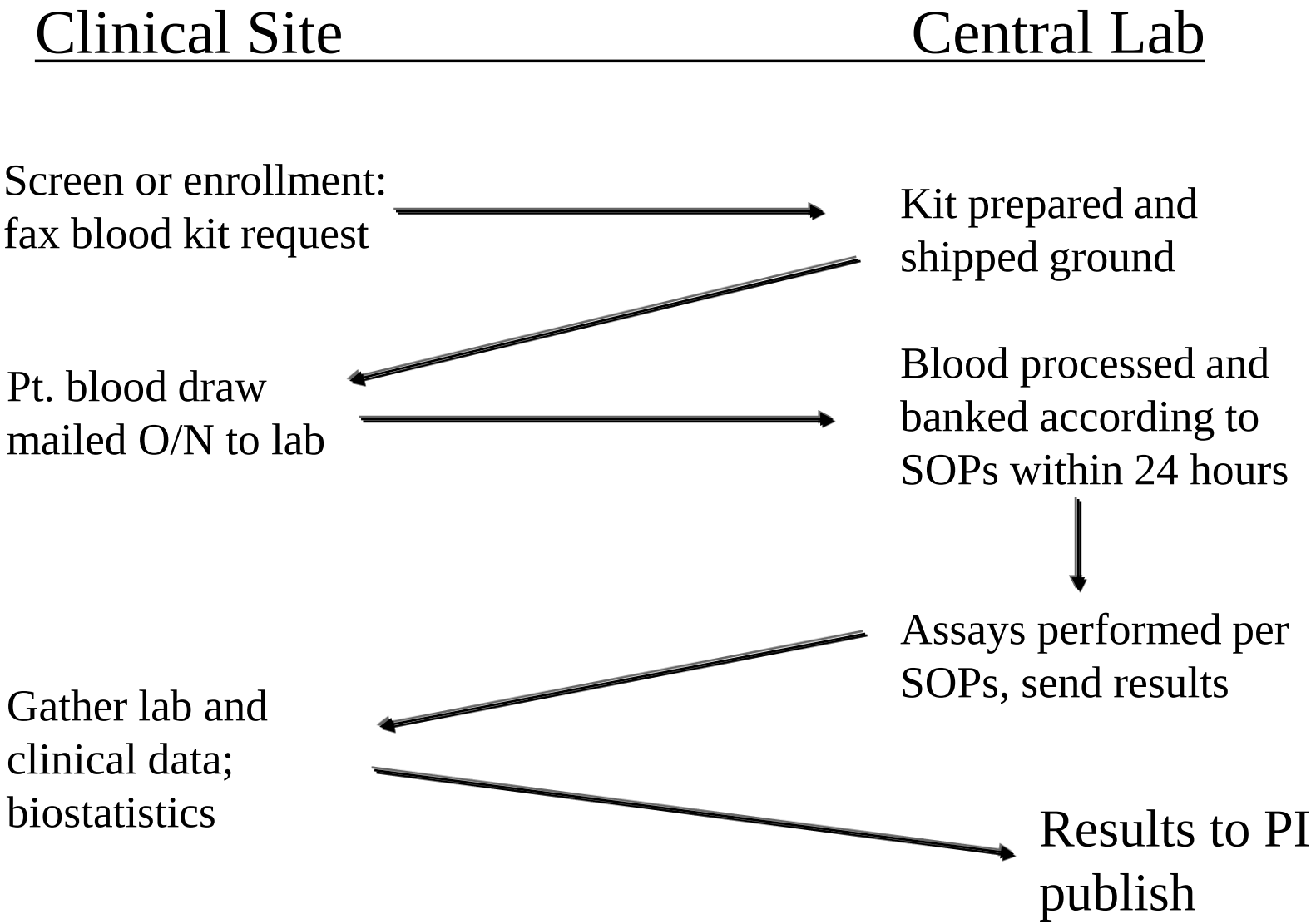
CLIA (Clinical Laboratory Improvements Amendments) rules:

Test Accuracy (close agreement to the true value),
Precision (agreement of independent results: same day, different day),
Reproducibility (intra-assay and inter-assay)

Reportable range (limits of detection)
Normal ranges (pools of healthy donors, accumulated patient samples),

Personnel competency testing
Equipment validation, monitoring
Reagent tracking

Central Immunology Laboratory



Standardized ELISPOT Assays

E4697 *(n=20, 2008-2009)*

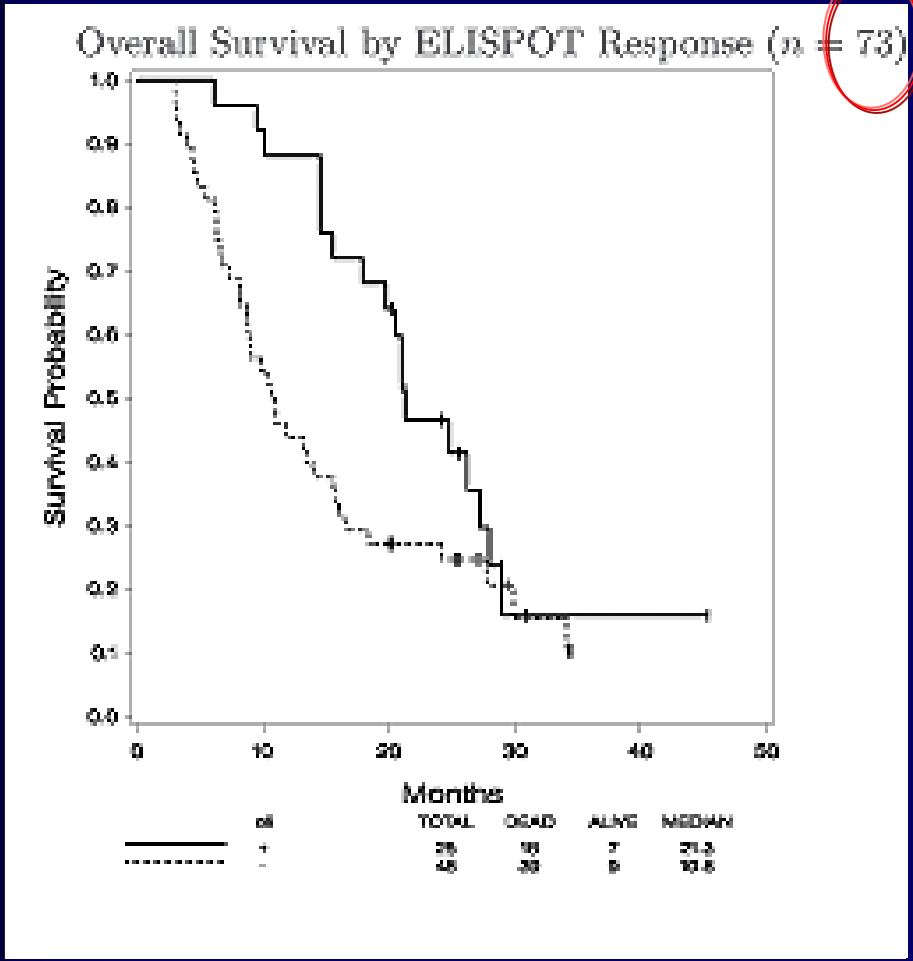
		spontaneous	PMA/I (+)/OKT3
Healthy control	ave.:	4.9 (54%CV)	304 (19.2%CV *intra-assay*) (48% CV inter-assay)
Patient	ave.:	0.7 (35%CV)	81 (38.7 %CV)

E1696 *(n=20, 2002-2003)*

		spontaneous	PMA/I (+)/PHA
Healthy control	ave.:	5.4 (56%CV)	284 (15.5%CV *intra-assay*) (51% CV inter-assay)
Patient	ave.:	19 (40%CV)	171 (18.8 %CV)

