Recommendations from the iSBTc/FDA/NCI Workshop on Immunotherapy Biomarkers

Lisa H. Butterfield, Ph.D.
Associate Professor of Medicine, Surgery and Immunology
Director, UPCI Immunologic Monitoring and Cellular Products Laboratory
University of Pittsburgh, UPCI
Member, iSBTc Biomarkers Validation Steering Committee

Presenter Disclosure Information

Lisa H. Butterfield, Ph.D.

The following relationships exist related to this presentation:

<No Relationships to Disclose>

Despite substantial efforts from many groups, we do not know which parameters of immune responses, and which assays used to assess these parameters are optimal for efficacy analysis.

Indeed, the tumor-specific cellular immune response promoted by immunization often has not correlated with clinical cancer regression despite the induced cytotoxic T cells detected in *in vitro* assays.

The major reason is that *objective clinical response rates are usually below 10%, preventing meaningful correlations of specific T cell response rates with clinical responses in small sized, early stage trials.*

Additionally: different assays chosen, assays performed differently, single parameters measured, in vitro stimulation.

To facilitate development of innovative immunotherapy approaches, there is a need to develop and validate tools to identify patients who can benefit (and are benefitting) from a particular form of immunotherapy.

The iSBTc, FDA and NCI partnered to address these issues for immunotherapy of cancer.

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Lisa H. Butterfield, A. Karolina Palucka, Cedrik M. Britten, Madhav V. Dhodapkar, Leif Håkansson, Sylvia Janetzki, Yutaka Kawakami, Thomas-Oliver Kleen, Peter P. Lee, Cristina Maccalli, Holden T. Maecker, Vernon C. Maino, Michele Maio, Anatoli Malyguine, Giuseppe Masucci, Graham Pawelec, Douglas M. Potter, Licia Rivoltini, Lupe G. Salazar, D.J. Schendel, Craig L. Slingluff, Jr., Wenru Song, David F. Stroncek, Hideaki Tahara, Magdalena Thurin, Giorgio Trinchieri, Sjoerd H. van Der Burg, Theresa L. Whiteside, Jon M. Wigginton, Francesco Marincola, Samir N. Khlief, Bernard A. Fox, Mary L. Disis

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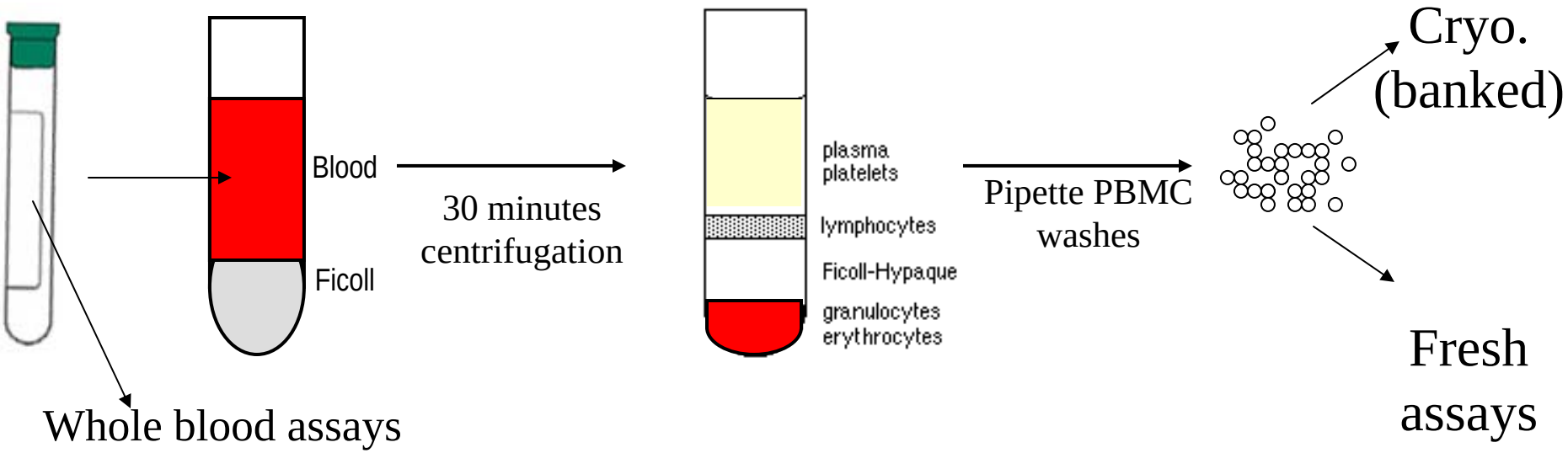
The work of the Immunotherapy Biomarkers Taskforce is addressing several challenges specific to immune-based therapies:

1. Processing and storage of blood samples to bank PBMC and serum for immunologic studies.
2. Characterization of cellular products for therapy
3. Assay standardization and harmonization before testing patient samples
4. Centralization of immunological monitoring
5. Standardized (or standardizable) assays which should be used for clinical trial antitumor immune response determination
6. How assay data should be analyzed for “responder” and “non-responder” identification
7. Reporting immunological monitoring data in publications
8. Validation of specific assays and/or analytes as biomarkers of clinical response
9. Novel assays in development for immunological testing of patients

1. Processing and storage of blood samples to bank PBMC and serum for immunologic studies.



Standardized Immunologic Monitoring Consortium protocols (published)

2. Characterization of cellular products for therapy

Challenge: A wide variety of cellular products are being tested for therapy of cancer, from minimally manipulated autologous blood products, to cultured cell lines, and antigen loaded, matured dendritic cells.

Autologous products can be highly variable between patients and are challenging to characterize and standardize such variability, often minimally characterized, can impact immune biomarkers.

Recommendations: Standardize and utilize multiple phenotypic and functional assay parameters specific to the cellular product.

Readers are encouraged to refer to FDA's Draft Guidance for Industry (released in Oct. 2008) Potency Tests for Cellular and Gene Therapy Products.

3. Assay standardization and harmonization

5. Standardized assays which should be used for clinical trial antitumor immune response determination

CLIA (Clinical Laboratory Improvements Amendments) rules:

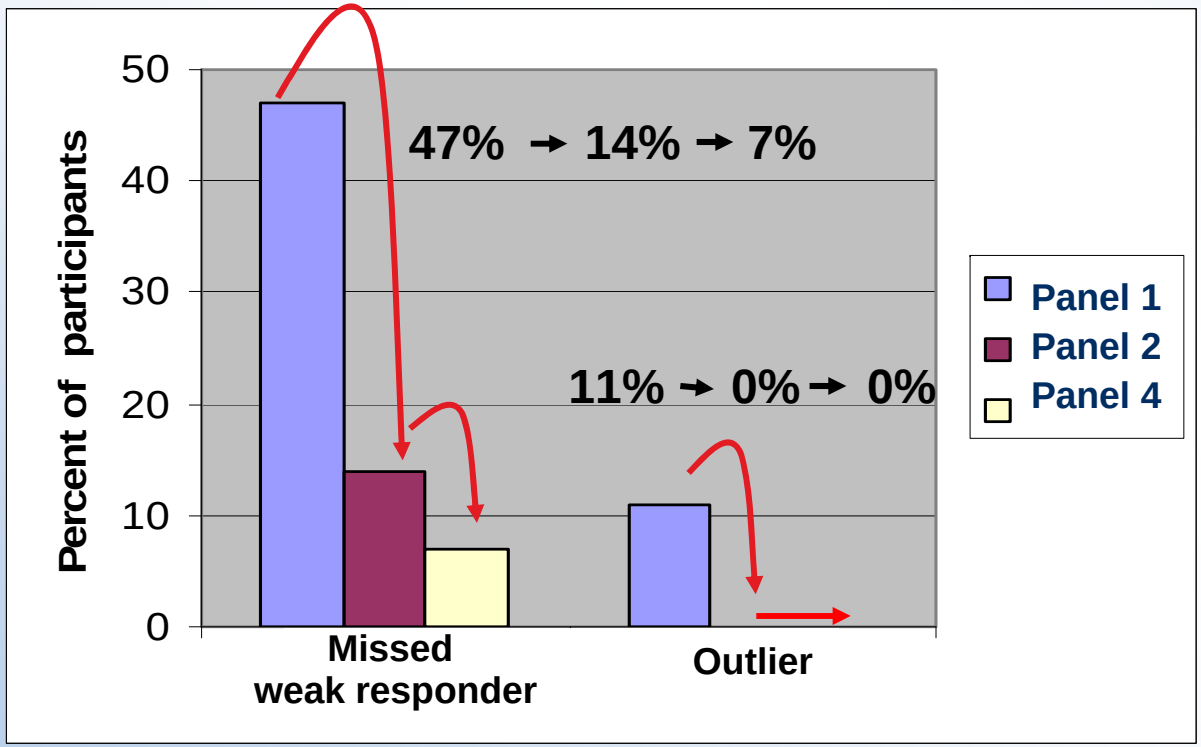
Test Accuracy (close agreement to the true value),
Precision (agreement of independent results: same day, different day),
Reproducibility (intra-assay and inter-assay)

