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

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Past President, SITC
Disclosures:

StemImmune/Calidi Scientific and Medical Advisory Board, April 6, 2017-present
SapVax Advisory Board meetings Nov. 15, 2017; Dec. 6, 2018
NextCure, Scientific Advisory Board, 2018-2020
Western Oncolytics, Scientific Advisory Board, 2018-present
Torque Therapeutics, Scientific Advisory Board, 2018-2020
Khloris, Scientific Advisory Board, 2019-present
Pyxis, Scientific Advisory Board, 2019-present
Cytomix, Scientific Advisory Board, 2019-present
Vir, Scientific Advisory Board meeting, Feb. 2020
DCprime, Scientific Advisory Board meeting, Nov. 2020
RAPT, Scientific Advisory Board, 2020-present
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.
At the core: T cell Activation

**ACTIVATION**
- Tumor releases antigens that bind with dendritic cell
- Dendritic cell presents antigens to activate T cell
- Vaccines promote response to additional antigens
- Adjunct therapy makes Tumor Microenvironment (TME) more friendly to T cells, etc.

**PROLIFERATION**
- Activated T cells rapidly multiply, creating an army of T cells
- Adoptive cell therapy increases number and activity of T cells

**RESPONSE**
- Activated T cells migrate back to the tumor to attack and kill cancer cells
- Adoptive cell therapy increases number and activity of T cells

Checkpoint blockade enhances priming

Vaccines promote response to additional antigens

Adoptive cell therapy increases number and activity of T cells
Cancer Immunoediting

Transformed cells

Elimination

“Danger signals”

Intrinsic tumor suppression
(senescence, repair, and/or apoptosis)

Carcinogens, Radiation, Viral infections, Chronic inflammation, Inherited genetic mutations

Equilibrium

Tumor antigens

NKR ligands

IL-12

IFN-γ

Antigens loss

MHC loss

Escape

Tumor dormancy and editing

Normal tissue

Innate & adaptive immunity

IFN-γ, IFN-α/β, IL-12, TNF, NKG2D, TRAIL, Perforin

Extrinsic tumor suppression

Intrinsically tumor suppression

NKR ligands

IL-6, IL-10

Galectin-1

TGF-β

IDO

 CTLA-4

PD-L1

IL-6, IL-10

TGF-β

IDO

CTLA-4

PD-L1

CTLA-4

PD-1

Tumor growth promotion

Schreiber, Science 2011
Cellular Communications and Heterogeneity

- Imaging Mass Cytometry/MINISAR FACS Imaging
- Luminex

CELL ENVIRONMENT
- What cells are in local stroma? Cytokines? etc?

CELL COMMUNICATION
- Molecular communication between cells

CELL FUNCTIONALITY
- Cells that release cytokines vs. living tumor cells

CELL SPECIFICITY
- Different kinds of cells, e.g., CD4+ T cell, CD8+ etc.

CELL TYPE
- T cells with different TCRs

METABOLOME
- Mass Spectrometry, Inter cellular Flow/CyTOF

PROTEOME
- PBMC RNA-Seq, Tumor RNA-Seq Nanostring

GENE EXPRESSION
- HLA Typing, Tumor Normal Exome Sequencing

EPIGENETICS
- ATAC-seq

GENETICS
The Key: Cancer Immunity Cycle

1. Release of cancer antigens (cancer cell death)
2. Cancer antigen presentation (dendritic cells)
3. Priming and activation (DCs + T-cells)
4. Trafficking of T-cells to tumors (CTLs)
5. Infiltration of T-cells into tumors (CTLs, endothelial cells)
6. Recognition of cancer cells by T-cells (CTLs, cancer cells)
7. Killing of cancer cells (immune and cancer cells)

Chen and Mellman, Immunity 2013
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…..
Patient-derived specimens used in immunologic monitoring

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

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

Digested tumor/TIL cell suspension

Tumor and lymphocytes

serum

PBMC

Freshly tested or cryopreserved for batch testing

Direct whole blood assays

obtain absolute counts and percentages
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

