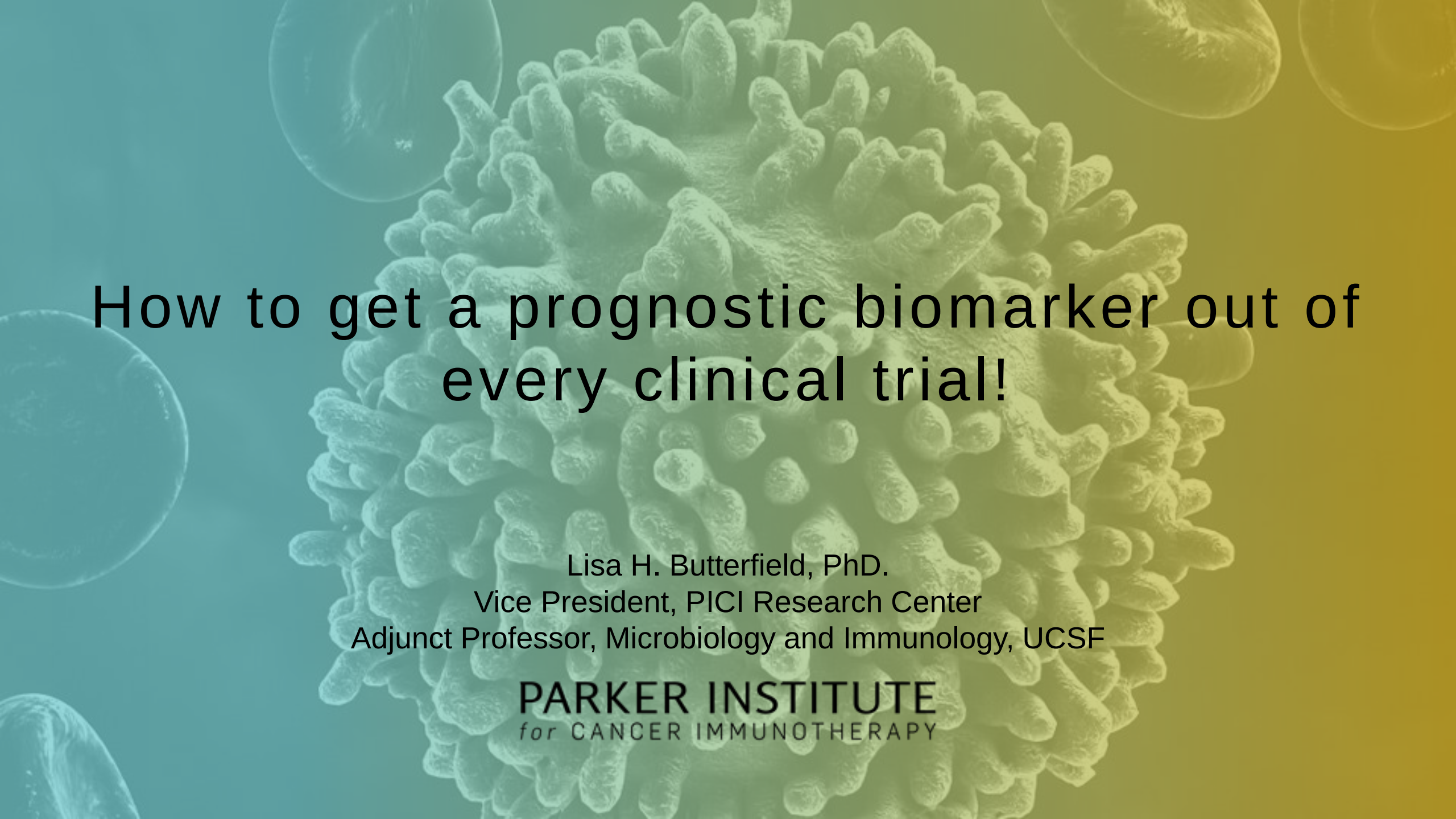


How to get a prognostic biomarker out of every clinical trial!

Lisa H. Butterfield, PhD.
Vice President, PICI Research Center
Adjunct Professor, Microbiology and Immunology, UCSF

PARKER INSTITUTE
for CANCER IMMUNOTHERAPY

Why don’t we have more useful Biomarkers?

There did not use to be populations of clinical trial objective *clinical* responders:

1. We need the right specimens saved under standardized conditions. Variably banked specimens give noisy data. Some trials bank non-viable tumor (FFPE), minimal blood (poorly functional PBMC) and plasma 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.

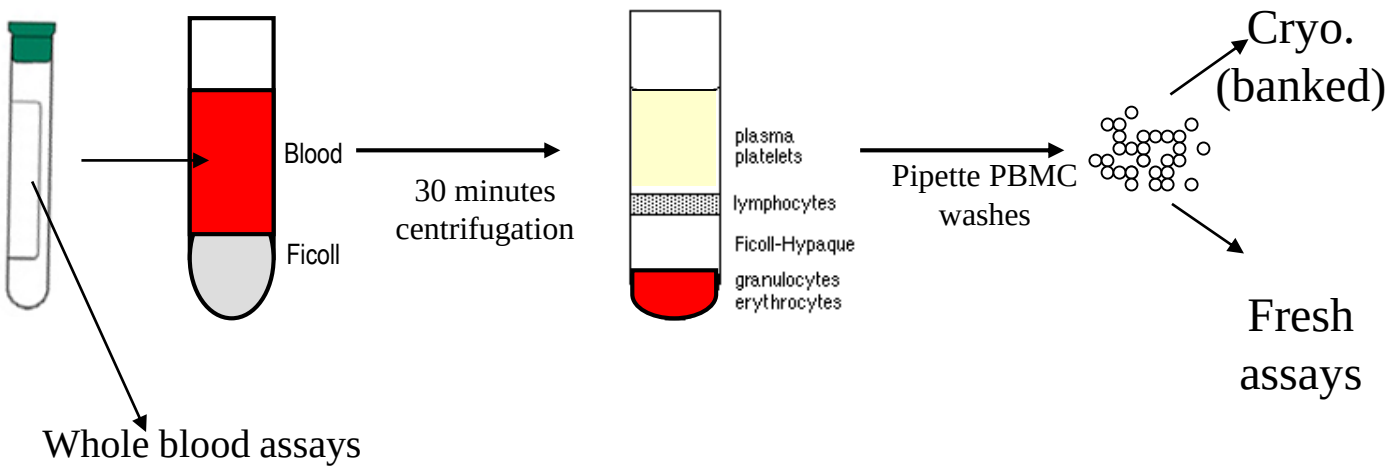
Grants focus on an investigator's one-dimensional analysis of samples and fail to provide funding for sample studies beyond that analysis.

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

Nature Biotechnology 08 October 2015

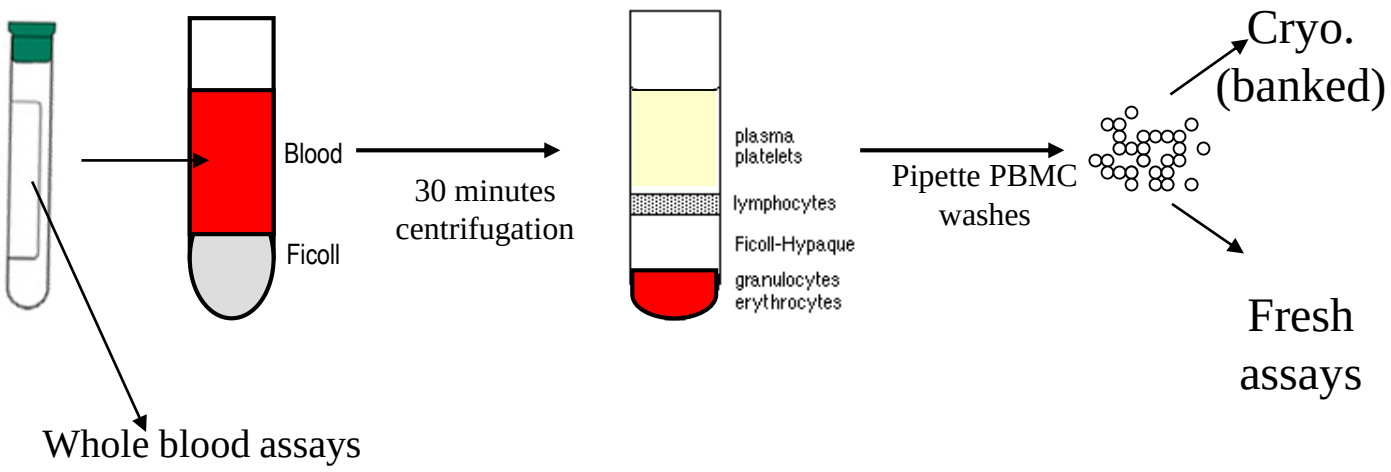
Peripheral Blood: easy to obtain at multiple time points

Separation of blood components on a Ficoll-Hypaque gradient.