Immune Response Correlates with Overall Survival

Multiple melanoma antigen peptide vaccine ± GM-CSF ± IFNα2b



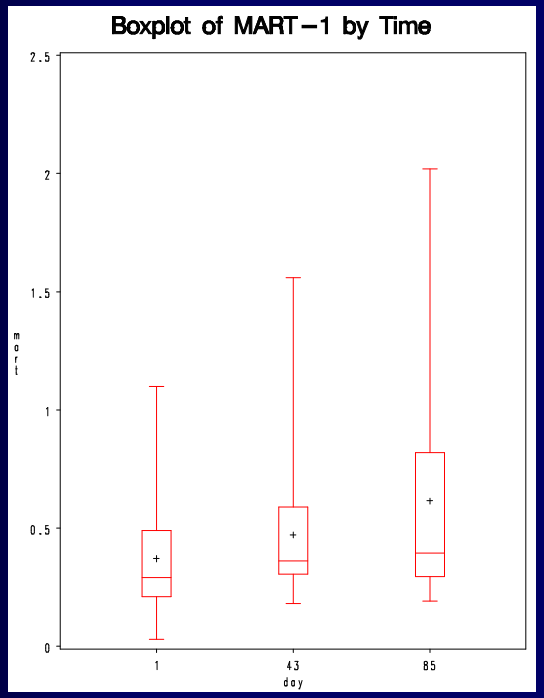
The Kaplan-Meier plot for OS by immune response status is shown for E1696 (Phase II).

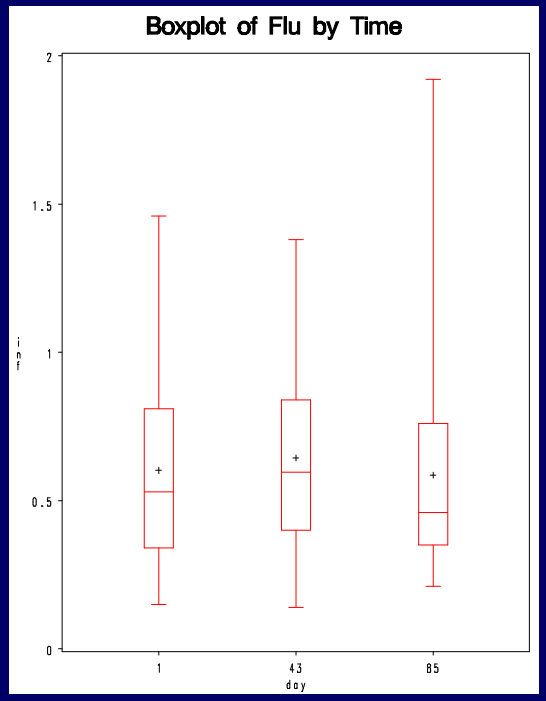
There was a significant difference in OS by immune response status. Immune responders lived longer than the non-immune responders (median OS 21.3 versus 10.8 months, *p*=0.033).

(Kirkwood, J.M., Clin. Cancer Res. 2009)

Immune Response: E1696

Melanoma antigen peptide-specific CD8+ T cells





%MART-1 CD8+ cells:	.29%	.36%	.39%	.53%	.53%	.43%
%effector cells:	10%	16%	18%	17%	17%	16%
		(p=0.048)			(p = ns)	

MHC Tetramer Analysis:

The frequency of vaccine peptide-specific CD8+ T cells was measured by MHC tetramers, showing *significant increases for all 3 melanoma antigen peptides* (not Flu).

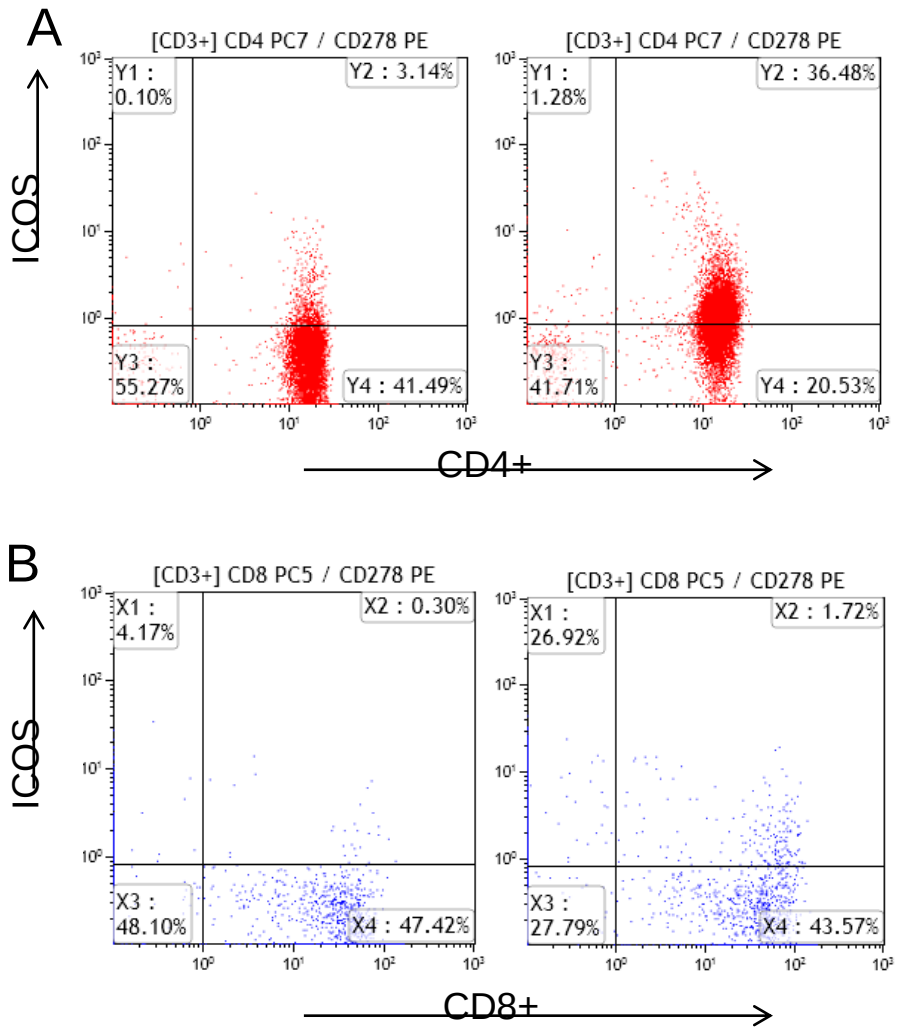
The MART-1 and gp100-specific cells *differentiated towards effector cells* with vaccination.

Schaefer, Int. J. Cancer, 2011

Multicenter, Randomized Phase II Trial of GM-CSF plus Ipilimumab (Ipi) vs. Ipi Alone in Metastatic Melanoma: E1608

