The iSBTc-FDA-NCI Taskforce on Immunotherapy Biomarkers

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Presenter Disclosure Information

Lisa H. Butterfield, Ph.D.

The following relationships exist related to this presentation:

<No Relationships to Disclose>

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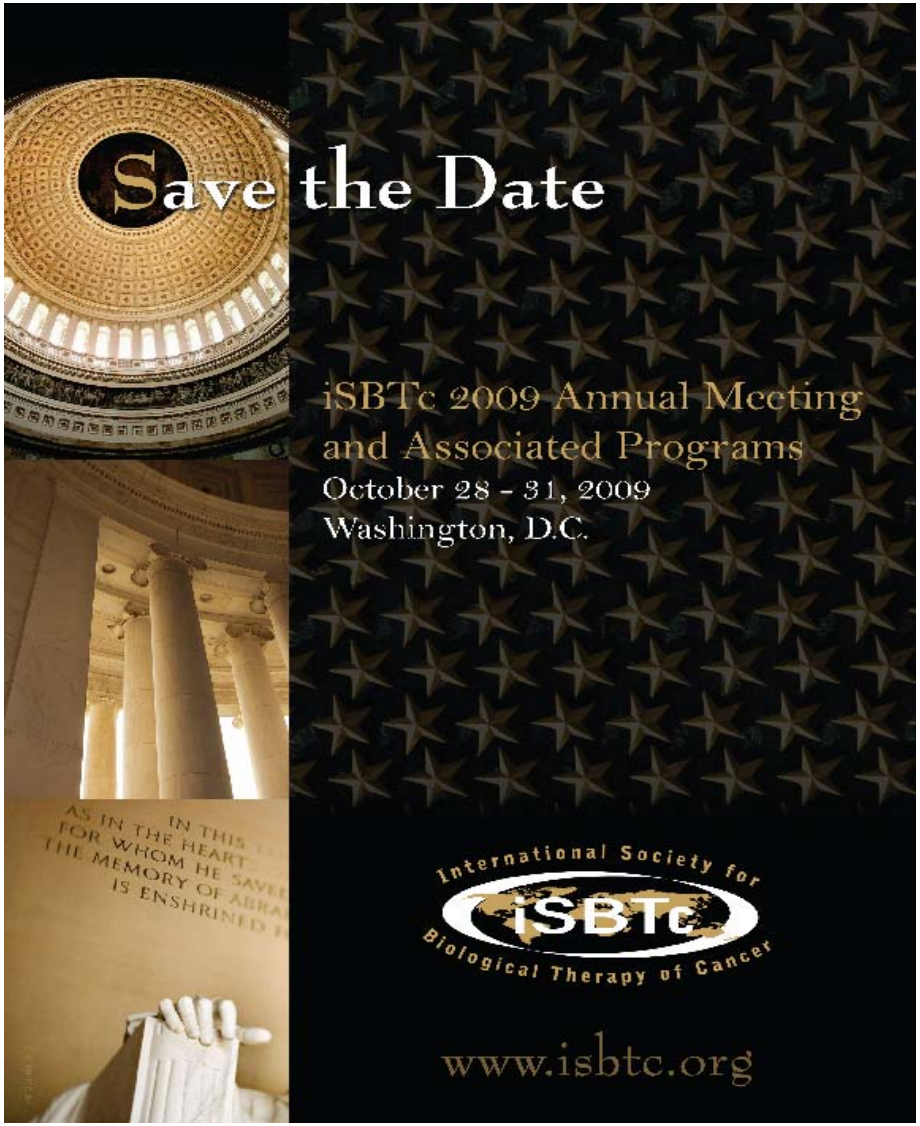
iSBTc-FDA-NCI Workshop on Prognostic and Predictive Immunologic Biomarkers in Cancer

October 28, 2009 ~ Washington, D.C.

iSBTc 24th Annual Meeting

October 29-31, 2009 ~ Washington, D.C.



iSBTc - FDA - NCI Workshop

Prognostic and Predictive Immunologic Biomarkers in Cancer

October 28, 2009 – Washington, D.C.