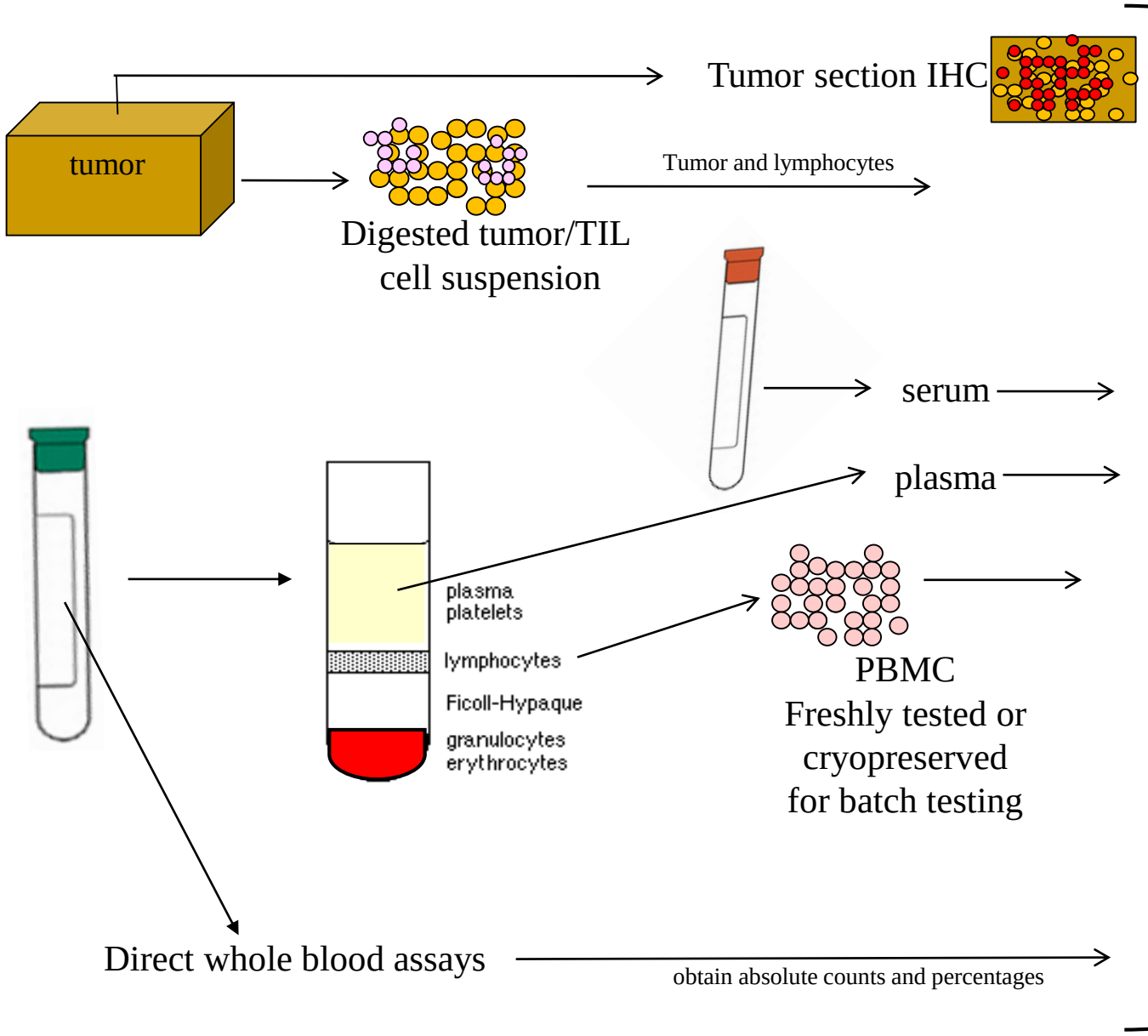


Limitations: for solid tumors, may not reflect what occurs at the tumor, and for leukemia/lymphoma, may be all tumor/blasts.

Peripheral Blood



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



Core, punch, FNA, surgical biopsy
Necrotic, fat, stroma…

TRADITIONAL TESTING:

Total lymphocyte subsets

Antigen-specific T cells
(CD4+, CD8+)

Antigen-specific antibodies

NK cells

Myeloid DC
Plasmacytoid DC

Cytokine/chemokines/
growth factors

Treg, MDSC

Frequency, phenotype,
function, activation,
suppression,
expression of key molecules
genetic polymorphisms
RNA expression

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.

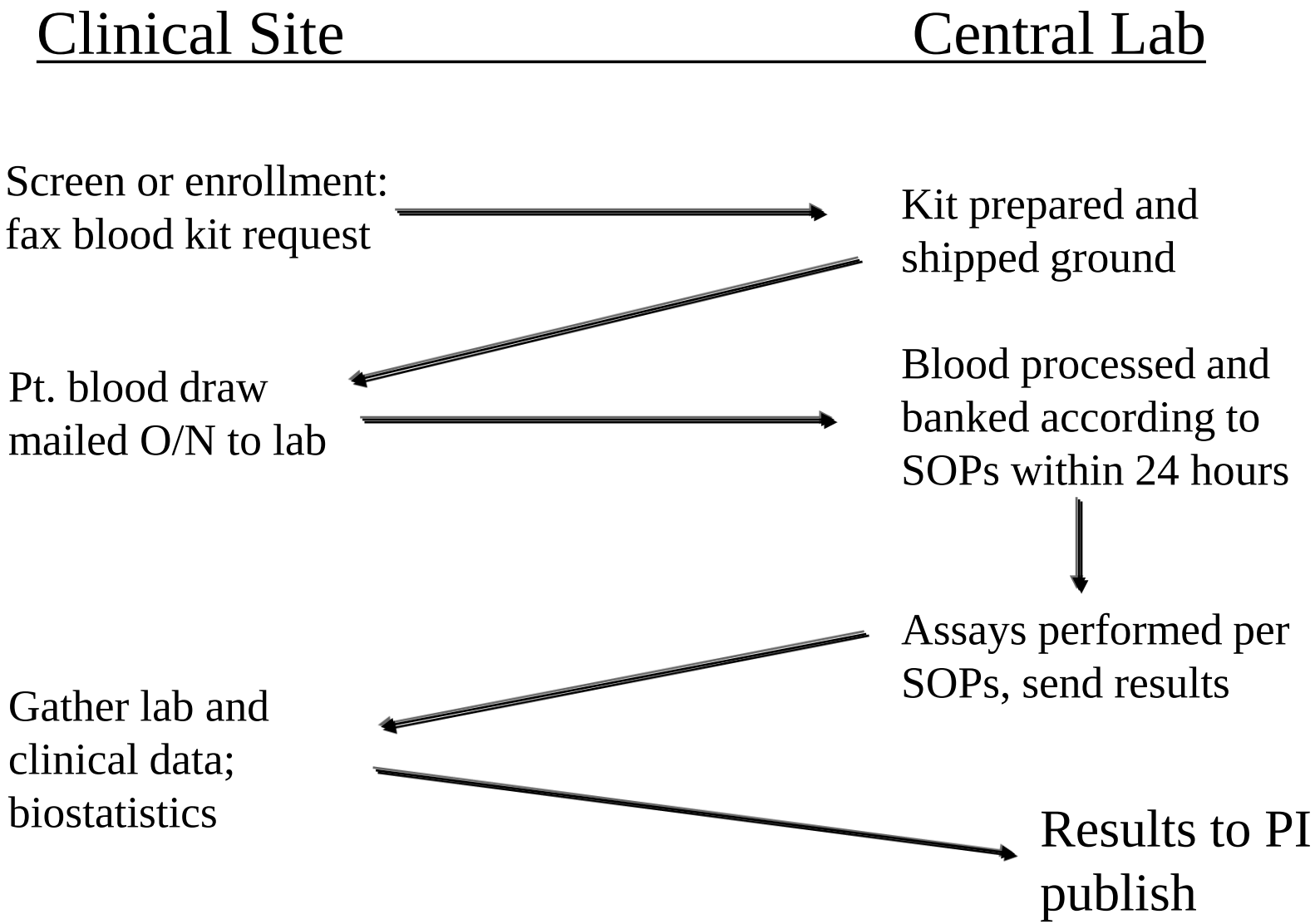
CLIA (Clinical Laboratory Improvements Amendments) rules:

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



Memorandum DATE: July 12, 2013

FROM: James A. Zwiebel, M.D.
Chief, Investigational Drug Branch, Cancer Therapy Evaluation Program
Division of Cancer Treatment and Diagnosis

Tracy Lively, Ph.D.
Deputy Associate Director, Cancer Diagnosis Program
Division of Cancer Treatment and Diagnosis

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.

Tried, true and very well standardized: the IFNγ ELISPOT assay

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)

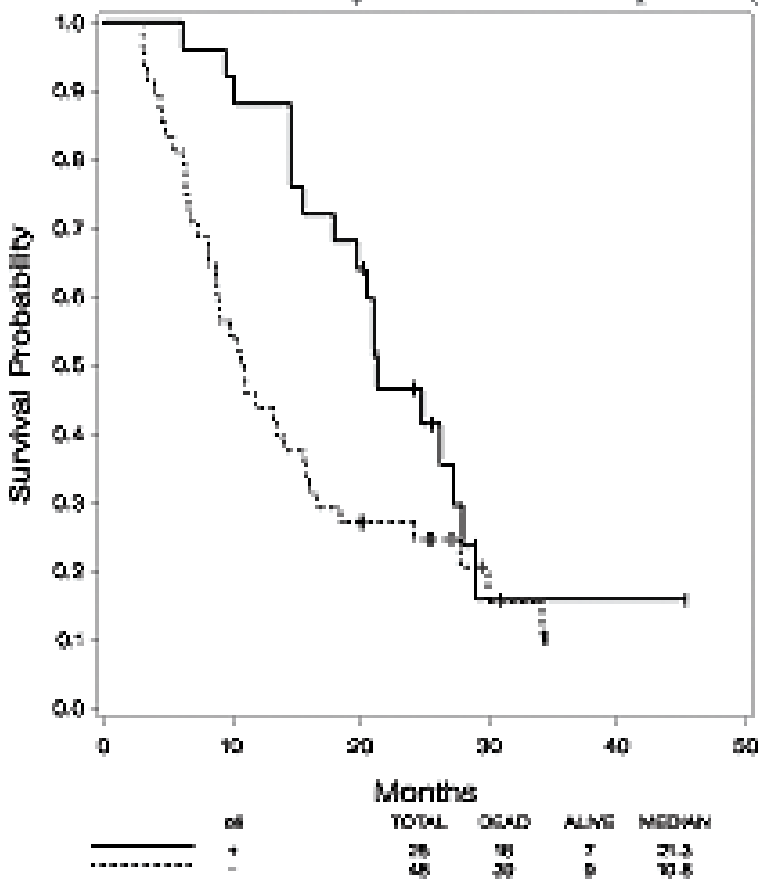
E1696 *(n=20, 2002-2003)*

		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

Fig 4: Overall Survival by ELISPOT Response (n = 73)