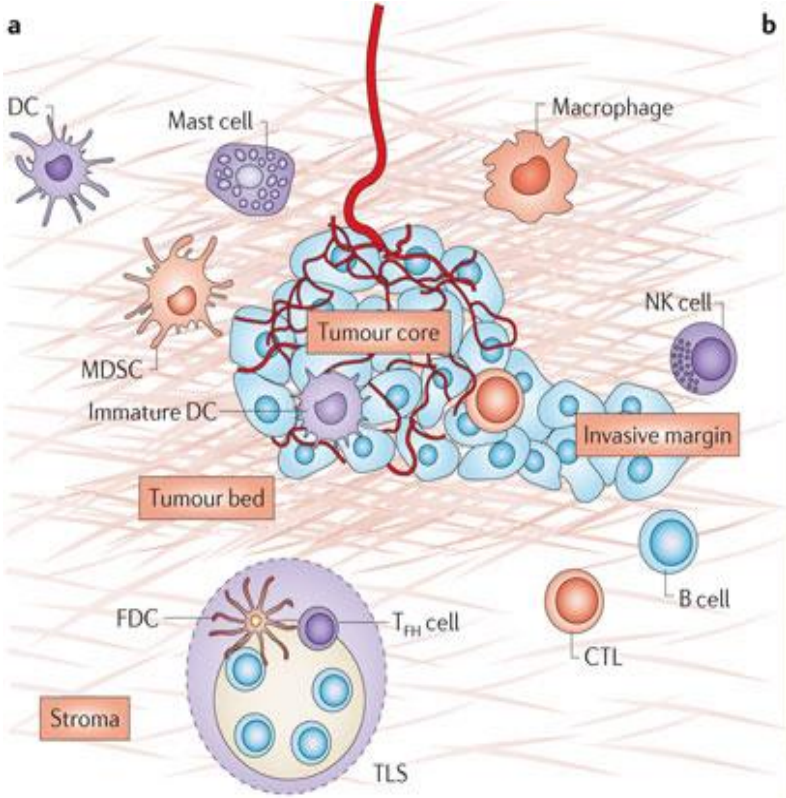
The addition of GM-CSF to ipilimumab significantly improves OS in patients with metastatic melanoma. Improved tolerability was seen in patients receiving GM-CSF.

Biomarkers: Increased ICOS on CD4+ and CD8+ T cells correlates with clinical outcome. Now being tested in other clinical trials.



Hodi FS, Rao UN, Butterfield, LH, Tarhini, AA, Kirkwood, JM, et al. Sargramostim plus ipilimumab for metastatic melanoma. (JAMA, 2014).

Immune Score



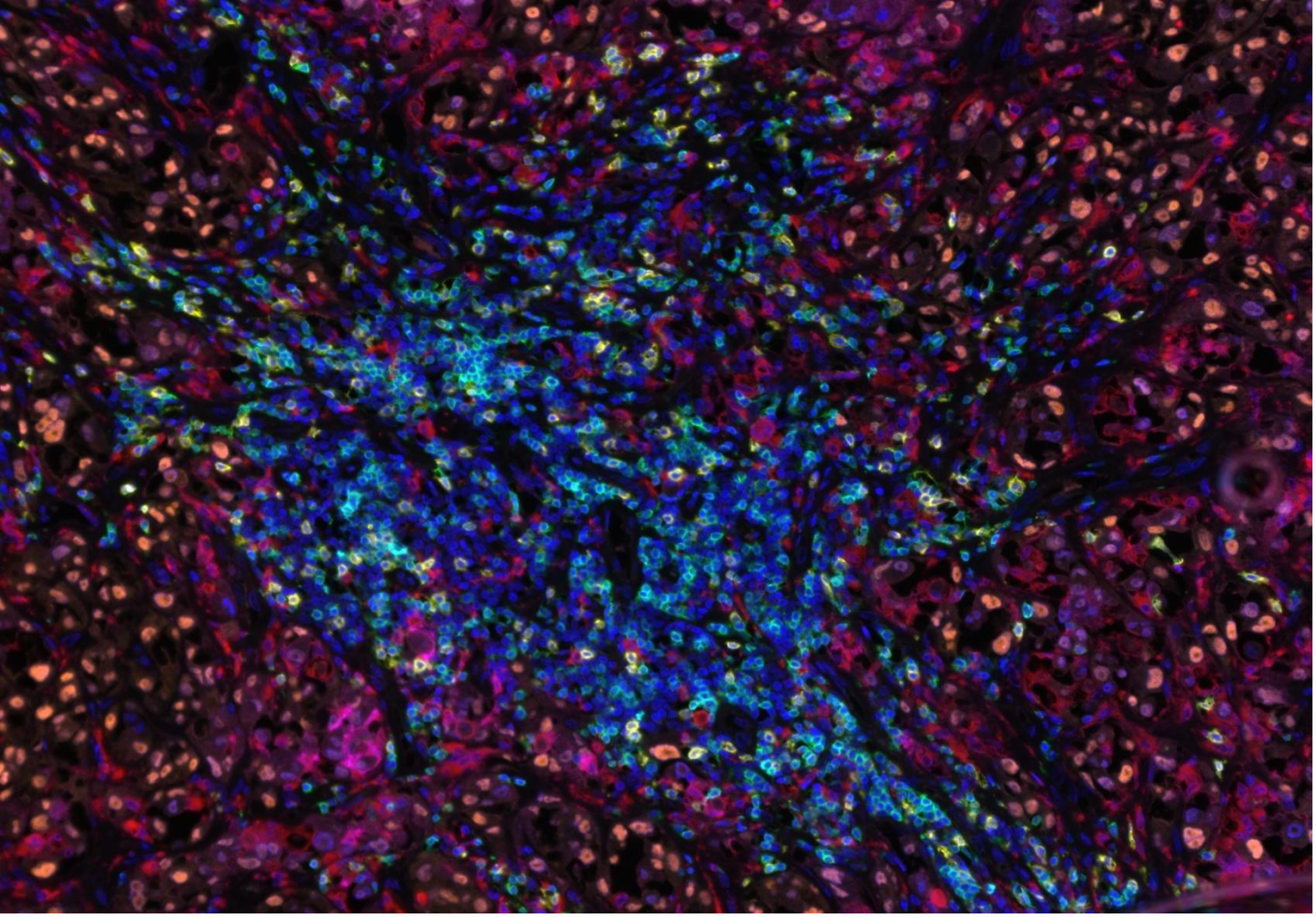
b

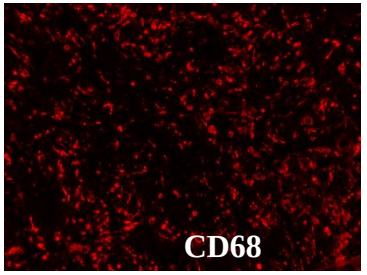
Immune contexture	Parameters: positive association with survival
Type	CTLs ($CD3^+CD8^+$)
	Memory T cells ($CD45RO^+$)
Location	Core of the tumour
	Invasive margin
Density	Number of cells per mm^2: 1, 10, 100, 1,000, 10,000 — $CD3^+_{CT}$, $CD3^+_{IM}$, $CD8^+_{CT}$, $CD8^+_{IM}$, $CD45RO^+_{CT}$, $CD45RO^+_{IM}$
Functional orientation	T_H1 cell-associated factors (IFNγ, IL-12, T-bet and IRF1)
	Cytotoxic factors (granzymes, perforin and granulysin)
	Chemokines (CX3CL1, CXCL9, CXCL10, CCL5 and CCL2)
	T_H17 cells, T_{Reg} cells and T_H2 cells have a variable effect on survival, depending on tumour type
TLS	Presence and quality

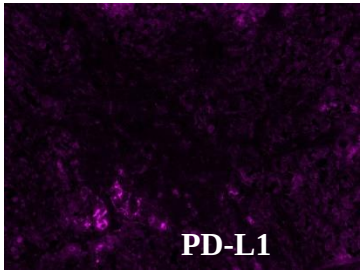
Nature Reviews | Cancer

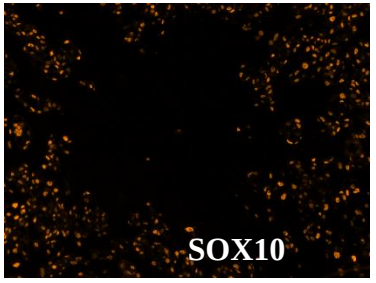
Tumor anatomy showing the features of the immune contexture, including the tumor core, the invasive margin, tertiary lymphoid structures (TLS) and the tumor microenvironment. The distribution of different immune cells is also shown.