Clinical Site

- Screen or enrollment:
  - fax blood kit request

Central Lab

- Kit prepared and shipped ground

- Blood processed and banked according to SOPs within 24 hours

- Assays performed per SOPs, send results

- Results to PI publish

Pt. blood draw mailed O/N to lab

Gather lab and clinical data; biostatistics
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.
SITC cancer immunotherapy resource document: a compass in the land of biomarker discovery
Siwen Hu-Lieskovan,1,2 Srabani Bhaumik,3 Kavita Dhodapkar,4,5 Jean-Charles J B Grivel,6 Sumati Gupta,7 Brent A Hanks,8 Sylvia Janetzki,9 Thomas O Kleen,10 Yoshinobu Koguchi,11 Amanda W Lund,12 Cristina Maccalli,6 Yolanda D Mahnke,13 Ruslan D Novosiadly,14 Senthamil R Selvan,15 Tasha Sims,16 Yingdong Zhao,17 Holden T Maecker,18 (JITC 2020)
Tried, true and very well standardized functional benchmark (albeit single functional parameter usually):
the IFNγ ELISPOT assay
## Standardized ELISPOT Assays

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

<table>
<thead>
<tr>
<th></th>
<th>Spontaneous</th>
<th>PMA/I (+)/OKT3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Control Ave.</td>
<td>4.9 (54% CV)</td>
<td>304 (19.2% CV intra-assay)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(48% CV inter-assay)</td>
</tr>
<tr>
<td>Patient Ave.</td>
<td>0.7 (35% CV)</td>
<td>81 (38.7 %CV)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Spontaneous</th>
<th>PMA/I (+)/PHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Control Ave.</td>
<td>5.4 (56% CV)</td>
<td>284 (15.5% CV intra-assay)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(51% CV inter-assay)</td>
</tr>
<tr>
<td>Patient Ave.</td>
<td>19 (40% CV)</td>
<td>171 (18.8 %CV)</td>
</tr>
</tbody>
</table>
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, \(p=0.033\)).

Peptide-specific and phenotypic: MHC multimer (tetra-, penta-…dextra-…):
count the cell, grab the cell, profile the cell
Immune Response: E1696
Melanoma antigen peptide-specific CD8+ T cells

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.
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 (mechanistic insights):**
Increased ICOS on CD4+ and CD8+ T cells correlates with clinical outcome. Now being tested in other clinical trials.

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
TME analysis: multiplex tissue staining:

Assessments of T cell density, location, and phenotype in baseline and on-treatment tumor samples provide important insights into the role of these cells in patients with cancer and immune checkpoint therapy.

It is apparent that complex immune monitoring approaches and robust computational solutions are needed to better characterize the tumor immune contexture.
Melanoma,
Vectra platform,
Phenoptics 2.0
Analytically validated.
High-throughput.
Melanoma, CODEX imaging platform

Cell 2020: “Coordinated Cellular Neighborhoods Orchestrate Antitumoral Immunity at the Colorectal Cancer Invasive Front” G. Nolan lab

The prevalence of somatic mutations across human cancer types.
Genetic basis for clinical response to CTLA-4 blockade in melanoma

More mutations = better checkpoint blockade response

TMB and other genetic determinants have demonstrated the potential to make immune checkpoint therapy more precise. Clinical data in support of the predictive value of TMB in the context of ICIs are encouraging but not fully conclusive, and challenges remain. It remains to be seen if tumor and/or bTMB can help identify patients who are likely to benefit from combination immunotherapies, including, but not limited to, angio-immunotherapy and chemoimmunotherapy combinations.