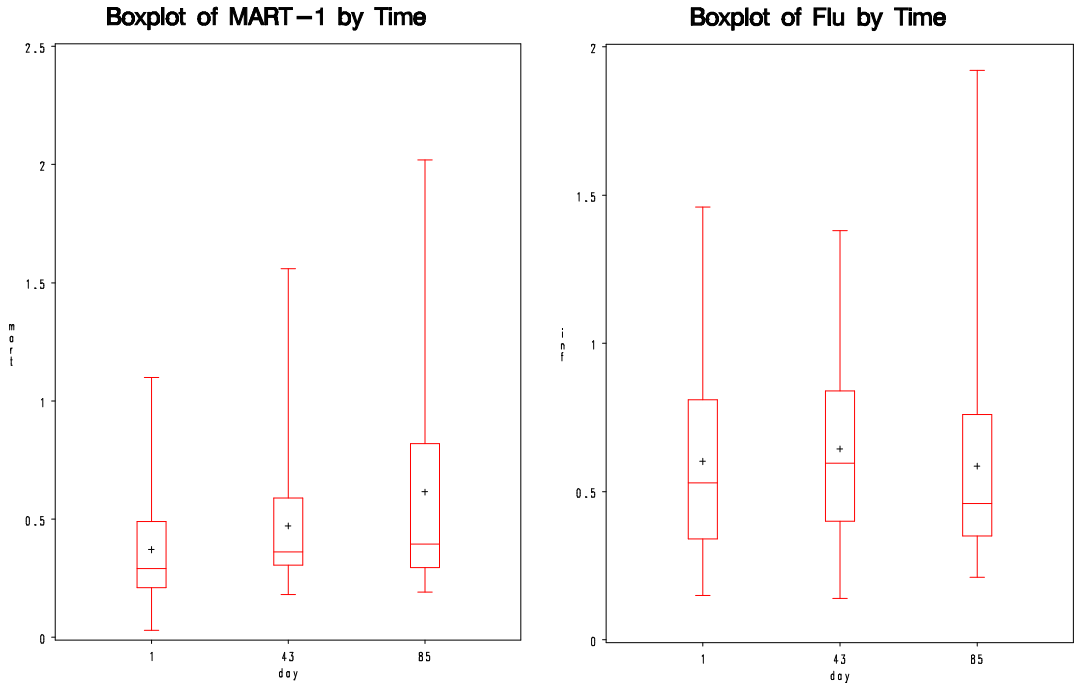
The Kaplan-Meier plot for OS by immune response status is shown for E1696 (Phase II).

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)

Peptide-specific and phenotypic: MHC multimer (tetra-, penta-…dextra-…)

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).

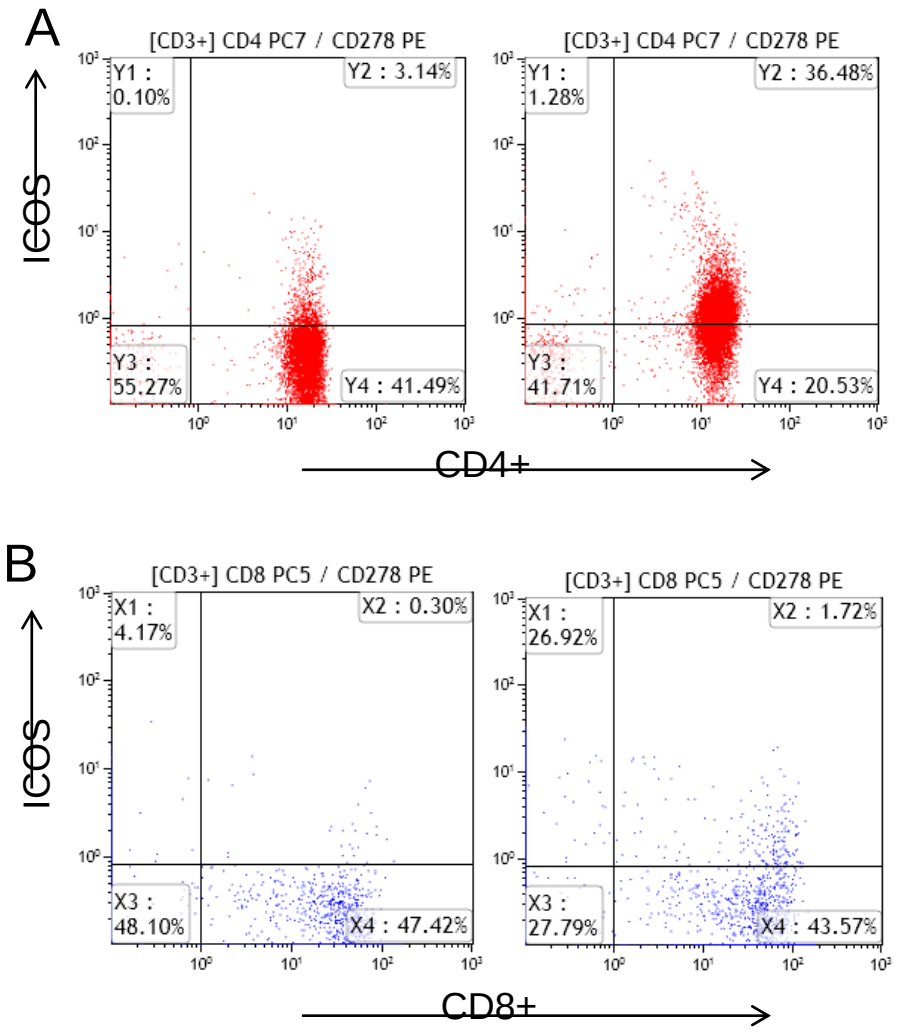
The MART-1 and gp100-specific cells *differentiated towards effector cells* with vaccination.

Immune cell phenotype

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

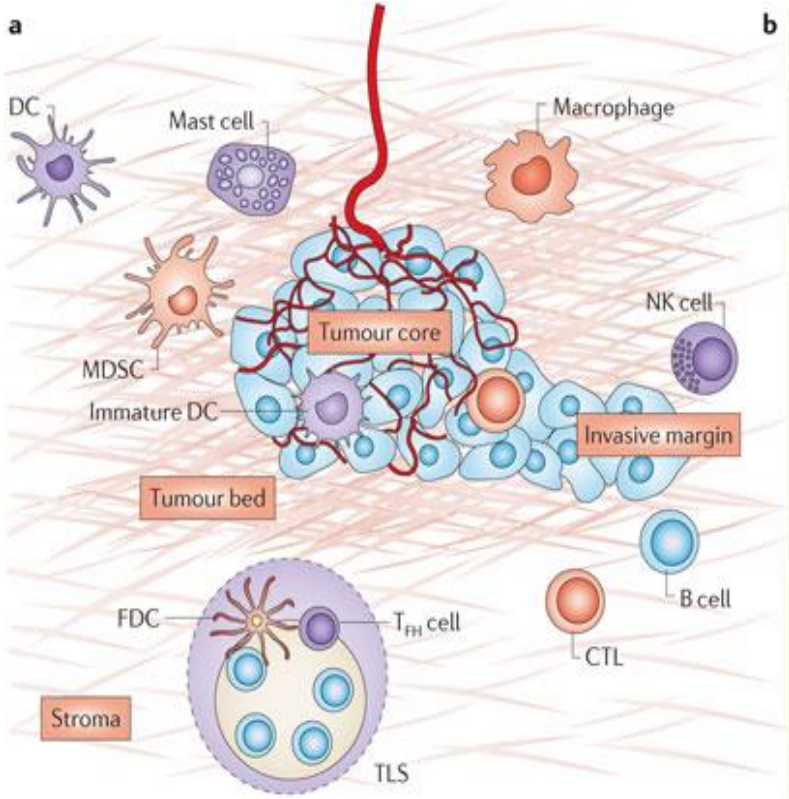
The addition of GM-CSF to ipilimumab significantly improves OS in patients with metastatic melanoma. Improved tolerability was seen in patients receiving GM-CSF.

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

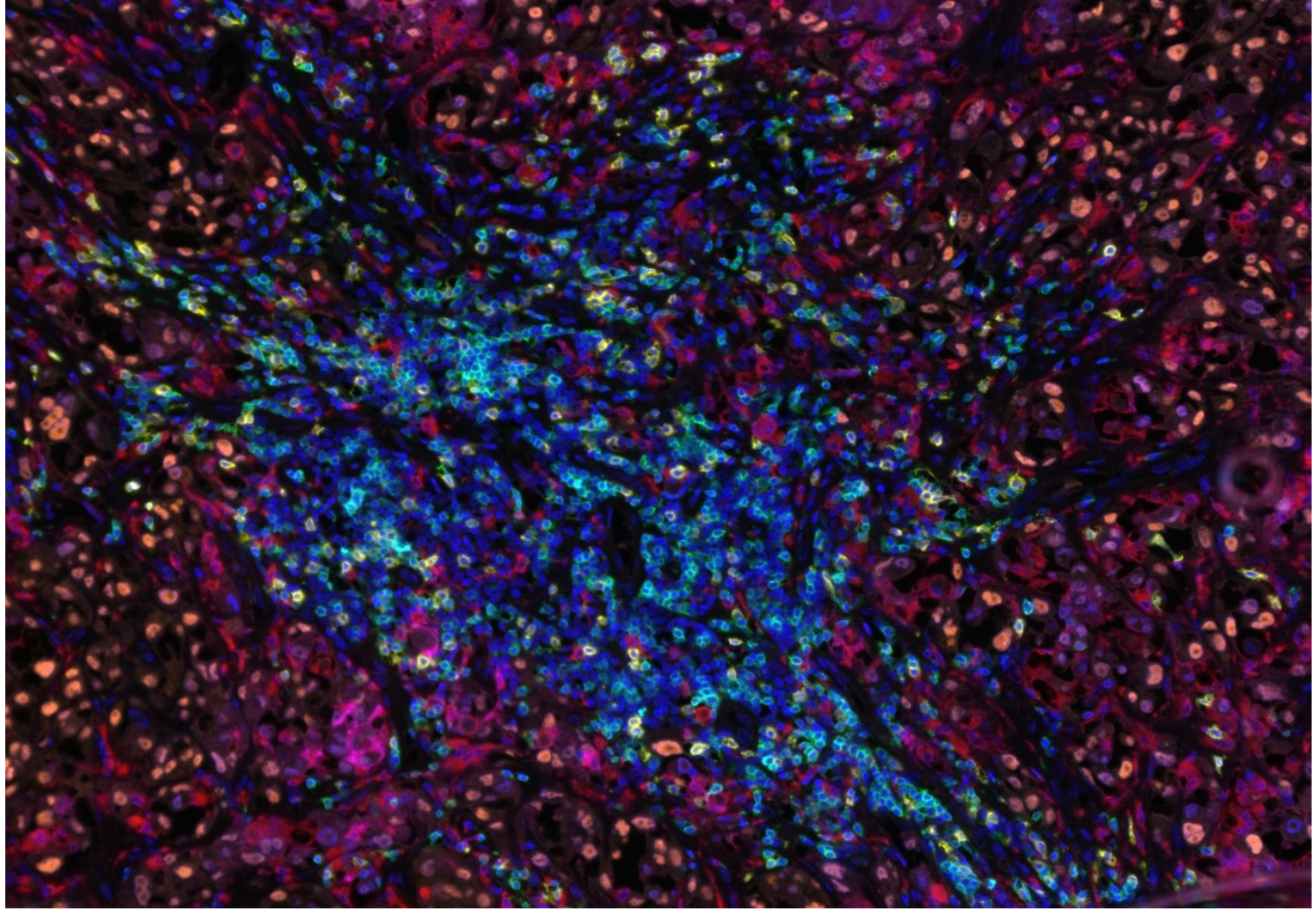
Immune contexture	Parameters: positive association with survival
Type	CTLs ($CD3^+CD8^+$)
	Memory T cells ($CD45RO^+$)
Location	Core of the tumour
	Invasive margin
Density	Number of cells per mm^2: 1, 10, 100, 1,000, 10,000 — $CD3^+_{CT}$, $CD3^+_{IM}$, $CD8^+_{CT}$, $CD8^+_{IM}$, $CD45RO^+_{CT}$, $CD45RO^+_{IM}$
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