CT, core of the tumor; DC, dendritic cell; FDC, follicular dendritic cell; IM, invasive margin; IRF1, interferon regulatory factor 1. ***J. Galon, W. Fridman***

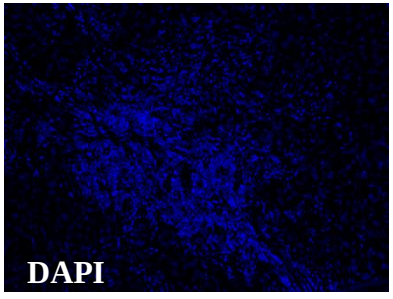


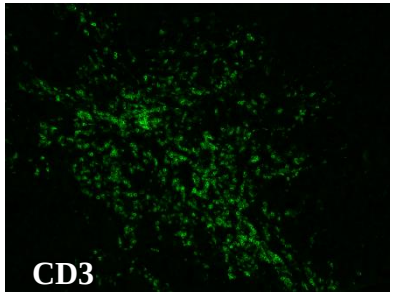


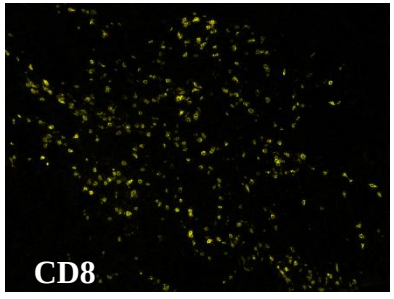


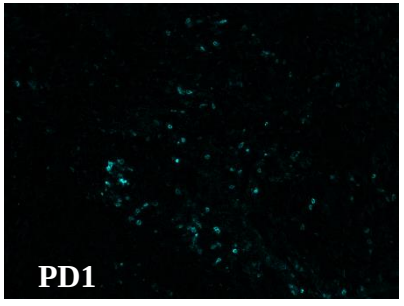


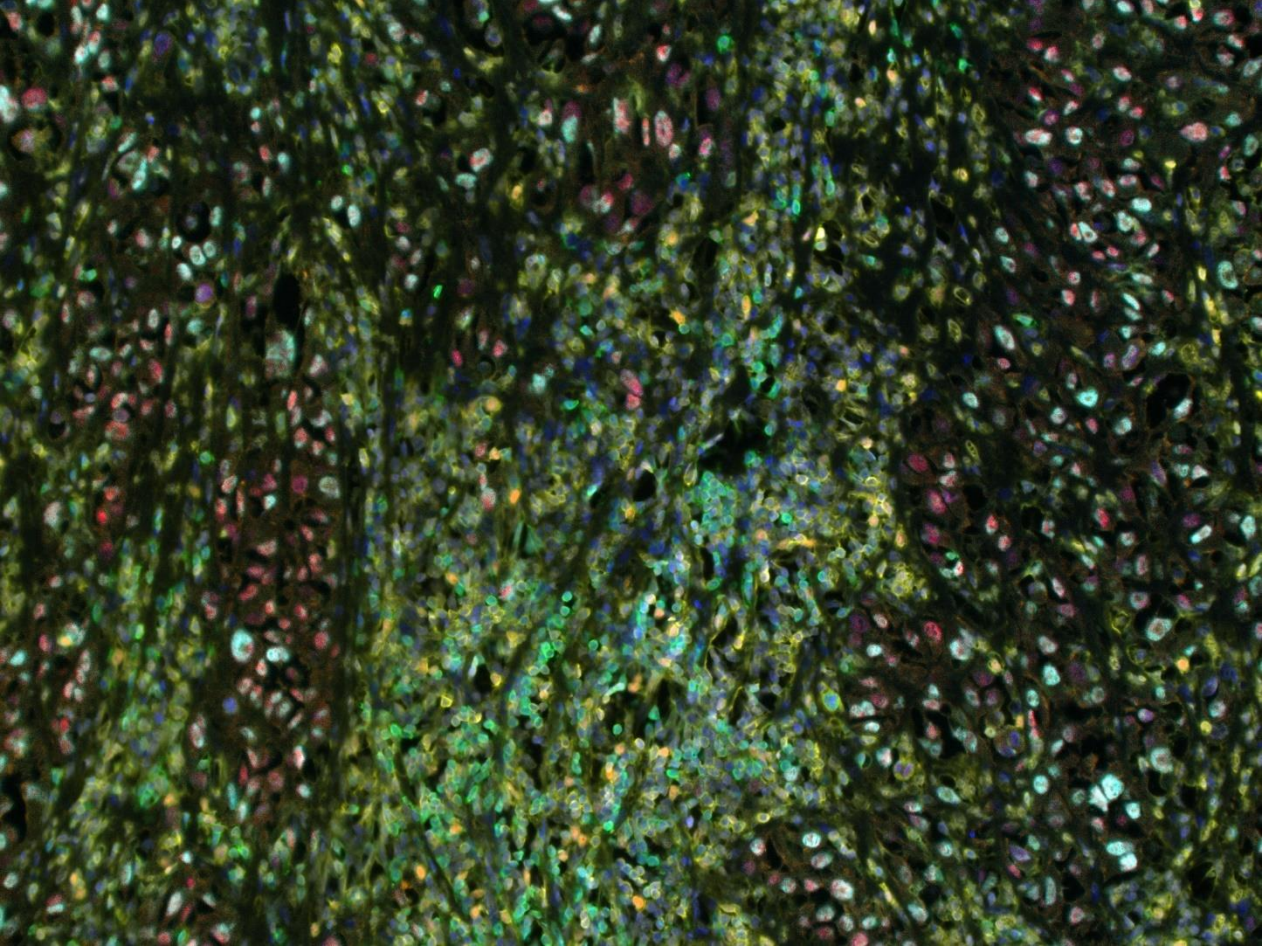
MELANOMA TISSUE

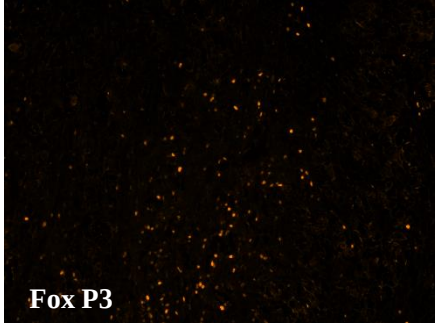


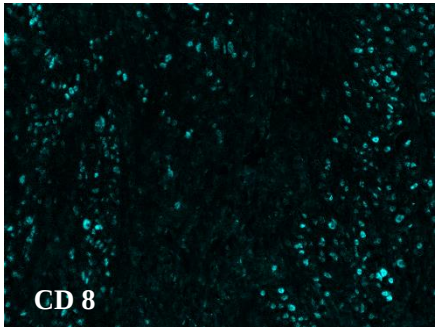


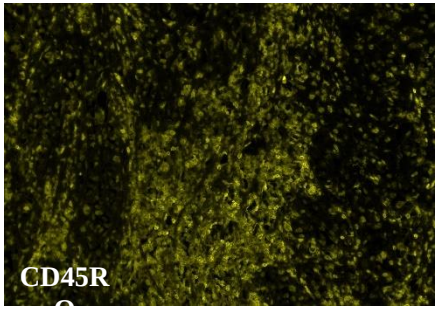