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

Personnel competency testing
Equipment validation, monitoring
Reagent tracking

Impact of Assay Harmonization: The CIC Elispot Proficiency Panel Program

Assay Harmonization guidelines deduced from panel results *(Janetzki et al., CII 2008, 57; 303)* and increasingly implemented by participating labs led to improved performance



Note: Harmonization does not impose strict standardization rules across all labs

CIC Cancer Immunotherapy Consortium

Which standardized assays are superior and should be validated?

IFNg/CD8+ ELISPOT of 20 spots/10e5

vs.

IFNg/CD8+ ELISPOT of 200 spots/10e5?

IFNg+IL-2+TNFa multi-functional CD8+ T cells

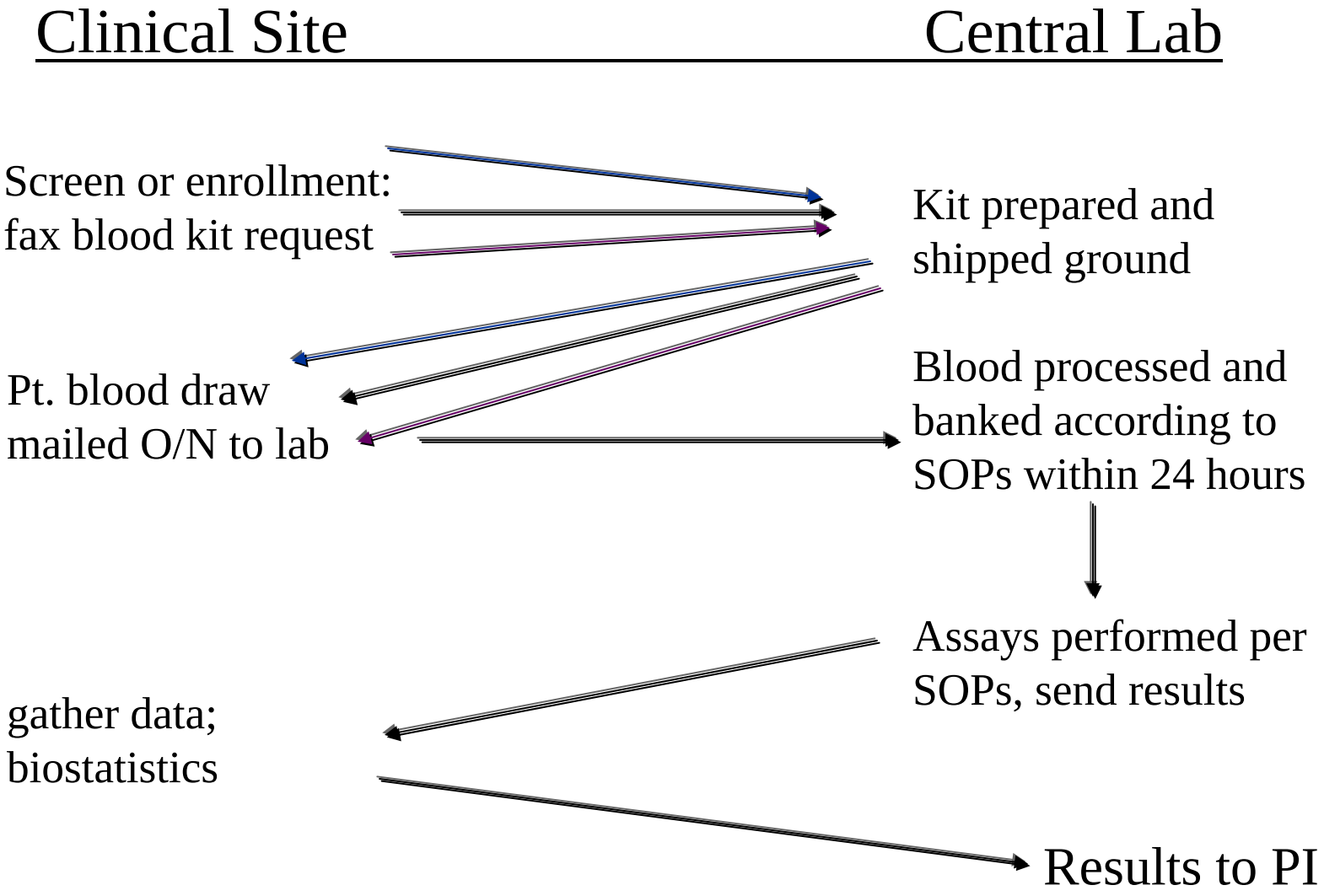
vs.