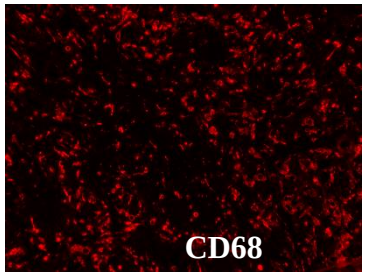
Nature Reviews | Cancer

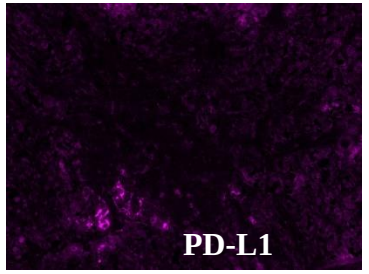
Tumor anatomy showing the features of the immune contexture, including the tumor core, the invasive margin, tertiary lymphoid structures (TLS) and the tumor microenvironment. The distribution of different immune cells is also shown.

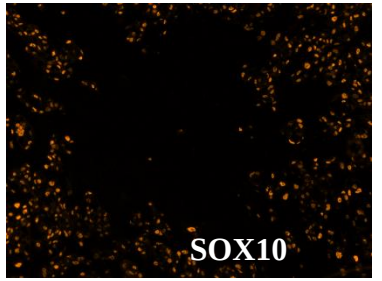
CT, core of the tumor; DC, dendritic cell; FDC, follicular dendritic cell; IM, invasive margin; IRF1, interferon regulatory factor 1. ***J. Galon, W. Fridman***