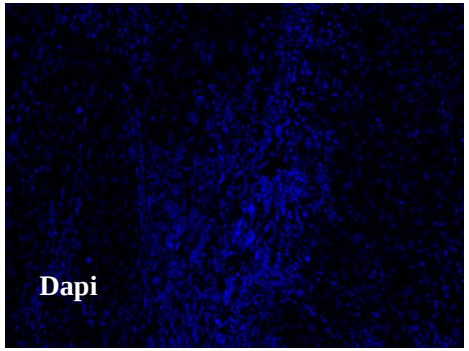


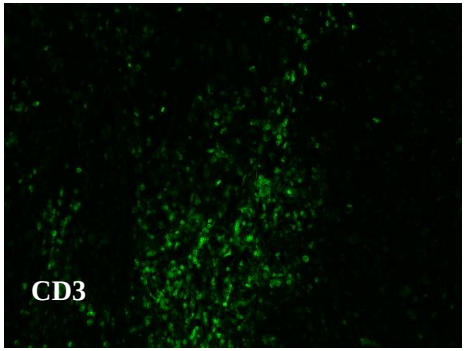


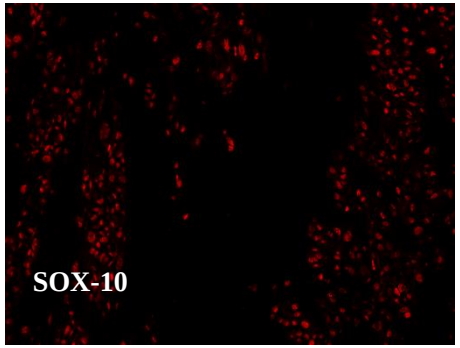






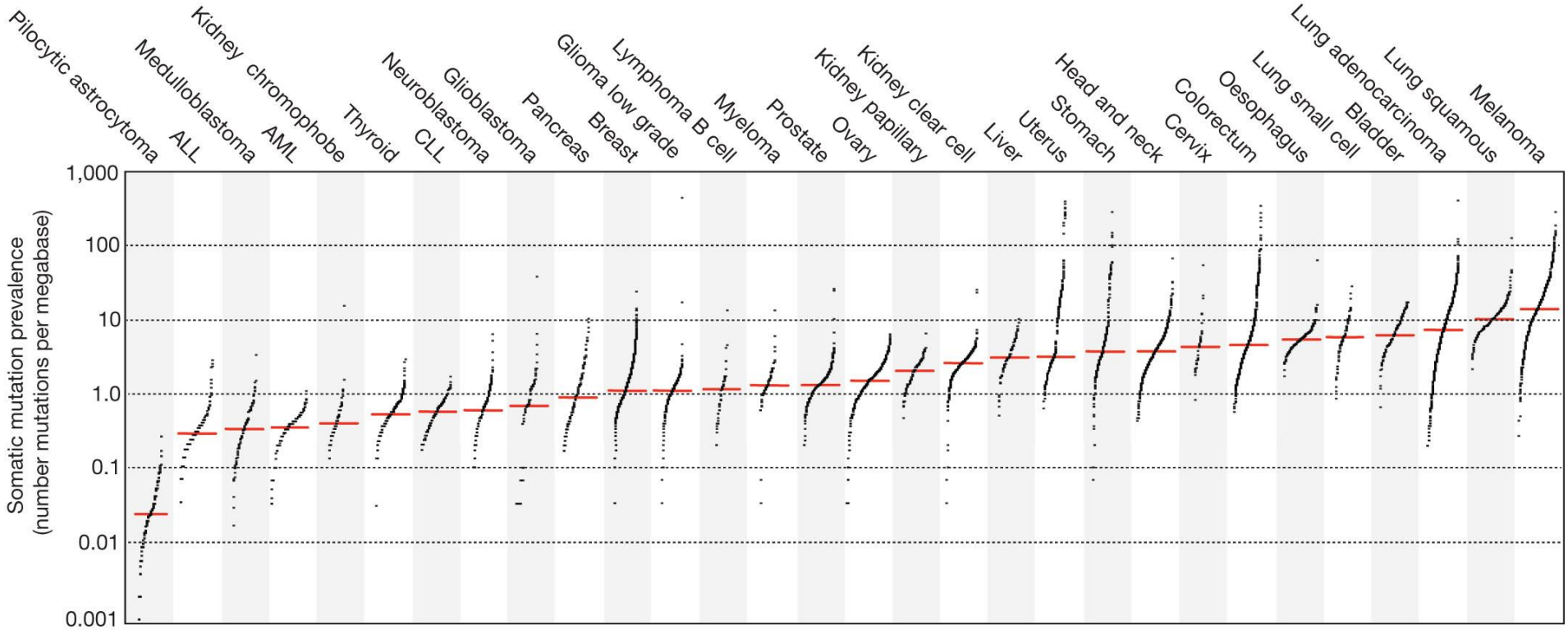






Melanoma Panel 2 in development

The prevalence of somatic mutations across human cancer types.



nature

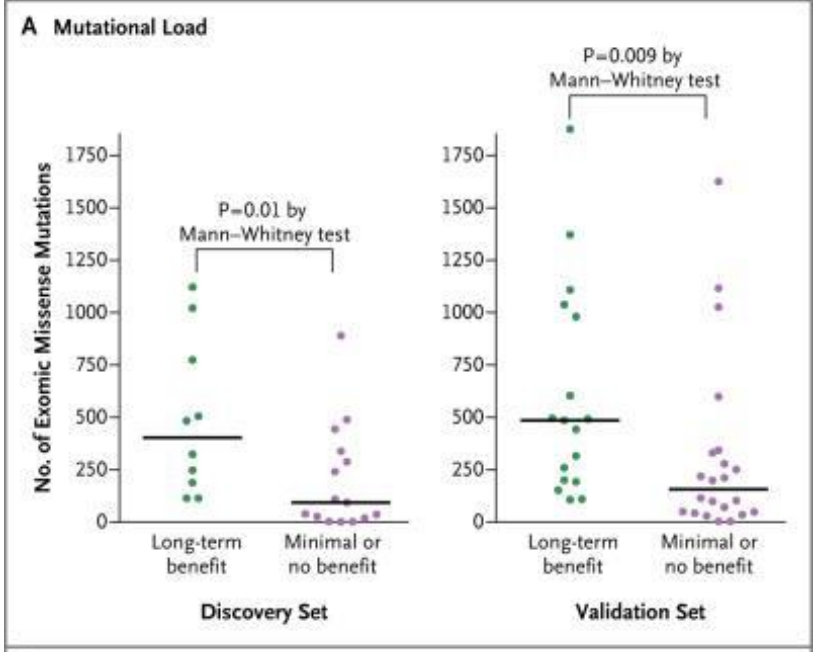
LB Alexandrov *et al. Nature* 000, 1-7 (2013) doi:10.1038/nature12477

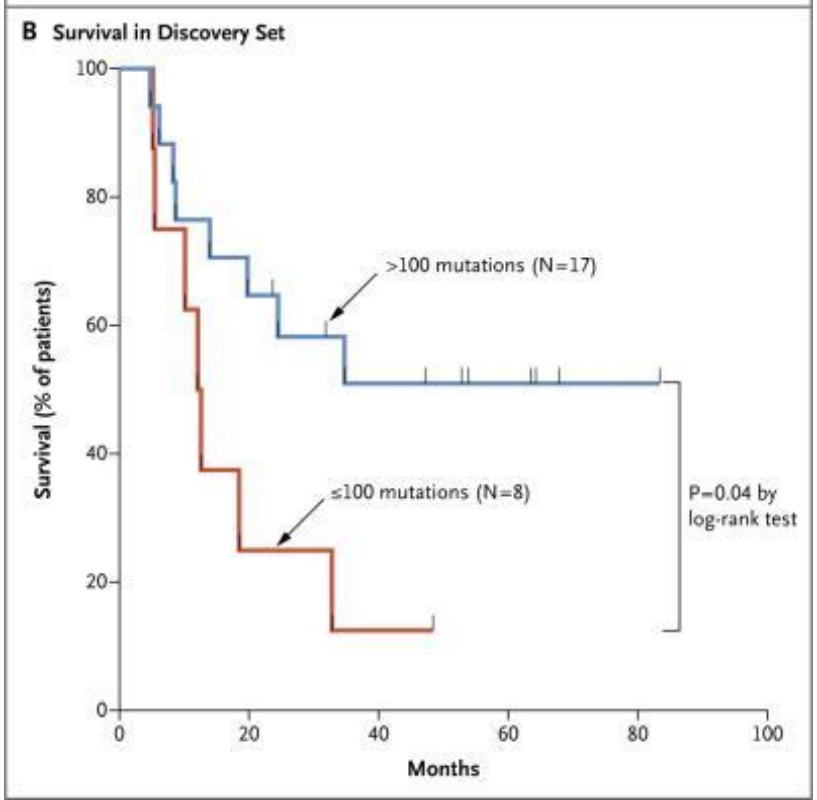
N Engl J Med. 2014 Dec

Genetic basis for clinical response to CTLA-4 blockade in melanoma.