Organizers:

Lisa H. Butterfield, PhD
University of Pittsburgh

Mary (Nora) L. Disis, MD
University of Washington

Samir Khleif, MD
National Cancer Institute

Francesco Marincola, MD
National Institutes of Health

Magdalena Thurin, MD
National Institutes of Health

Workshop Topics:

- **A**ssessing the Immunologic Signature of Clinical Response
- **C**entralization of Immunologic Monitoring
- **D**etermining Potency of Immunologic Therapy
- **N**ovel Marker Identification
- **S**tandardization and Validation of Immunologic Biomarkers

Participating Organizations:

***iSBTc** ***CIMT** ***FDA** ***NIBIT** ***NCV-network**
***BDA** ***CVC** ***NCI** ***NIH**

www.isbtc.org

The iSBTc-FDA-NCI Workshop will focus on:

•Immunologic monitoring assays
•Novel methodologies for assessing the immune landscape in cancer.

•Standardization of assays
•Assay validation
•Potency assays
•Clinical utility of novel technologies

•Recommendations on how to incorporate these into the clinical arena.

Specific Goals:

- Establish a "best practices" protocol for the collection and storage of clinical samples for the assessment of immunologic outcomes in clinical trials of immune based therapies.
- Define minimum quality standards for laboratories developing immunologic biomarkers to be used for clinical trial analyses.
- Establish a consensus for performance characteristics of the most common immunologic assays to be used for the evaluation of immune based therapies and discuss standards for the reporting of immunologic results.
- Evaluate and determine key measures of potential potency of cell based immunotherapeutic products.

Participating Organizations:

- Association for Immunotherapy of Cancer (CIMT)
- Biotherapy Development Association (BDA)
- Cancer Vaccine Consortium (CVC) of the Cancer Research Institute (CRI)
- Food and Drug Administration (FDA)
- Italian Network for Tumor Biotherapy (NIBIT)
- Japanese Society of Cancer Immunology (JSCI)
- National Cancer Institute (NCI)
- National Institutes of Health (NIH)
- Nordic Center for Development of Antitumour Vaccines (NCV-network)

Which interventions are superior and should be moved forward?

Issues:

1.“In house” developed assays.

2. Many assays chosen may correlate with successful treatment, but not with clinical outcome.

3. Might

vaccine A: IFNg ELISPOT of 20 spots/10e5

REALLY be superior to

vaccine B: IFNg ELISPOT of 200 spots/10e5?

Published Resources (1976-present):

Cryopreservation of human lymphocyte function measured by in vitro assays.
Oldham, Dean, Cannon, Ortaldo, Dunston, Applebaum, McCoy, Djeu, Herberman
Int. J. Cancer, **1976**
Methods are described by which cryopreserved cells can be utilized in a number of in vitro assays. Highly reproducible activity was recovered on a per lymphocyte basis in lymphocyte cytotoxicity but with a definite decrement in the percentage recovery. Both for longitudinal studies of immune function and for standardization of these assays in one or more laboratories such cryopreserved cells are of immense value and should be widely utilized.

Impact of Cryopreservation on tetramer, CFC and ELISPOT
Maecker, Moon, Bhatia, Ghanekar, Maino, Payne, Kuus-Reichel, Chang, Summers, Clay, Morse, Lyerly, DeLaRosa, Ankerst, Disis
BMC Immunology **2005** 6:17.
Peptide responses correlate well between fresh and cryopreserved.

”iSBTc-FDA-NCI Workshop on Prognostic and Predictive Immunologic Biomarkers in Cancer”

October 28, 2009 ~ Washington, D.C.

- 7:45 am – 7:50 am Welcome and Introduction

Bernard A. Fox, PhD – Earle A. Chiles Research Institute

- 7:50 am – 8:10 am FDA Perspectives on Biomarkers

Steven Kozlowski, MD – Food and Drug Administration, Office of Biotechnology Products

- 8:10 am – 8:30 am Perspectives of the NCI

James W. Jacobson, PhD – Cancer Diagnosis Program, DCTD, NCI

Session 1: Standardization and Validation of Immunologic Biomarkers

Co-Chairs: Mary (Nora) Disis, MD & Sylvia Janetzki, MD

- Standardization of Immune Biomarkers: Lessons from the HIV Field

Alan L. Landay, PhD – Rush University Medical Center

- Immune Monitoring Consortium Experience

Mary (Nora) Disis, MD – University of Washington

- Multi-Institution Trials and the Eastern Cooperative Oncology Group

Lisa H. Butterfield, PhD – University of Pittsburgh

- Discussion

Moderator: Sylvia Janetzki, MD – ZellNet Consulting

Immunology Quality Assessment Program(IQA)

The IQA is a resource designed to help Immunologists evaluate and enhance the integrity and comparability of immunological laboratory determinations performed on patients enrolled in multi-site HIV/AIDS investigations (therapeutic, vaccine, prevention, etc.).