Multiplex tissue staining: Vectra

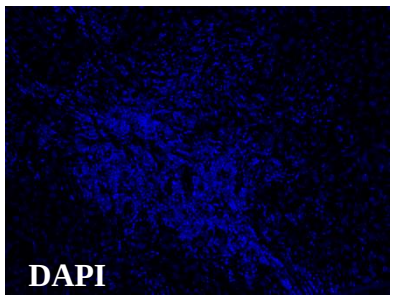


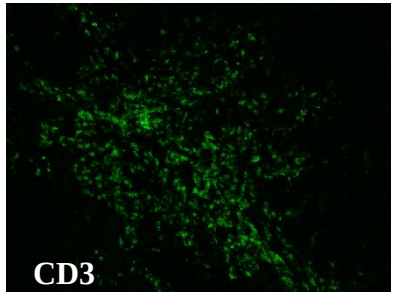


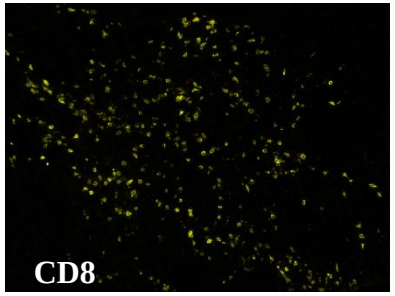


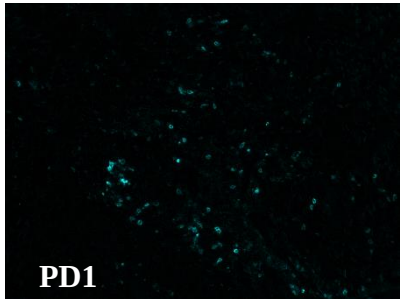


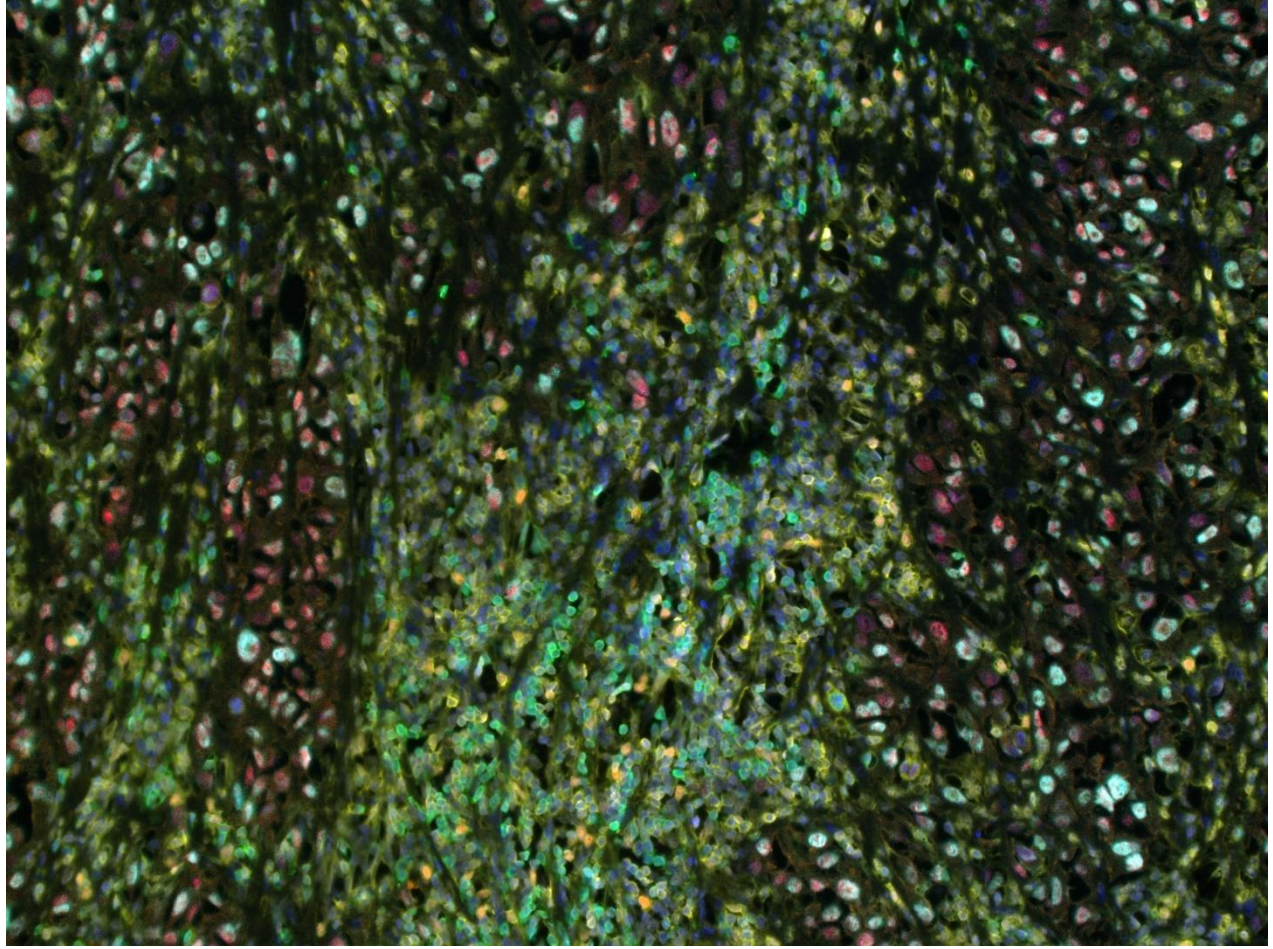
MELANOMA TISSUE

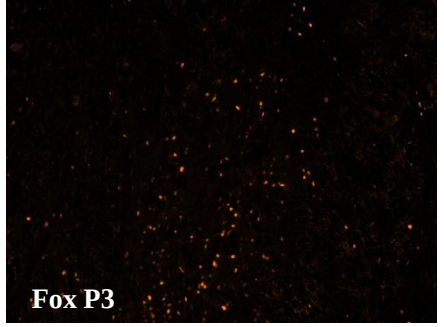


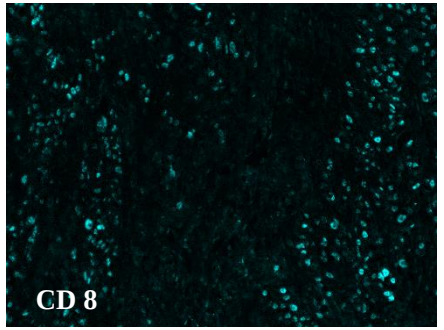


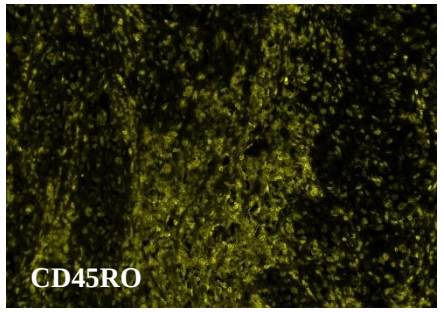


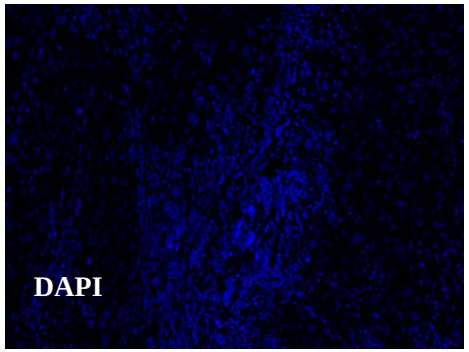


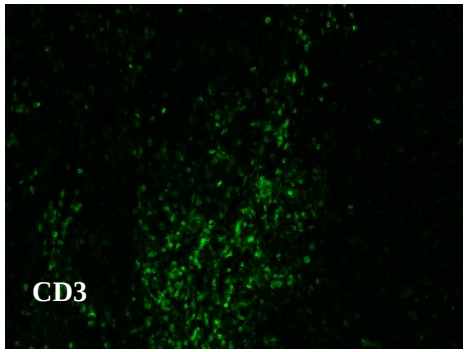


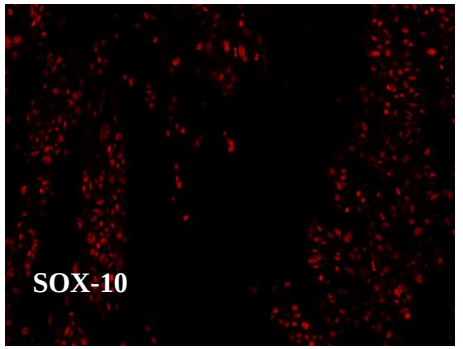






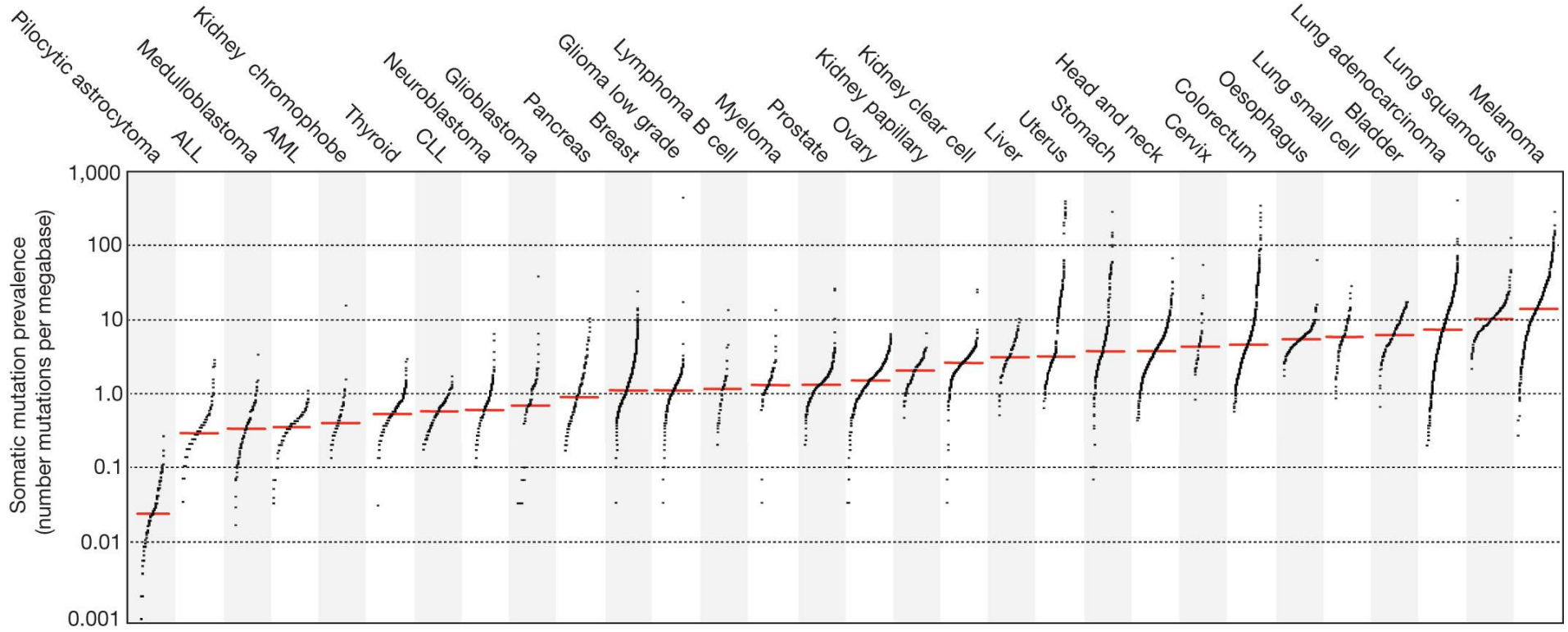






Melanoma

The prevalence of somatic mutations across human cancer types.



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

Tumor Mutational Burden (TMB) and Friends of Cancer Research

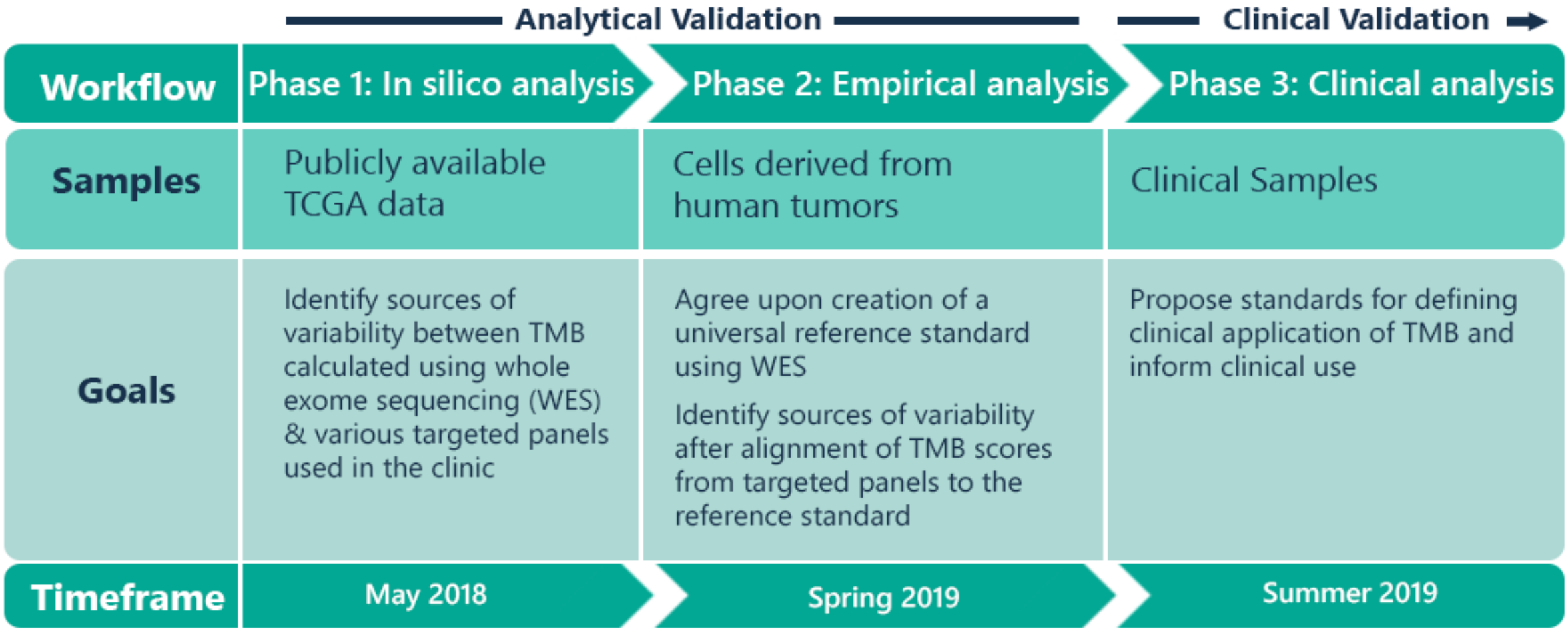
<u>The Definition:</u> Tumor mutational burden (TMB) measures the quantity of mutations found in a tumor. This type of biomarker is currently under study to evaluate whether it may help predict the likelihood a patient with cancer will benefit from immuno-oncology (IO) therapies.

<u>The Problem:</u> Currently, there is a lack of standardization for TMB calculation and reporting. Different tests may report different measurements, and since there is currently no one way of calculating TMB it is difficult to use as a biomarker. To achieve consistency and accurate reporting across tests, it is imperative to create some sort of standardization to arrive at clinically-meaningful results, which will support informed decision-making for patients.

The Solution: There needs to be a standardized way of calculating and reporting TMB. Friends of Cancer Research (Friends) will convene stakeholders across all health sectors to review the current methods of TMB calculation and reporting and create a consensus solution on how best to standardize them.

The group will propose analytical and clinical validation studies to support a standardized method of TMB measurement, which will help improve patient care through consistent TMB reporting in a clinical setting despite differences in the testing panel used. Ultimately, this project will help ensure consistent identification of patients who are likely to respond to IO therapies.