Snyder A[1], Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, Walsh LA, Postow MA, Wong P, Ho TS, Hollmann TJ, Bruggeman C, Kannan K, Li Y, Elipenahli C, Liu C, Harbison CT, Wang L, Ribas A, Wolchok JD, Chan TA.

More mutations = better checkpoint blockade response





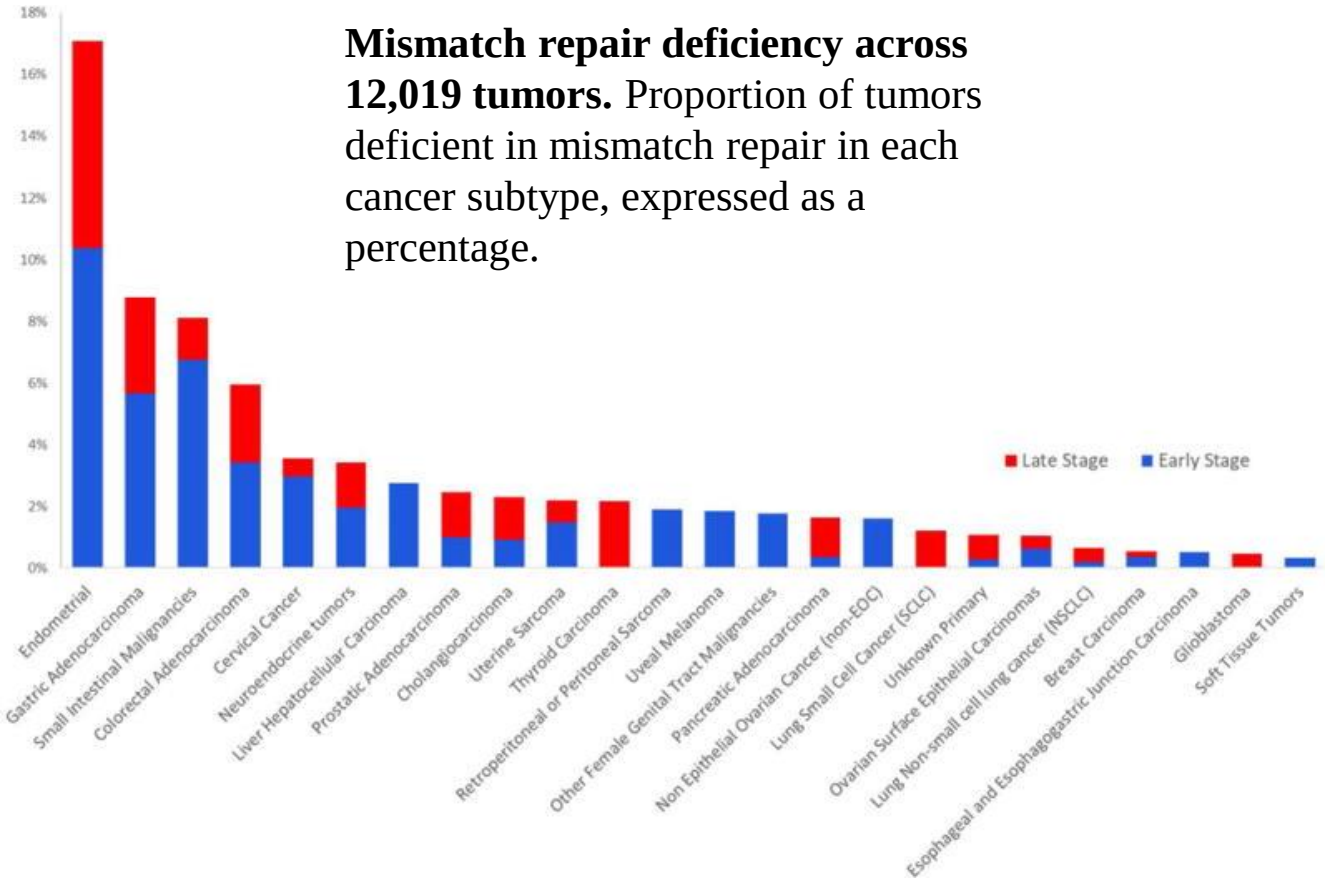
N Engl J Med. 2015 Jul

Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer.

Brahmer J[1], Reckamp KL, Baas P, Crinò L, Eberhardt WE, Poddubskaya E, Antonia S, Pluzanski A, Vokes EE, Holgado E, Waterhouse D, Ready N, Gainor J, Arén Frontera O, Havel L, Steins M, Garassino MC, Aerts JG, Domine M, Paz-Ares L, Reck M, Baudelet C, Harbison CT, Lestini B, Spigel DR.

BACKGROUND:
Patients with advanced squamous-cell non-small-cell lung cancer (NSCLC) who have disease progression during or after first-line chemotherapy have limited treatment options. This randomized, open-label, international, phase 3 study evaluated the efficacy and safety of nivolumab, a fully human IgG4 programmed death 1 (PD-1) immune-checkpoint-inhibitor antibody, as compared with docetaxel in this patient population.
RESULTS:
The median overall survival was 9.2 months (95% confidence interval [CI], 7.3 to 13.3) with nivolumab versus 6.0 months (95% CI, 5.1 to 7.3) with docetaxel. The risk of death was 41% lower with nivolumab than with docetaxel (hazard ratio, 0.59; 95% CI, 0.44 to 0.79; P<0.001). At 1 year, the overall survival rate was 42% (95% CI, 34 to 50) with nivolumab versus 24% (95% CI, 17 to 31) with docetaxel. The response rate was 20% with nivolumab versus 9% with docetaxel (P=0.008). The median progression-free survival was 3.5 months with nivolumab versus 2.8 months with docetaxel (hazard ratio for death or disease progression, 0.62; 95% CI, 0.47 to 0.81; P<0.001). **The expression of the PD-1 ligand (PD-L1) was neither prognostic nor predictive of benefit.** Treatment-related adverse events of grade 3 or 4 were reported in 7% of the patients in the nivolumab group as compared with 55% of those in the docetaxel group.
CONCLUSIONS:
Among patients with advanced, previously treated squamous-cell NSCLC, overall survival, response rate, and progression-free survival were significantly better with nivolumab than with docetaxel, regardless of PD-L1 expression level.

Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade



Mismatch repair deficiency across 12,019 tumors. Proportion of tumors deficient in mismatch repair in each cancer subtype, expressed as a percentage.

The genomes of cancers deficient in mismatch repair contain exceptionally high numbers of somatic mutations. We evaluate the efficacy of PD-1 blockade in patients with advanced mismatch repair-deficient cancers across 12 different tumor types. The large proportion of mutant neoantigens in mismatch repair-deficient cancers make them sensitive to immune checkpoint blockade, regardless of the cancers' tissue of origin.

Le, D. et al. Science. 2017 Jul

TME Gene Expression Profiles

Baseline gene expression in the tumor microenvironment, using RNA isolated from FFPE tumor tissue samples from patients undergoing treatment with pembrolizumab in clinical trials using multiple distinct tumor types.

