Biomarker integration in clinical trials for immunotherapies

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What are the biomarker questions:

1. **Prediction:**
   Who should be enrolled in this trial?

2. **Prognostication:**
   Who is benefitting from this therapy (in time to change course if needed)?

3. **Mechanism:**
   What worked well/not well about this intervention?
   Did the vaccine induce anti-tumor immunity?
   Did the TIL kill the tumor?
   Was immune suppression reversed? Why or why not?
   Was the signaling pathway blocked?
Where do we still need biomarkers?

**IL-2.** Used since 1984, the Surgery Branch reported on ...patients treated with high-dose bolus IL-2 with metastatic melanoma or renal cancer, ...409 consecutive patients: 15% incidence of objective regressions ...with metastatic melanoma (7% were complete) and a 19% overall response rate ...with metastatic renal cancer (9% were complete). Twenty-seven of the 33 completely responding patients (82%) remained in CR ...and appeared to be cured.

Rosenberg, 2014

The **toxicities** associated with high-dose IL-2 are severe but reversible; such toxicities sometimes included hemodynamic complications that required hospitalization in specialized or intensive care units.

**IFNα.** Three large meta-analyses have evaluated the survival benefits of adjuvant IFN-α, at various dose levels, durations, and routes of administration. ... highly significant RFS benefits (HR, 0.83; \( p = 0.000003 \)) and OS benefits that were less significant (HR, 0.93; \( p = 0.1 \)) *(one year regimen)*.

Attempts to identify a subset of patients likely to benefit from adjuvant treatment with IFN-α have failed to discover clinical or demographic features of true therapeutic predictive value.

Tarhini, Gogas, Kirkwood, 2012
How would Immunotherapy Biomarkers Help?

Avoid toxicity and toxicity treatment

Avoid ineffective therapies for specific patients

Understand mechanisms of action and how to build on them

Rational design of combinations
Why don’t we have more useful Biomarkers?

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

2. Immune assays can be costly; testing small numbers don’t give robust, reproducible signals; guessing at 1-2 assays may miss the mark (testing IFNγ from PBMC and Treg frequency in blood, and tumor MDSC suppression or a complex mRNA signature was critical......)
…conference, “Immune Profiling in Health and Disease,” …showcased high-throughput technologies…They are helping to unravel how and why these patients respond differently to cancer immunotherapy…. 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….
Approaches to Immunologic Monitoring

1. We ran some hospital labs for toxicity (CBC, ALC…), and one other single analyte assay tied to the strategy (IFNγ ELISPOT for the peptide in our vaccine; PDL-1 in tumors by IHC).

2. We ran several standardized cellular and humoral assays around our strategy (flow for phenotyping (effectors and suppressors), ELISPOTs, antibody arrays, mostly from blood).

3. We ran a couple of hot new technologies that have not been run in many trials before (TCR sequencing, multi-spectral IF, whole exome sequencing, epigenetics, whole genome sequencing with mutational analysis…).
Patient-derived specimens used in immunologic monitoring

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

Direct whole blood assays
  - PBMC
    - Freshly tested or cryopreserved for batch testing
    - Obtain absolute counts and percentages
  - Tumor section IHC
  - Tumor and lymphocytes
    - Digested tumor/TIL cell suspension
    - Serum
    - Plasma
      - Plasma platelets
      - Lymphocytes
        - Ficoll-Hypaque granulocytes erythrocytes
## Recommendations from the iSBTc-SITC/FDA/NCI Workshop on Immunotherapy Biomarkers

<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>
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<td>Newest, non-standardized technology</td>
<td>Sufficient blood/tissue to interrogate the samples <em>now</em>, as well as <em>later</em>, to generate new hypotheses</td>
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Tumor Antigen Responses/Vaccine Responses

**Vaccines**
- Peptides
- Proteins
- Virus
- DNA

**Vaccine Effects**
- Tumor ablation
- Chemotherapy
- Radiotherapy
- Small molecules
- Oncolytic virus

**Tumor Cells**
- +/- adjuvants or cytokines

**Tumor lysate**
- +/- adjuvants

**Dendritic Cells**
- + boost or electroporation

**Immunologic Monitoring**
- Blood
- Tumor

Butterfield, BMJ, 2015
Single or Combinations

Vaccines

- Peptides
- Proteins
- Viruses
- DNA
- Prime-Boost
- Dendritic Cells
- Tumor lysates
- Tumor cells

+ Standard of care
  - Ablation, Chemotherapy
  - Radiation, Small Molecules

Checkpoint Blockade
  - CTLA-4, PD-1, PD-L1, TIM-3...