TMB Harmonization Project Overview

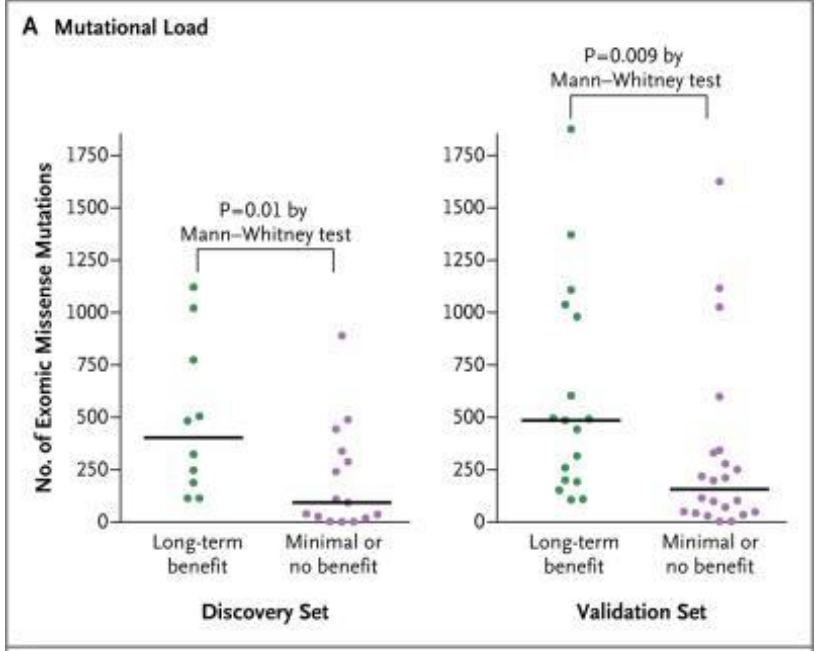


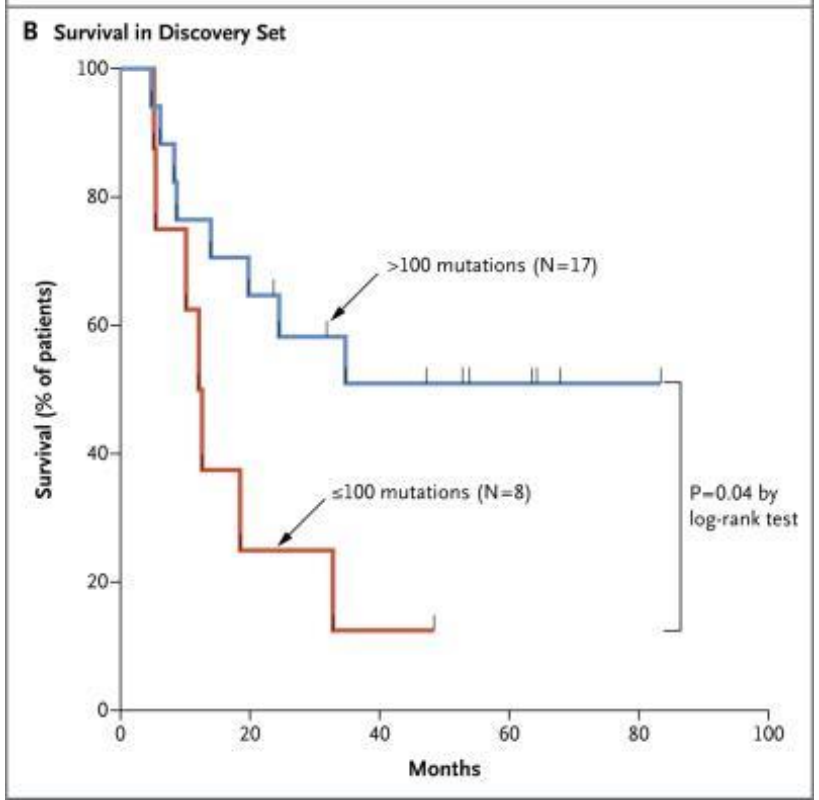
N Engl J Med. 2014 Dec

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

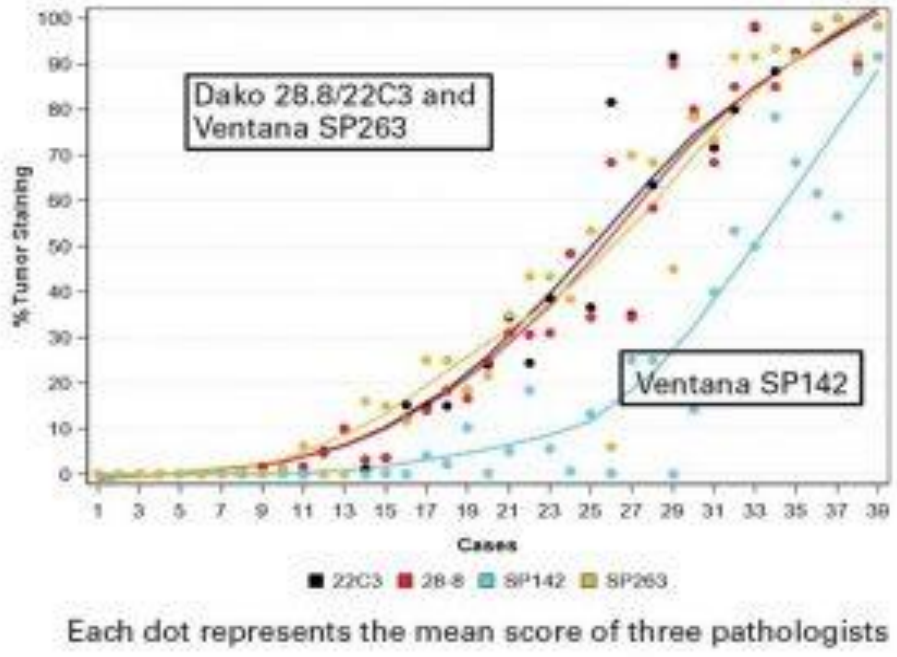
Snyder A[1], Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, Walsh LA, Postow MA, Wong P, Ho TS, Hollmann TJ, Bruggeman C, Kannan K, Li Y, Elipenahli C, Liu C, Harbison CT, Wang L, Ribas A, Wolchok JD, Chan TA.

More mutations = better checkpoint blockade response





Consistency in PD-L1 staining by IHC on tumor cells: The Blueprint Project



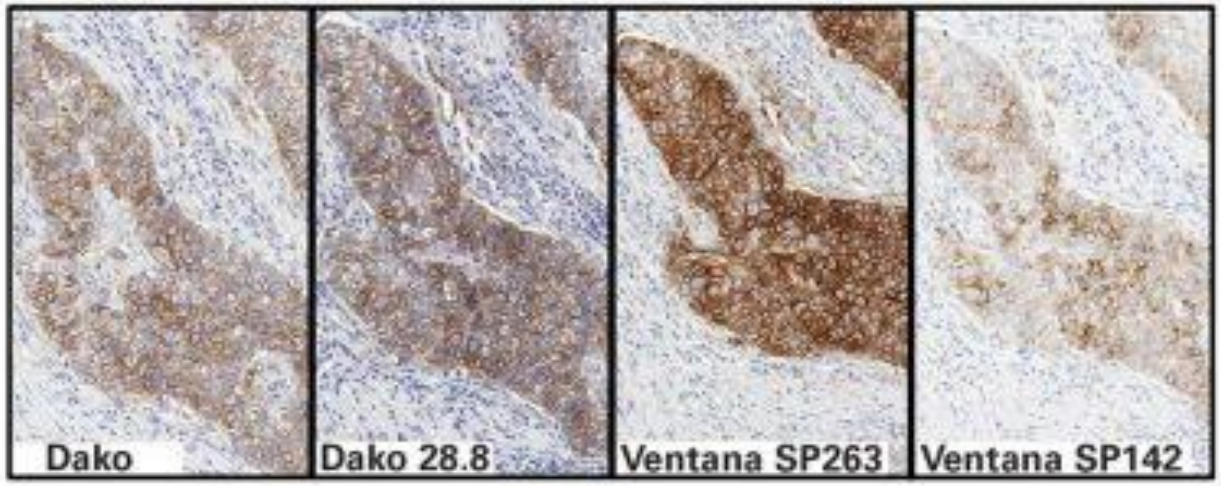


Fig. 1. PD-L1 Expression on Tumor Cells Hirsch FR, McElhinny A, Stanforth D, et al. PD-L1 Immunohistochemistry Assays for Lung Cancer: Results from Phases 1 of the Blueprint PD-L1 IHC Assay Comparison Project. J Thor Oncol. 2017;12(2) 208-222

In an effort to provide consistency in this area, members of the IASLC pathology panel and other IASLC members proposed a comparative study of the performance of the various PD-L1 IHC assays.

The study was encouraged by the U.S. FDA as well as by the AACR and the ASCO.

A steering committee consisting of representatives from multiple pharmaceutical companies (AstraZeneca, Genentech/ Roche, Bristol-Myers Squibb, Merck Pharmaceuticals, and, later, Pfizer and Merck MSD), and the two diagnostic companies (Ventana and Dako) was established, and the study was performed by members of the IASLC Pathology Committee and coordinated by IASLC.

Thus far, the PD-L1 Blueprint Project has underscored that **interchangeability of the three assays (Dako 28-8/22C3 and Ventana SP263) is a possibility**. However, the clinical cutoffs chosen for positive status/high expression levels versus negative status/ low expression levels might be the determining factor in clinical associations, rather than the actual choice of assays.

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.

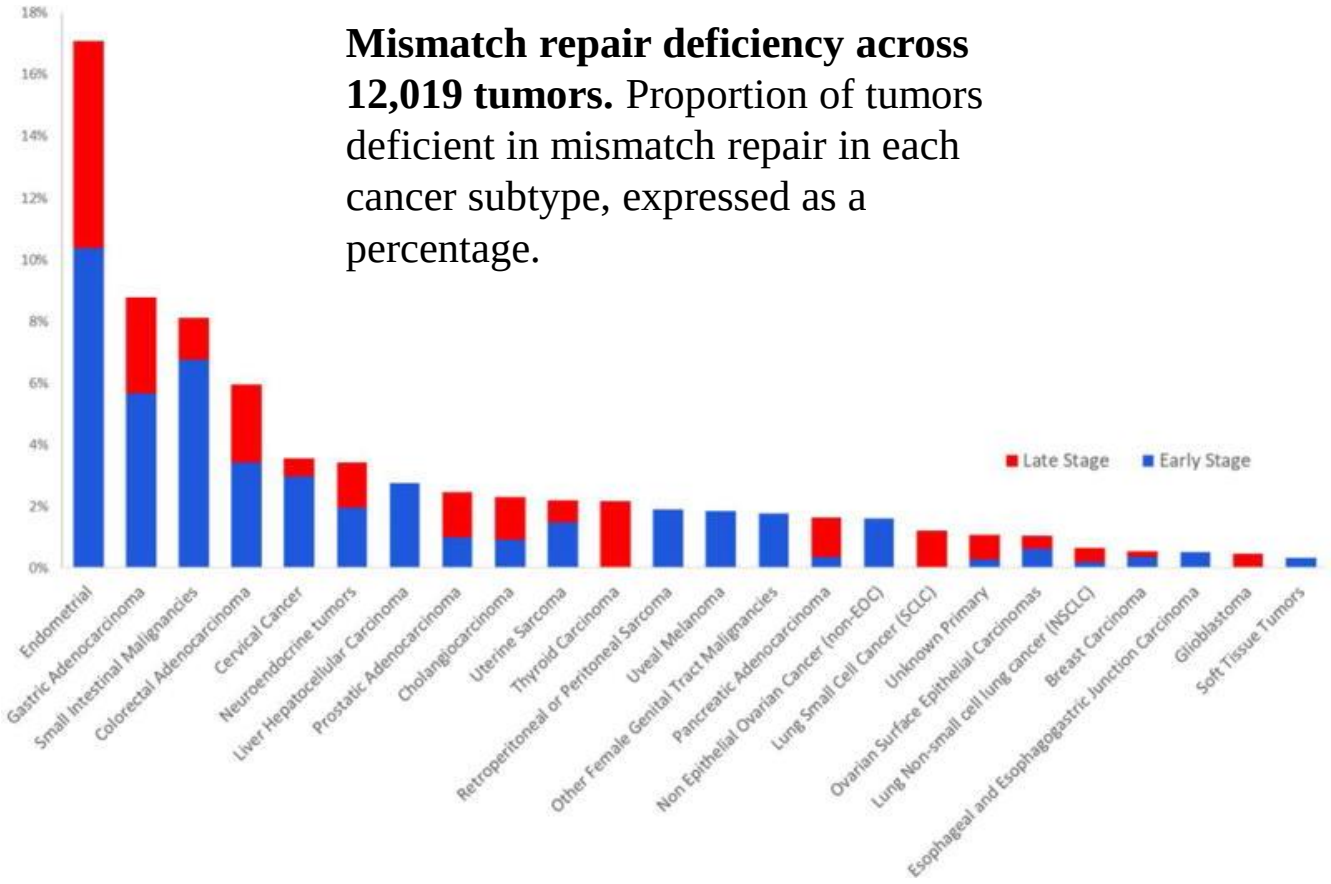
Status of Testing for High-Level Microsatellite Instability/Deficient Mismatch Repair in Colorectal Carcinoma