We report validation of the hypothesis that immune-related gene signatures can predict clinical response to PD-1 checkpoint blockade. Signatures related to IFN-γ signaling and activated T cell biology were initially delineated in a small pilot melanoma cohort, then confirmed and refined in a larger independent cohort of patients with melanoma. The cross-tumor predictive value of these signatures was demonstrated by testing in head and neck squamous cell carcinoma (HNSCC) and gastric cancer cohorts, followed by a modeling exercise to determine a final T cell–inflamed gene expression profile that predicted response across 9 different cancer cohorts to arrive at a final signature, forming the basis of a clinical-grade assay for evaluation of clinical utility in select ongoing pembrolizumab clinical trials (18).

Our data definitively confirm that a T cell–inflamed microenvironment, characterized by active IFN-γ signaling, cytotoxic effector molecules, antigen presentation, and T cell active cytokines, is a common feature of the biology of tumors that are responsive to PD-1 checkpoint blockade. Moreover, these data demonstrate that a focused set of genes can be used to identify this PD-1 checkpoint blockade–responsive biology and predict clinical response across a wide variety of tumor types.

Ayers et al., JCI, 2017

IFN-γ–related mRNA profile predicts clinical response to PD-1 blockade

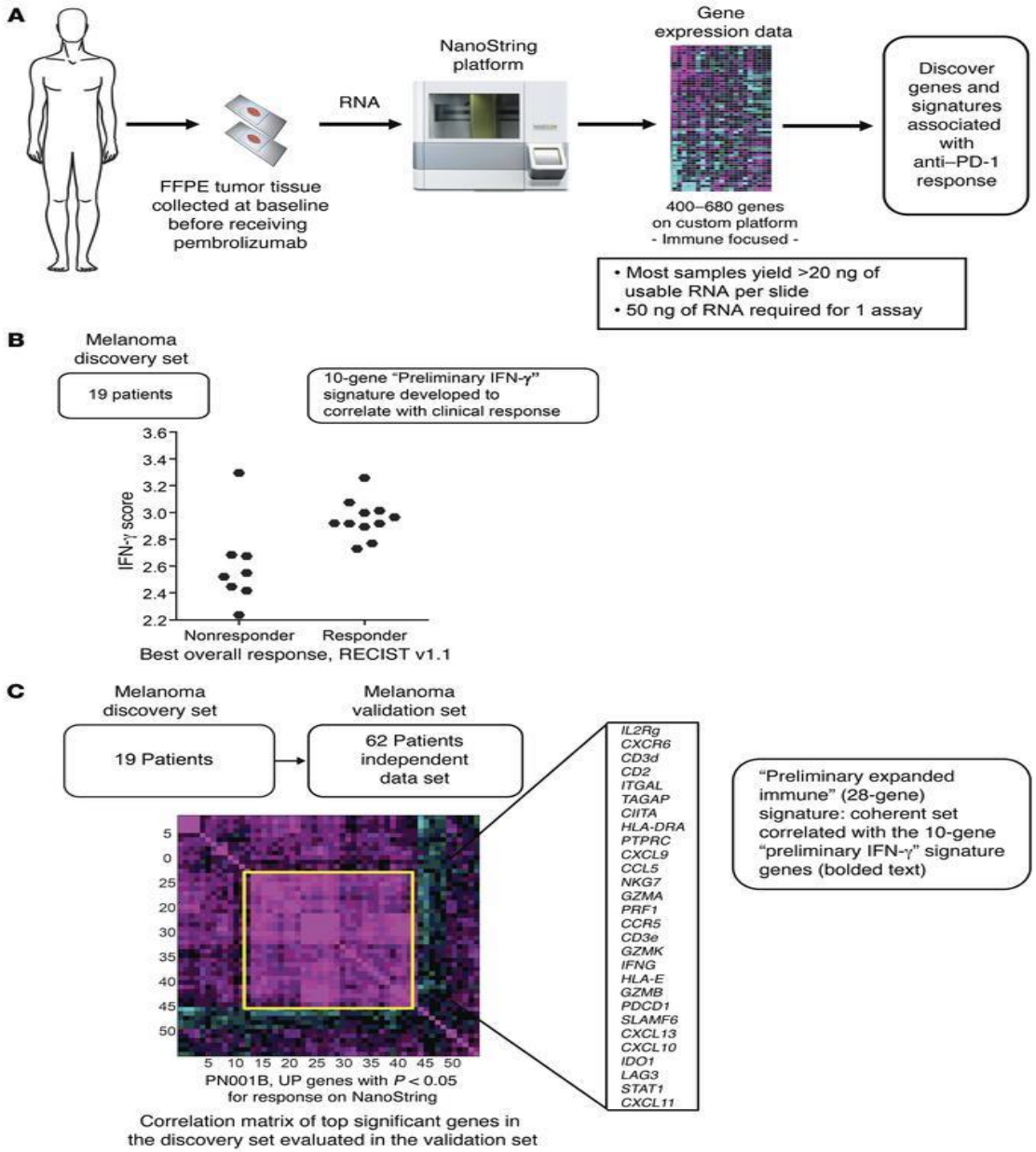
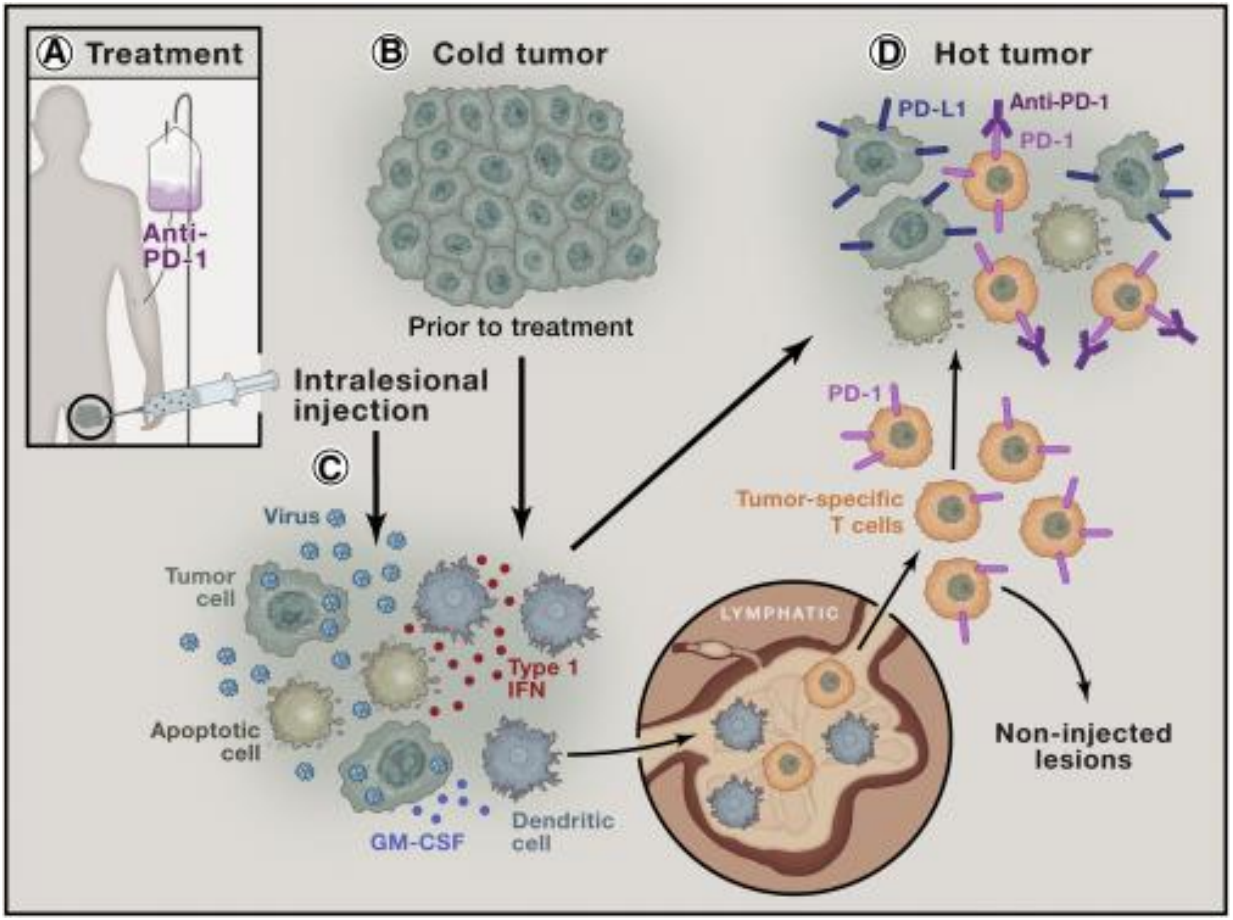