IFNg+TNFa vs. TNFa expressing CD8+ T cells?

Highest IFNg ELISA results after 7+ day IVS?

Broadest immunity to antigens/determinant spreading?

4. Centralization of immunological monitoring



6. How assay data should be analyzed for "responder" identification

7. Reporting immunological monitoring data in publications

Obtain multiple pre-therapy samples to assess pre- therapy variability of the biomarker.

Require positive responses at two consecutive post-therapy time points; this is useful for limiting post-therapy variability.
Consider using clinical response to refine definition of immune response.

When immune response is the primary outcome of interest in a trial, use non-parametric techniques (such as the Wilcoxon signed-ranks test) to assess response of entire sample of patients as a group.

No one knows how big an increase in the frequency of antigen-specific T cells between two time points should be to be considered a biologically relevant response.

Always include: the QA/QC performed, reference populations included, all testing of reagents and controls, at least some selected examples of truly representative raw data and the assay performance characteristics.

9. Novel assays in development for immunological testing of patients

We recommend that both RNA and DNA samples as well as sera and plasma be banked under standardized conditions for later testing in multiplex, molecular assays (from blood and the tumor, and to study the microenvironment).

Improved collection of tumor and TIL are crucial for understanding the impact of different therapeutic approaches.

Sufficient blood be drawn to allow for the planned testing of the primary hypothesis being investigated in the trial, such that additional baseline and post-treatment blood is banked for testing novel hypotheses.

Variability:

1. Patient
2. Blood draw
3. Processing/cryo/thaw
4. Cellular product
5. Assay choice
6. Assay conduct
7. Assay analysis
8. Data reporting
9. Next cool new assay

Recommendations:

1. Save DNA/RNA/cells/tumor; include healthy donor control
2. Standardized procedures
3. Standardized procedures
4. Functional assays to characterize/develop potency
5. Standardized, functional
6. SOP
7. Appropriate biostatistical methods
8. Full details, controls, QA
9. Sufficient blood/tissue to interrogate the samples *now*, as well as *later*, to generate new hypotheses.

While specific immune parameters and assays are not yet validated, we recommend:

1. Following standardized (accurate, precise and reproducible) protocols
2. Use of functional assays for the primary immunologic readouts of a trial (to address hypothesis being tested)
3. Consideration of central laboratories for immune monitoring of large, multi-institutional trials
4. Standardized testing of several phenotypic and potential potency assays for any cellular product
5. When reporting results, the QA/QC, examples of truly representative raw data and the assay performance characteristics should be included
6. To promote broader analysis of multiple aspects of immunity, in addition to cells and serum, RNA and DNA samples should be banked (under standardized conditions) for later testing
7. Sufficient blood should be drawn to allow for the planned testing of the primary hypothesis being addressed, *and* for testing novel hypotheses (or generating new hypotheses) that arise in the field

ELISPOT standardization , story of successful standardization

Paul V. Lehmann, MD, PhD – Cellular Technology Limited

Harmonization of Immunologic Monitoring Across Institutions

Cedrik M. Britten, MD – Association for Cancer Immunotherapy (CIMT)

Panel Discussion:

Sylvia Janetzki, MD (Cancer Immunotherapy Consortium)

Michael Kalos, PhD (University of Pennsylvania)

Banking PBMC and serum for immunologic studies

Assay standardization before testing patient samples

Assay harmonization across institutions/laboratories

Standardizable assays which should be used for clinical trial antitumor immune response determination

Publication of immunological monitoring data