~83 Participating Laboratories

- **6 Shipments per year**
- **Included in shipment 5 Whole Blood samples**
- **Samples are shipped overnight priority via Federal Express in Ambient Temperature**

Standardization of Immune Biomarkers: Lessons from the HIV Field
Alan L. Landay, PhD – Rush University Medical Center

NCCLS consensus process for global harmonization

Performance of single cell immune response assays:

Submitted for comment Sept. 2003, published v. 23, n. 25, October 2004 (proposed guideline), v. 24, n. 29 (approved guideline) **2005**.

A. Landay, T. Fleisher, K. Kuus-Reichel, V. Maino, N. Reinsmoen, K. Weinhold, T. Whiteside, J. Altman

ELISPOT
Intracellular cytokine staining
MHC Tetramer

Issues: Specimen handling, transport, preparation, QA, test validation approaches, data acquisition analysis and reporting.

Open Access Protocols

Protocol for Isolation, Cryopreservation, and Thawing of PBMCs

Description

Cryopreserved PBMCs are a common specimen source for studies of immunological responses to vaccines, immunotherapies, etc. The health and viability of cells recovered post-cryopreservation are of course critical to the success and accuracy of immunological assays performed on them. We have developed this protocol to help standardize PBMC isolation and cryopreservation techniques, specifically for the assessment of thawed cells by cytokine flow cytometry.

Cryopreservation of PBMCs

The following protocol for freezing PBMCs uses a final concentration of 10% dimethylsulfoxide (DMSO) and 11.25% protein (human serum albumin) in cRPMI. Cryoprotectants, such as DMSO, reduce the amount of ice present during freezing and reduce solute concentration, thus reducing ionic stress. However, these compounds can themselves cause osmotic injury since they are hypertonic and can cause damage during their addition or removal.

1. Resuspend PBMCs (from **Isolation** section of **Processing of Fresh PBMCs**, above) at 1 x 10^7 viable lymphocytes/ml in 4°C 12.5% HSA in RPMI medium, in a 50 ml conical polypropylene tube.
2. While *gently* swirling the tube, add dropwise enough 4 C 2X freezing medium to double the volume of the cell suspension.
3. Immediately place the tube on ice. Avoid any further mixing or agitation of the cells. Slowly remove the cell suspension into a pipet and dispense 1 ml per cryovial on ice.
4. Place the cryovials in a pre-cooled Mr. Frosty-style freezing container that has been filled with 70% isopropanol according to the manufacturer's instructions. Place the freezing container at –80°C.

Thawing of PBMCs

If PBMCs are not thawed properly, viability and cell recovery can be compromised; and cells may not perform optimally in functional assays. In general, cells should be thawed quickly but diluted slowly to remove DMSO. Cells with DMSO intercalated into their membranes are very fragile, and must be pelleted and handled gently.

1. Warm cRPMI to 22°-37°C in a 37°C water bath before beginning thawing procedure.
2. Transfer the cryovial from liquid nitrogen to a 37°C water bath. If liquid nitrogen has seeped into the cryovial, loosen the cap slightly to allow the nitrogen to escape during thawing.