Table 2. IFN-γ and expanded immune gene signatures

IFN-γ	Expanded immune gene signature	
IDO1	*CD3D*	*IL2RG*
CXCL10	*IDO1*	*NKG7*
CXCL9	*CIITA*	*HLA-E*
HLA-DRA	*CD3E*	*CXCR6*
STAT1	*CCL5*	*LAG3*
IFNG	*GZMK*	*TAGAP*
	CD2	*CXCL10*
	HLA-DRA	*STAT1*
	CXCL13	*GZMB*

Ayers et al., JCI, 2017

T-VEC oncolytic virus + PD-1 Blockade



Phase 1b trial testing oncolytic virotherapy with T-VEC on cytotoxic T cell infiltration and therapeutic efficacy of the anti-PD-1 antibody pembrolizumab. Twenty-one patients with advanced melanoma were treated with T-VEC followed by combination therapy with pembrolizumab. Confirmed objective response rate was 62%, with a complete response rate of 33% per immune-related response criteria. Patients who responded to combination therapy had increased $CD8^+$ T cells, elevated PD-L1 protein expression, as well as IFN-γ gene expression on several cell subsets in tumors after T-VEC treatment. Response to combination therapy did not appear to be associated with baseline $CD8^+$ T cell infiltration or baseline IFN-γ signature.

Ribas, et al. CELL Volume 170, Issue 6, 7 September 2017, p1055

Approaches to addressing inherent variability in immunologic monitoring of clinical trials

Source of Variability	Recommendation
Patient	Save DNA/RNA/cells/tumor to understand host variation include healthy donor control
Blood draw	Standardized tubes and procedures
Processing/cryopreservation/ thaw	Standardized procedures and reagents
Cellular product	Phenotypic and functional assays to characterize the individual product, development of potency assays
Assay choice	Standardized functional tests
Assay conduct	Standardized operating procedures (SOPs)
Assay analysis	Appropriate biostatistical methods
Data reporting	Full details, controls, quality control/assurance (QA/QC) MIATA guidelines
Newest, non-standardized technology	Sufficient blood/tissue to interrogate the samples *now*, as well as *later*, to generate new hypotheses

Recommendations from the iSBTc-SITC/FDA/NCI Workshop on Immunotherapy Biomarkers, CCR 2011

Immunotherapy Biomarkers Task Force: 2015-2018

GROUP 1: "Immune monitoring assay standardization and validation—update" *Leaders: Magdalena Thurin, PhD and Giuseppe Massucci, MD*

GROUP 2: "New developments in biomarker assays and technologies"
Leader: Jianda Yuan, MD

GROUP 3: "Assessing Immune Regulation and Modulation Systematically (high throughput approaches)" *Leader: David Stroncek, MD*

Group 4: "Baseline Immunity, tumor immune environment and outcome prediction" *Leader: Sacha Gnjatic, PhD*

Taskforce Contributions to the field:

1. Preamble/overview commentary (JITC March 2015)
2. Recommendations/white paper 1/WG (WG2 JITC Mar. 2016)
3. Biomarker Technology short reports (1/month in JITC x 12)
4. Clinical trial analysis project: standard cellular/cytokine assays and high throughput molecular analyses—ongoing (CTLA-4 +/- GM-CSF)
5. Summary meeting: April 1st 2016
6. Workshop for next projects: May 2018
7. Next: Data sharing, Immunoscore images, multispectral imaging

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Society for Immunotherapy of Cancer

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SUBJECT: Guidelines for Biomarker Assays Used in CTEP-Sponsored, Early Phase Clinical Trials Performed Under CTEP IND
TO: Investigators and Company Collaborators

Briefly, markers are **integral** when they are essential for conducting the study as they *define eligibility, stratification, disease monitoring or study endpoints*.

Markers are considered **integrated** when they actually are *testing a hypothesis based on preexisting data* and not simply generating hypotheses. Such integrated markers need to be performed ideally on all patients in a trial and *the assay should already have been tested in human subjects with the disease in question and demonstrated reproducible analytic qualities*.

In contrast, **exploratory biomarkers** may not be performed on all subjects in a trial, and collection of these exploratory markers by investigators participating in the trial may be voluntary.

BIOMARKER STUDY Evaluation Guidelines

Purpose and Background As part of its Prioritization and Scientific Quality Initiatives, the Clinical Trials Working Group (CTWG) of NCI recommended establishing a **funding mechanism and prioritization process for essential correlative biomarker studies that are incorporated into the fundamental design of a clinical trial.** The objective of this initiative is to ensure that the most important biomarker studies can be initiated in a timely manner in association with clinical trials. The primary purpose of this funding mechanism is to support integral and/or integrated biomarker studies embedded in large (≥100 patients), randomized phase 2 treatment trials or in any randomized phase 3 clinical trials conducted by NCI National Clinical Trials Network (NCTN) Groups and NCI Community Oncology Research Program (NCORP).

Two types of biomarker studies are eligible – Integral and Integrated

Anticipated/planned INTEGRATED biomarker study applications …..must be submitted within three (3) months of the PI receiving notification by the respective CTEP/DCP PIO, that the concept was approved.

INTEGRAL Studies - Defined as assays that must be performed in order for the trial to proceed. Integral studies are inherent to the design of the trial from the onset and must be performed in real time for the conduct of the trial. **Integral biomarkers require a CLIA-certified lab**. Studies that will be conducted in the future on stored specimens are not eligible for BIQSFP funding, except if the results are critical to the stated primary or secondary objectives of the trial.

BIQSFP proposals for funding of INTEGRAL biomarker studies must be submitted concurrently with the parent concept. Integral studies will have the highest priority.

For in vitro tests, describe the current status of studies defining the accuracy, precision, reportable range, reference ranges/intervals (normal values), and failure rate of the assay as it is to be performed in the trial (e.g., performance of test on specimens intended to be used in the clinical trial). Describe the use of positive and negative controls, calibrators, and reference standards for clinical assays. Describe any critical pre-analytic variables.

Combination Approaches and Biomarkers

1. Standardized specimens
2. Greater emphasis on tumor biopsies
3. Include “hit the target” assessments
4. Include previously identified candidate biomarkers to confirm in new setting
5. Include high throughput hypothesis-generating technologies
6. Innovative data analysis

Conclusions

Biomarkers for prediction, prognostication and mechanism-of-action in cancer immunotherapy are still largely exploratory, although exciting signals are being validated (analytically and clinically). Biomarkers identified in tissue might ultimately be testable in blood.

New high throughput technologies can yield important insights:

Could "multiple TAA T cell responses" in blood = "determinant spreading" from "*in vivo* cross-presentation" = "greater TCR diversity" in blood, driven in part by "higher mutation loads" in tumors with "IFNγ signatures" showing they are permissive for immune infiltration?

Common mechanisms: PD-L1 on tumors, Tumor Mutation Burden, CD8+ T cell infiltrate, IFNγ gene signature