S. Hamilton, JAMA Oncol. 2018

Diagnostic approaches have evolved since the early 1990s, from relying exclusively on clinical criteria to incorporating pathologic features, PCR-based MSI testing, and immunohistochemistry for loss of MMR component expression. Tumor types can be grouped into categories based on the frequency of MSI, from colorectal (20%) and endometrial (22%–33%) to cervical (8%) and esophageal (7%) to skin and breast cancers (0%–2%).

Dudley et al., CCR 2016

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



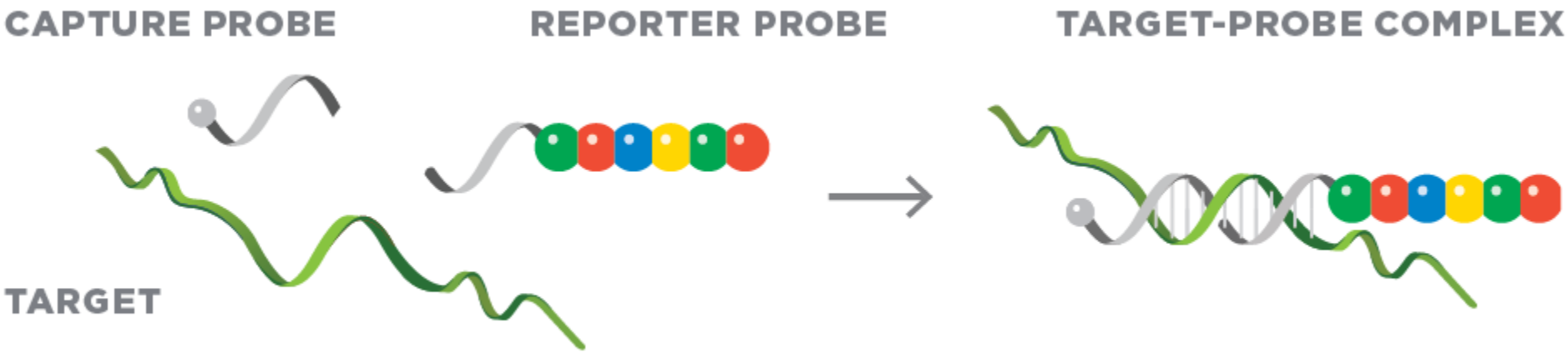
Mismatch repair deficiency across 12,019 tumors. Proportion of tumors deficient in mismatch repair in each cancer subtype, expressed as a percentage.

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

HIGHLY MULTIPLEXED SINGLE MOLECULE COUNTING

NanoString's patented molecular barcodes provide a true digital detection technology capable of highly multiplexed analysis



nanoString™

Tumor Microenvironment (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

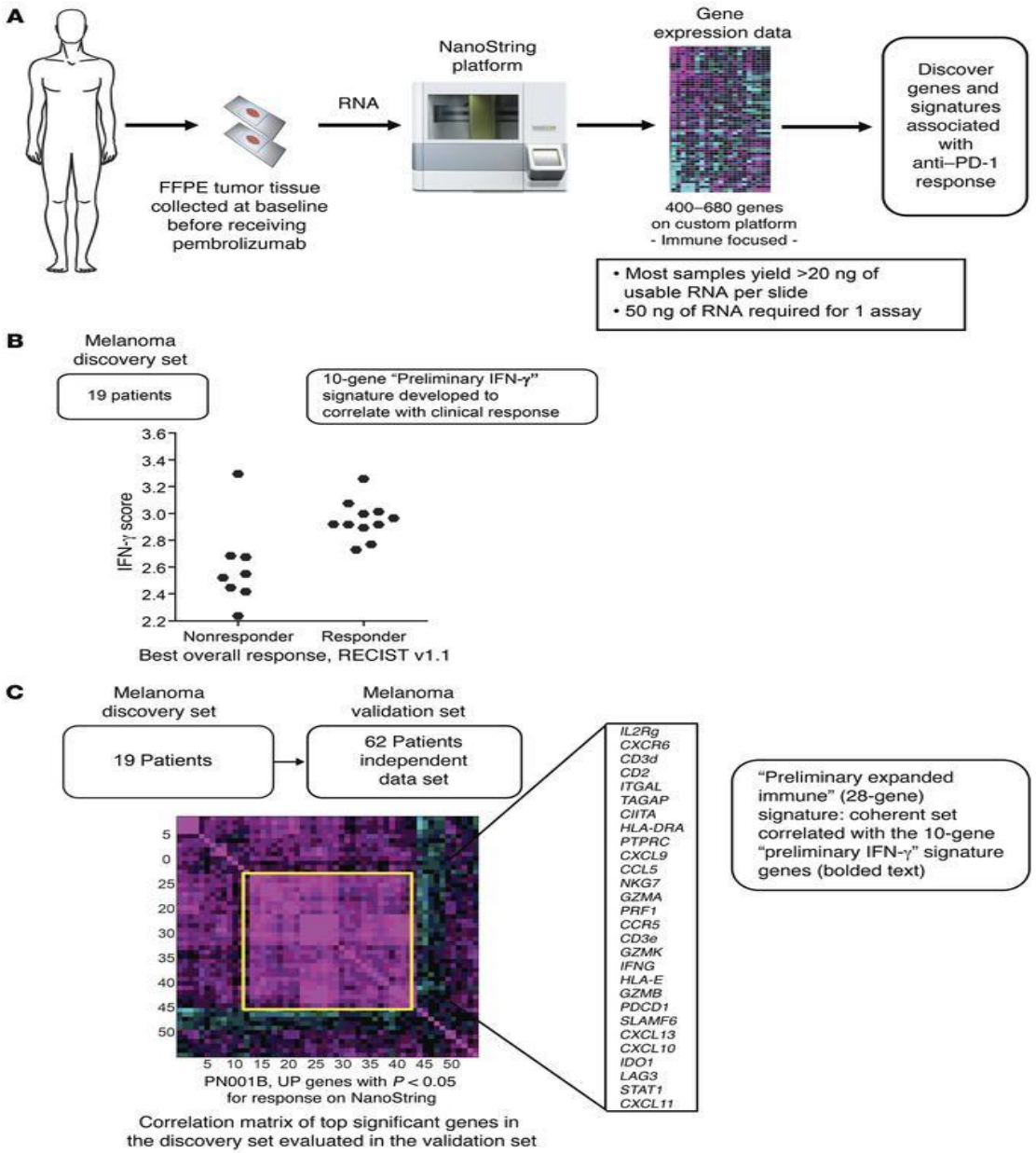


Table 2. IFN-γ and expanded immune gene signatures

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

Serum (or supernatant) profiling by Luminex

Screening 65-plex:
APRIL, BAFF, BLC, CD30, CD40L, ENA-78, Eotaxin, Eotaxin-2, Eotaxin-3, FGF-2, Fractalkine, G-CSF, GM-CSF, Gro a, HGF, IFN-a, IFN-g, IL-10, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-18, IL-1a,IL-1b, IL-2, IL-20, IL-21, IL-22, IL-23, IL-27, IL-2R, IL-3, IL-31, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, I-TAC, LIF, MCP-1, MCP-2, MCP-3, M-CSF, MDC, MIF, MIG, MIP1a, MIP-1b, MIP-3a, MMP-1,NGF beta, SCF, SDF-1a, TNF b, TNF-a, TNF-R2, TRAIL, TSLP, TWEAK, VEGF-A

BTLA; GITR; HVEM; IDO; LAG-3; PD-1; PD-L1; PD-L2; TIM-3; CD28; CD80; CD137; CD27; CD152 soluble checkpoints
(Thermo-Fisher/Affymetrix ProcartaPlex)

Soluble Checkpoints/Costimulatory Molecules

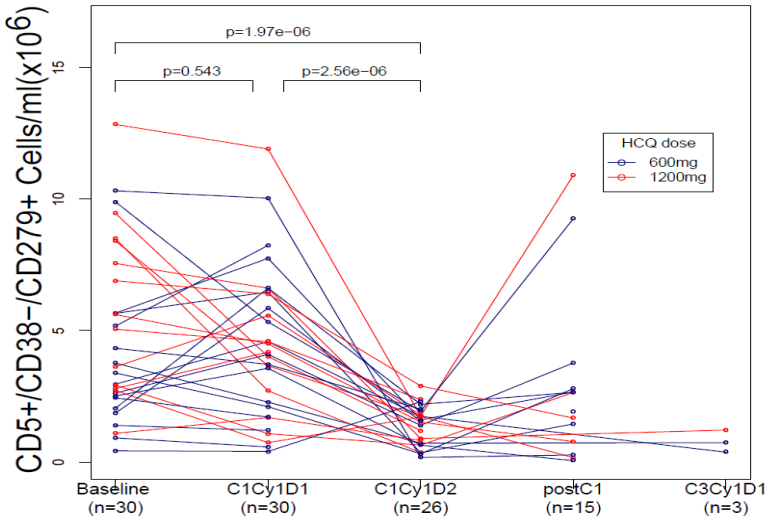
Safety and activity of hydroxychloroquine and aldesleukin in metastatic renal cell carcinoma: A cytokine working group phase II study (ASCO 2018 poster)