Disis et al, J Immunol Meth, 2005
Maecker et al, BMC Immunol, 2005

Session 2: Determining Potency of Immunologic Therapy

Co-Chairs: A. Karolina Palucka, MD, PhD & Theresa L. Whiteside, PhD

- Assessing Dendritic Cell Vaccines

A. Karolina Palucka, MD, PhD – Baylor Institute for Immunology Research

- Mechanisms of Immune Suppression and Regulatory T Cells

Theresa Whiteside, PhD – University of Pittsburgh Cancer Institute

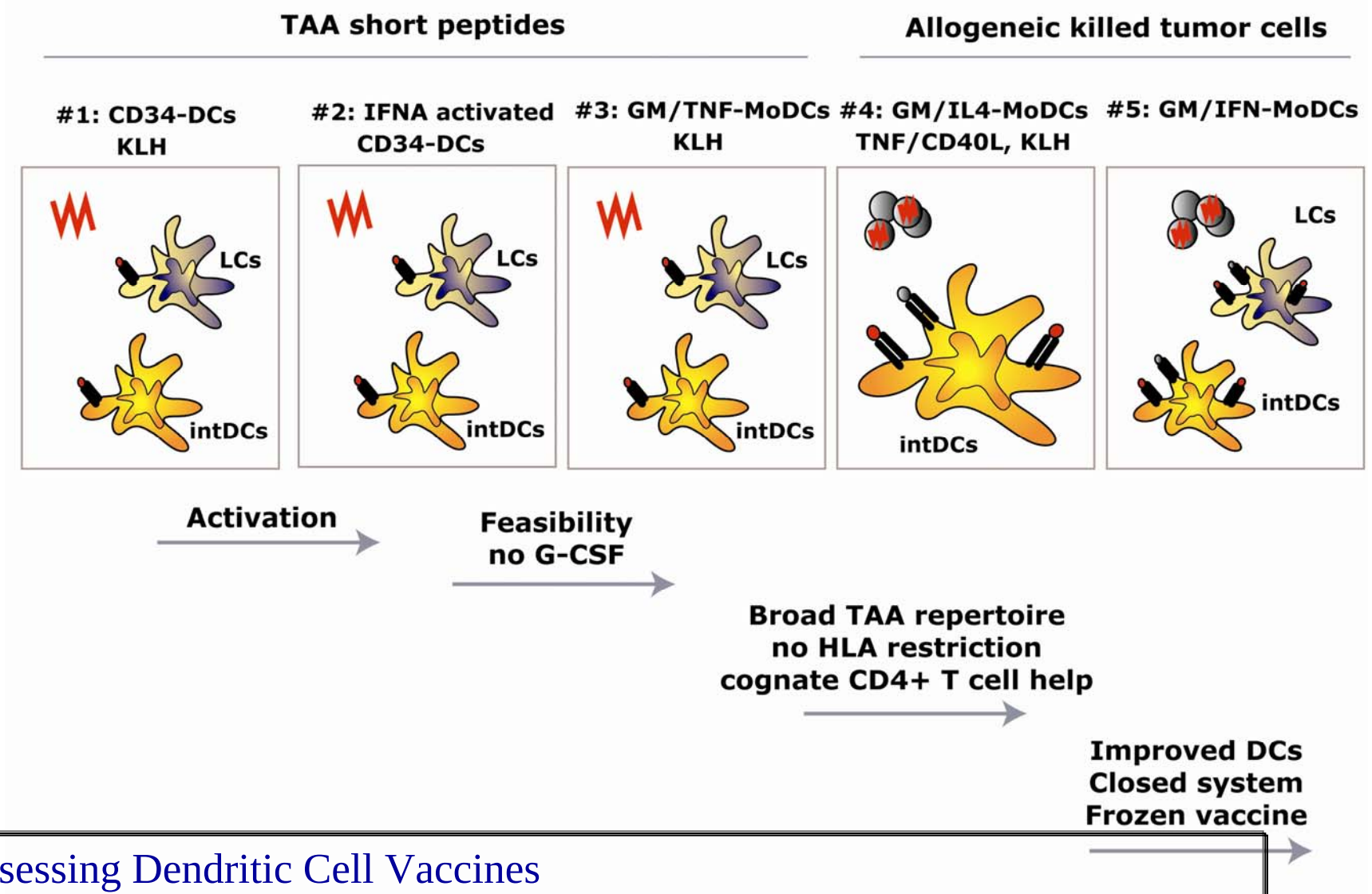
- Use of Molecular Assays to Assess Cellular Therapies

David Stroncek, MD – Cell Processing, DTM, CC, NIH

- Discussion

Moderator: Licia Rivoltini, MD – Fondazione IRCCS Istituto Nazionale dei Tumori

BIIR DENDRITIC CELL VACCINE TRIALS:
FIRST GENERATION TRIALS IN METASTATIC MELANOMA



Assessing Dendritic Cell Vaccines

A. Karolina Palucka, MD, PhD – Baylor Institute for Immunology Research

Session 3: Assessing the Immunologic Signature of Clinical Response

Co-Chairs: Cedrik M. Britten, MD – Johannes Gutenberg University

Vernon C. Maino, PhD – BD Biosciences

- T Cell Response Signatures in Breast Cancer vs. Chronic Infection

  Holden T. Maecker, PhD – Stanford University
- Immunity to Glycolipid and Stem Cell Antigens in Human Cancer

  Madhav Dhodapkar, MD – Yale University
- Summary of the US-Japan Workshop on Immunotherapy Markers in Oncology

  Hideaki Tahara, MD, PhD – University of Tokyo

- Discussion

Moderator: Lupe G. Salazar, MD – University of Washington

Features of a T cell response signature

- Magnitude and Breadth
  - Total frequency of Ag-specific T cells
  - Breadth of epitope responses
- Functional properties
  - Cytokine production
  - Degranulation or lytic capacity
  - Fraction of Ag-specific cells that are functional
- Phenotypes
  - Markers of memory and effector differentiation
  - Markers of exhaustion (PD-1, etc.)
  - Perforin, granzymes, etc.