Additionally, the variability in the current methods of TMB assessment may complicate therapeutic decisions in the clinic. This highlights the need for standardization and harmonization of TMB analysis and reporting across assays and laboratories.
# TMB Harmonization Project Overview

**Friends of Cancer Research**

## Analytical Validation

<table>
<thead>
<tr>
<th>Workflow</th>
<th>Samples</th>
<th>Goals</th>
<th>Timeframe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase 1: In silico analysis</strong></td>
<td>Publicly available TCGA data</td>
<td>Identify sources of variability between TMB calculated using whole exome sequencing (WES) &amp; various targeted panels used in the clinic</td>
<td>SITC 2018, ms</td>
</tr>
<tr>
<td><strong>Phase 2: Empirical analysis</strong></td>
<td>Cells derived from human tumors</td>
<td>Agree upon creation of a universal reference standard using WES Identify sources of variability after alignment of TMB scores from targeted panels to the reference standard</td>
<td>AACR 2020, ms</td>
</tr>
<tr>
<td><strong>Phase 3: Clinical analysis</strong></td>
<td>Clinical Samples</td>
<td>Propose standards for defining clinical application of TMB and inform clinical use</td>
<td>coming</td>
</tr>
</tbody>
</table>
Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade

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 makes them sensitive to immune checkpoint blockade, regardless of the cancers' tissue of origin.

Le, D. et al. Science. 2017 Jul
The expression of the PD-1 ligand (PD-L1) was neither prognostic nor predictive of benefit. 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.

PD-L1 IHC has demonstrated clinical utility by allowing patient selection and enrichment for clinical benefit from single-agent treatment with anti-PD-1 checkpoint inhibitors.

A number of PD-L1 IHC tests were independently codeveloped to support specific anti-PD-(L)1 programs, and the lack of standardization between these IHC requires harmonization of these assays in the clinic, as well as consensus on the scoring algorithms and cut-off levels to define positive PD-L1 status across various tumor types.

While PD-L1 IHC tests allow for enrichment of patients who are likely to derive clinical benefit from anti-PD-(L)1 agents, their clinical utility is less clear in the context of combination immunotherapies (eg, nivolumab/ipilimumab, angio-immunotherapy, and chemoimmunotherapy) which, based on currently available data, appear to be efficacious irrespective of tumor PD-L1 status.
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
NanoString’s patented molecular barcodes provide a true digital detection technology capable of highly multiplexed analysis.
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.
IFNγ–related mRNA profile predicts clinical response to PD-1 blockade

Table 2. IFN-γ and expanded immune gene signatures

<table>
<thead>
<tr>
<th>IFN-γ</th>
<th>Expanded immune gene signature</th>
</tr>
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<tbody>
<tr>
<td>IDO1</td>
<td>CD310</td>
</tr>
<tr>
<td>CKCL10</td>
<td>ID01</td>
</tr>
<tr>
<td>CKCL9</td>
<td>CIITA</td>
</tr>
<tr>
<td>HLA-DR4</td>
<td>C0E21</td>
</tr>
<tr>
<td>HLA-E</td>
<td>CXCR6</td>
</tr>
<tr>
<td>STAT1</td>
<td>CCL5</td>
</tr>
<tr>
<td>IFNG</td>
<td>GZMK</td>
</tr>
<tr>
<td>CD2</td>
<td>TAGAP</td>
</tr>
<tr>
<td>HLA-DR4</td>
<td>CCL13</td>
</tr>
<tr>
<td>LAG3</td>
<td>CKCL13</td>
</tr>
<tr>
<td>GZMB</td>
<td>GZMBB</td>
</tr>
</tbody>
</table>

Ayers et al., JCI, 2017
Serum (or supernatant) profiling

**Luminex:** Screening 65-plex (cytokines/chemokines/growth factors, also 14+ soluble checkpoints and costimulatory molecules; pg/ml)

**Olink:** **Target 96:** Targeted protein biomarker discovery 96-plex panels, qPCR readout;  
**Explore 1536:** Measure 1,536 proteins. Readout on NGS

**Phage display:** rationally designed libraries encompassing the entire human proteome have been implemented. With next-generation sequencing as a readout, researchers can quantify the enrichment of millions of individual phage clones simultaneously and identify sequences that bind to the target or antibody of interest (“phage immunoprecipitation and sequencing (PhIP-Seq)”)
**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. Appleman¹, Daniel P. Normolle¹, Theodore F. Logan², Paul Monk³, Thomas Olencki³, David F. McDermott⁴, Marc S. Ernstoff⁵, Jodi K. Maranchie¹, Rahul Parikh¹, David Friedland¹, Mary Jo Buffo¹, Shuyan Zhai¹, Herbert Zeh¹, Xiaoyan Liang¹, Lisa H. Butterfield¹, Michael T. Lotze¹