Leonard J. Appleman[1], Daniel P. Normolle[1], Theodore F. Logan[2], Paul Monk[3], Thomas Olencki[3], David F. McDermott[4], Marc S. Ernstoff[5], Jodi K. Maranchie[1], Rahul Parikh[1], David Friedland[1], Mary Jo Buffo[1], Shuyan Zhai[1], Herbert Zeh[1], Xiaoyan Liang[1], Lisa H. Butterfield[1], Michael T. Lotze[1]

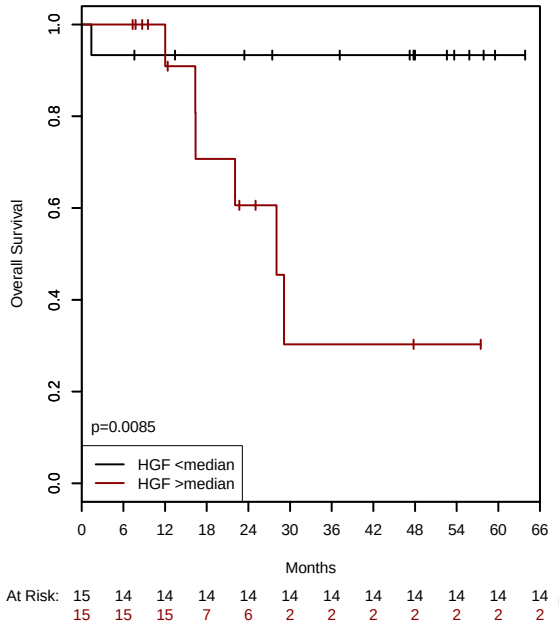
Overall Survival (OS) was compared to each baseline biomarker. Test 1) split markers at the median, and then used a log-rank test to compare the dichotomized biomarker to OS 2) a proportional hazards (Cox) model. Those with at least one p-value <0.01 from 64 cytokines **and 14 checkpoints:**

	Median split	--------Proportional Hazards--------	
	p-value	Hazard Ratio (95% CI)	p-value
sLAG-3	0.8506	1.022 (1.0033,1.042)	0.0087
HGF	0.0085	1.010 (0.999,1.021)	0.0360
sCD-30	0.0066	1.0005 (0.999,1.001)	0.34

PD1+ CD5+ Cells Migrate Out of Circulation with IL-2



Decreased OS in Patients with Increased HGF



UPMC | HILLMAN CANCER CENTER

Themes Emerge

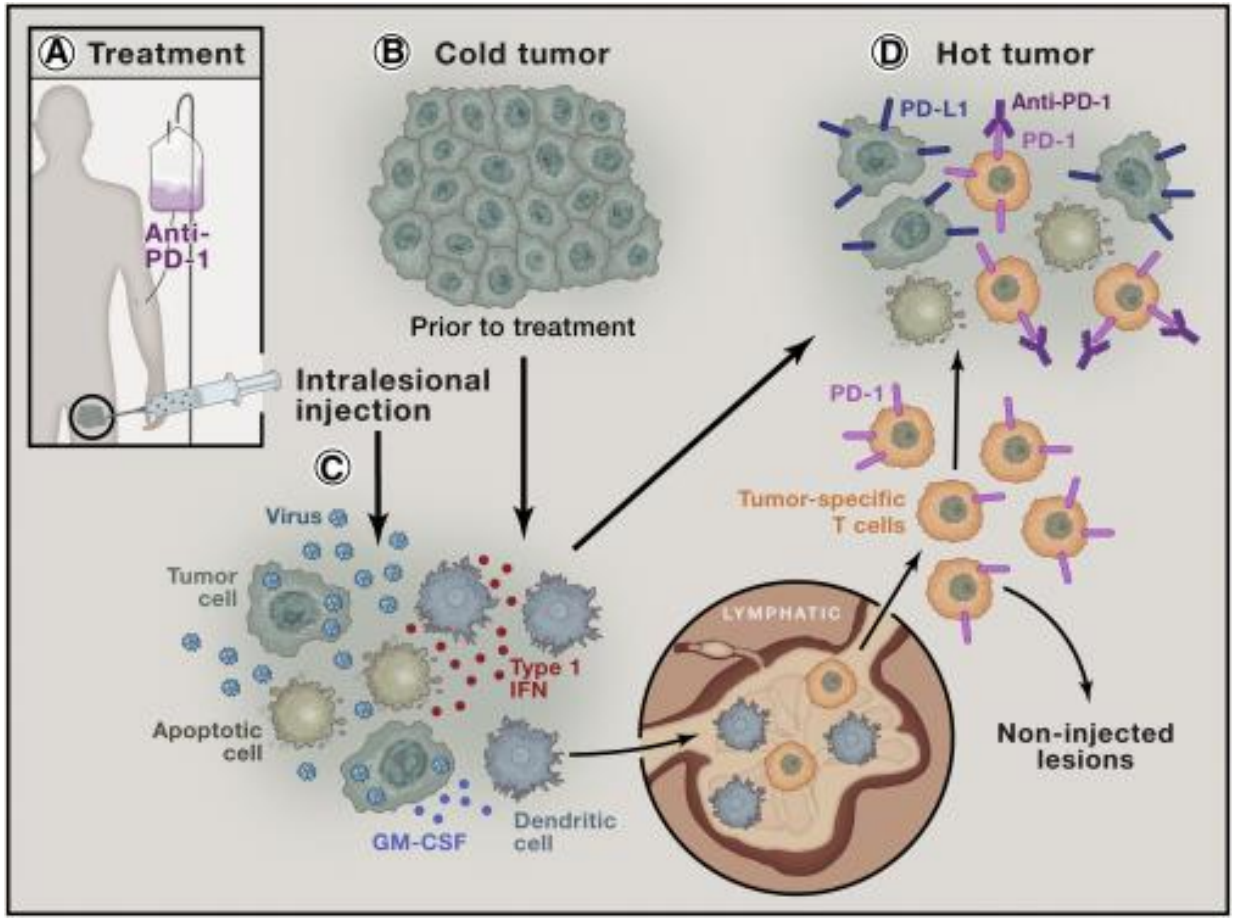
Biomarkers for prediction, prognostication and mechanism-of-action in cancer immunotherapy are still largely exploratory, although exciting signals are being validated (analytically and clinically). Biomarkers identified in tissue might ultimately be testable in blood.

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 (TMB), CD8+ T cell infiltrate, IFNγ (or related type 1 T cell response) gene expression signature

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

Biomarker Development

- Biomarkers will help predict or guide combination treatment
- Biomarkers will help identify the right population for the right combination treatment/options
- Biomarker research is critical in the future

SITC Activities in Biomarkers

- **Problem Based Workshops**
  - Biometrics: Identifying Biomarkers and Surrogates of Tumors in Patients (2003, Live meeting and publication)
  - iSBTc-FDA-NCI Workshop on Prognostic and Predictive Immunologic Biomarkers in Cancer (2009, Live meeting and publication)
  - Immuno-Oncology Biomarkers, 2010 and Beyond (2010, Live meeting)
  - Immuno-Oncology Biomarkers, 2010 and Beyond: Perspectives from the iSBTc Biomarker Task Force (2010, Live meeting and publication)
  - Immunotherapy Biomarkers – Overcoming the Barriers (2016, Live meeting and publication)
  - Immuno-Oncology Biomarkers: Today's Imperatives for Tomorrow's Needs (2017, Live event)
  - Immuno-Oncology Biomarkers: State of the Art featured researchers from a broad representation of the field to identify the next steps for action in immunotherapy biomarker development

Continued

- **SITC Hot Topic Sessions at the SITC Annual Meeting**
  - Predictive Biomarkers in Checkpoint Blockade: Is PD-L1 Tumor Expression Necessary? (2013)
  - Biomarkers Debate (2015)
- **SITC Scientific Project Working Groups and Task Forces**
  - Biomarkers Steering Committee and Task Forces (2008-2010 & 2014-present, Live Meetings and publications)
  - Task Force Groups: Immune Monitoring, Biomarkers Assays, Immune Regulation, Baseline Immunity
- **Journal for ImmunoTherapy of Cancer**
  - Online society owned journal, open access
  - Section on Immunotherapy Biomarkers

Continued

- **Publications**
  - SITC/iSBTc Cancer Immunotherapy Biomarkers Resource Document: Online resources and useful tools – a compass in the land of biomarker discovery (2011)
  - Recommendations from the iSBTc-SITC/FDA/NCI Workshop on Immunotherapy Biomarkers (2011)
  - Validation of Biomarkers to Predict Response to Immunotherapy in Cancer (2016)
  - Novel technologies and emerging biomarkers for personalized cancer immunotherapy (2016)
  - Systematic evaluation of immune regulation and modulation (2017)
  - Identifying baseline immune-related biomarkers to predict clinical outcome of immunotherapy (2017)
  - Significance and implications of FDA approval of Pembrolizumab for biomarker-defined disease (2018)

Continued

- **Collaborations/Memberships**
  - Member of FNIH Biomarkers Consortium (2010-2011, 2017-2019)
- **New Guidelines Webinars**
  - In July 2018, a new series of webinars dedicated to presentation and discussion of recently published SITC Cancer Immunotherapy Guidelines. The webinars allow clinicians to engage with leading experts in the field, ask questions of treatment recommendations in a specific disease state, guidelines concerning biomarker utilizations and more.

Approaches to addressing inherent variability in immunologic monitoring of clinical trials

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

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

Immunotherapy Biomarkers Task Force: 2015-2019

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 (2019)

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.

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