Immunotherapy
  - Cytokines (IL-2, IFNα, GM-CSF)
  - Co-stimulation (4-1BB, OX40, CD40)
  - Adoptive Transfer of Effectors (T, NK)

Suppression Reduction
  - Lymphodepletion
  - Treg, MDSC reduction/inhibition
  - Myeloid cell modulation
Prognostic Signals: Ipilimumab Biomarker

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

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

Biomarkers: Increased ICOS on CD4+ and CD8+ T cells correlates with clinical outcome.


Previous work from P. Sharma (MD Anderson), in other trials.
Mechanism of Action signal: Determinant Spreading

Vaccine-induced, Adoptively transferred, Spontaneously activated T cells

Tumor antigens

Tumor lysis
Endogenous antigen release

Antigen cross presentation by endogenous APC. T cell activation against antigenic specificities

Assay?
Basic mechanisms used by T-regulatory cells

- **Inhibitory cytokines**
  - Membrane-tethered TGFβ
  - IL-35
  - IL-10

- **Cytolysis**
  - Granzyme A or granzyme B
  - Perforin pore
  - Apoptotic effector T cell

- **Metabolic disruption**
  - Through gap junctions
  - CD25
  - IL-2
  - Death due to cytokine deprivation

- **Targeting dendritic cells**
  - CTLA4
  - CD80/CD86
  - LAG3
  - MHC class II
  - IDO
  - Inhibition of DC maturation and function

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

Table depicting the parameters of the immune contexture that predict a good prognosis.

J. Galon, W. Fridman

“Cancer classification using the Immunoscore: a worldwide task force” JTM 2012
Myeloid-derived Suppressor Cells (MDSC)

- MDSCs suppress antitumor immunity
  - Impair innate immunity
  - Suppress T-cell activation
  - Limited antigen presentation due to the expansion of MDSCs at the expense of DCs

What’s new in Immune Biomarkers:

New areas of biology impacting immune response
Metabolism, microbiome, signaling pathway modulation

New technologies and high throughput approaches
Mass cytometry, exome sequencing, TCR diversity, epigenetics

New and old drugs impacting immunity:
Chemotherapy, Radiation, Ablation, signal transduction pathway inhibition

Bioinformatics, complex data analysis, and new biological samples
What is limiting immune biomarkers:

**Funding.** R21/R01 grants to fund biomarker assessments rarely successful.

CTEP won’t allow “exploratory” biomarker inclusion in protocols, can’t bank specimens for undefined, exploration of biomarkers. “Integral” or “Integrated” only.

NCTN no longer supports multiple, specialized specimen banks. Institutional funds limited to maintain them and expand them.

Knowledge of and access to older/existing banks for companies wanting to validate new technologies and candidate biomarkers.
Immunotherapy Biomarkers Task Force
History/Background

Previously:
Society Workshops: Immunologic Monitoring
2002 Keilholz Workshop summary, 2005 Lotze Workshop summary, state-of-the-art and recommendations

2008: assembled current Steering Committee:
Preamble ms JTM ’08;
SITC Workshop 2009 and meeting report JTM ’09
Taskforce meeting at the NIH 2010 and “Recommendations” paper (CCR ’11) and Resources document (JTM ’11)

Biomarkers Task Force: Steering Committee:
Lisa Butterfield, PhD
   Nora Disis, MD
   Bernie Fox, PhD
   Samir Khleif, MD
   Francesco Marincola, MD
Immunotherapy Biomarkers Task Force: 2015

Biomarkers Task Force: Working Groups:

GROUP 1: “Immune monitoring assay standardization and validation—update”  
*Leaders: Magdalena Thurin, PhD and Guiseppe 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 (Steering Committee, JITC March 2015)
2. Recommendations/white paper 1/WG (WG2 published Mar. 2016)
3. Biomarker Technology short reports 1/month in JITC x 12 mo.
4. Clinical trial analysis project: standard cellular/cytokine assays and high throughput molecular analyses
5. Summary meeting: **April 1st 2016, NIH**
E1608-Sidra Biomarker Project

Refinement of Peripheral Blood Correlative Analysis by Flow Cytometry and Serum Luminex (IMCPL) and New Genomics/Transcriptomics (Sidra)

L. Butterfield/F. S. Hodi; F. Marincola, E. Wang, D. Bedognetti (Sidra)

1) **Effector cell, antigen presenting cell and regulatory cell flow cytometry analysis; Melanoma antigen-specific T cell assays** (using non-HLA-restricted peptide pools to stimulate CD4+ and CD8+ T cells); **Treg and MDSC** measures; and **serum cytokine/chemokine/growth factor** measures.

2) Sidra analysis aims to define novel biomarkers of response to treatment by using integrated high-throughput gene expression and genotype profiling:

**PBMC Gene Expression Analysis and PBMC Genotype Analysis**

Data generated by the present analysis will be evaluated in the now open trial assessing the combination of Ipilimumab + Nivolumab +/- GM-CSF (EA6141, F. S. Hodi, PI).