**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:

<table>
<thead>
<tr>
<th>Median split</th>
<th>Proportional Hazards</th>
</tr>
</thead>
<tbody>
<tr>
<td>sLAG-3</td>
<td>0.8506 1.022 (1.0033,1.042) 0.0087</td>
</tr>
<tr>
<td>HGF</td>
<td>0.0085 1.010 (0.999,1.021) 0.0360</td>
</tr>
<tr>
<td>sCD-30</td>
<td>0.0066 1.0005 (0.999,1.001) 0.34</td>
</tr>
</tbody>
</table>

PD1+/CD38-/CD5+ Cells Migrate Out of Circulation with IL-2

Decreased OS in Patients with Increased HGF

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[Graph showing Overall Survival over time with comparison of HGF above median and below median.]
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 “IFNy signatures” showing they are permissive for immune infiltration?

Common mechanisms: PD-L1 on tumors, Tumor Mutation Burden (TMB), CD8+ T cell infiltrate, IFNy (or related type 1 T cell response) gene expression signature (related but not the same and not completely overlapping with each other)
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.
Biomarkers

Who should be enrolled?
Who will benefit and why?
Who will experience an adverse event/toxicity and why?

*Predictive, prognostic, mechanism of action*
# Addressing inherent variability in immunologic monitoring of clinical trials

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

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

- **Chair:** Carlo B. Bifulco, MD; **Co-Chair:** Janis M. Taube, MD, MSC

- **White Paper 1** - “Best practices for Multiplex IHC/IF Staining and Validation, and Future Directions”

- **White Paper 2** - “Best practices for Multiplex IHC/IF Image analysis, Harmonization Efforts, and Data Sharing”
Emerging Biomarker Themes

Multiple TAA T cell responses
Epitope spreading
Greater TCR diversity

High mutation loads
IFNγ signatures
Tumor clonal TCR expansion

Multiple antigens, polyclonal response
Immune infiltrated tumor, active cellular infiltrate

PD-L1 on tumors (+/-infiltrate): expression cut-off?
TMB: which measure? Cut-off?
CD8+ T cell infiltrate/”ImmunoScore” (CD3/CD8/CD45RO)
Gene expression signatures: validation?
Focus Areas

**CAR-T and Cell Therapy: The Next Wave**
To engineer a smarter army of next-gen cell therapies that seek out specific targets and attack cancer — again and again.

**Checkpoint Inhibitors: Overcoming Resistance**
To uncover why some patients respond to checkpoint inhibitors for cancer while others don’t. If we know when and how immunotherapy resistance arises, we can prevent or even reverse it.

**Tumor Antigen Discovery: Targeting Cancer**
To find the “red flags” that show us where cancer is hiding. By pinpointing these antigens that fire up our immune system, we can create more effective personalized anti-cancer therapies.

**Tumor Microenvironment**
To infiltrate a solid tumor’s defenses. How can we break down the tumor microenvironment that walls off cancer from immunotherapy treatments?
The Parker Translational Suite: Deep Immune Profiling

Patient Samples

- Discovery Samples
- Tumor
- Blood

Clinical Metadata

- Germline WES
- Tumor WES
- TCRseq (tumor & blood)
- RNAseq (tumor & blood)
- Multiparameter Imaging (Including PDL1)
- Immune Profile CyTOF
- Multiplex Cytokine (plasma/serum)
- Stool/Microbiome
- Emerging Technologies

Harmonized methods of collection and processing at a central biorepository

Computation Deep Learning

- HLA Determination (MHC Class I and II)
- Neo-epitope Prediction
- Tumor Genome/TMB
- TME Gene Expression Signature
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 (and lots of candidate biomarkers!)

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