T Cell Response Signatures in Breast Cancer vs. Chronic Infection
Holden T. Maecker, PhD – Stanford University

Session 4: Centralization of Immunologic Monitoring

Co-Chairs: Alan L. Landay, PhD & Anatoli M. Malyguine, MD, PhD

- Immunological Monitoring of Cancer Vaccine Trials – What to Measure, How and Why?

Anatoli M. Malyguine, MD, PhD – SAIC-Frederick, Inc., NCI-Frederick

- Systems Biology Approaches

Rafick-Pierre Sékaly, PhD – University of Montreal, VGTI Florida

- Some Statistical Issues in Design and Analysis of Vaccine Clinical Trials in Cancer Patients

Douglas M. Potter, PhD – University of Pittsburgh

The Laboratory of Cell Mediated Immunity (LCMI) is part of the Applied & Developmental Research Support Program, SAIC-Frederick, Inc., located on the NCI-Frederick campus, Frederick, MD

Michael W. Baseler, Ph.D.
Director
Applied & Developmental Research Support Program

Other laboratories

William C. Kopp, Ph.D.
Deputy Director
Applied & Developmental Research Support Program
Head
Clinical Support Laboratory

Anatoli Malyguine, M.D., Ph.D.
Head
Laboratory of Cell-Mediated Immunity

Clinical Monitoring

- Sample login and processing
- Cell Culture
- Magnetic bead cell enrichment
- EBV Transformation
- Nucleic acid extraction and quantification

Flow Cytometry

- Immunophenotyping
- Cell Cycle Analysis
- Bioassays – antibody binding and titration assays
- Viability and apoptosis

Lymphokine Testing

- ELISA assays for cytokines, soluble receptors
- Multiplex cytokine assays

Cytokine bioassays
Proliferation assay and recertification testing

Clinical monitoring

- Peptide ELISPOT assay
- Whole protein ELISPOT assay
- Tumor ELISPOT assay
- Granzyme B ELISPOT assay
- Cytokine induction assay
- Proliferation assay
- CTL induction and cytotoxicity assay

Basic research support

- Murine IFN-γ, IL-2, IL-5, IL-17, CM-CSF, MCP-1 and Granzyme B ELISPOT assays
- Non-human primates IFN-γ ELISPOT

Assay development, optimization and validation

IFNγ ELISPOT RESPONSE-- SUGGESTIONS

- Get as many pre-tx samples (at different times) for analysis as possible; useful for limiting pre-tx variability
- Tighten response criteria: require positive responses at 2 consecutive post-tx time points; useful for limiting post-tx variability
- True immune response is continuous, not binary. Different definitions of binary response are arbitrary & will correlate differently with clinical outcome.

Session 5 – Novel Marker Identification

Francesco Marincola, MD –NIH & Peter P. Lee, MD

- High Throughput Technology and Predictive Immune Monitoring

Peter P. Lee, MD – Stanford University

- System Immunology from the Bottom-Up

Damien Chaussabel, PhD – Baylor Institute for Immunology Research

- Trans-NIH Center for Human Immunology: Goals and Progress

Giorgio Trinchieri, M.D., NIH

- Application of Proteomics to Biomarker Discovery: New Challenges, New Technology

Lance Liotta, MD, PhD – George Mason University

- Defining Immunologic Health

Mark M. Davis, PhD – Stanford University School of Medicine

PLATFORM FACILITIES

Flow Cytometry

- clinical assays adapted for immune system cells
- intracellular cytokines and phosphoproteins for function
- circulating cytokines
- tetramer staining
- flow-based imaging

-Omics

- high throughput sequencing-based analysis of transcriptome
- high density SNP arrays for genetic associations
- epigenomic analysis using ChIP-Seq
- high throughput sequencing ("$1000 genome")
- proteomics (shared with PSIIM)

Clinical Protocol

- "deep" phenotyping of healthy humans ("immunome") and patients
- intensive assessment of limited numbers of subjects
- observation and intervention studies; new protocols and supplements to existing studies

Computational/Systems Biology

- informatics to link all of the above
- modeling to interpret and hypothesize

The Immune Profiling Arsenal

- High throughput molecular profiling platforms to study the human immune system “in nature”.
  - Polychromatic flow cytometry
  - RNA profiling (**mRNA**, miRNA, RNAseq)
  - SNP arrays (soon genome sequence)
  - Multiplex serum chemokines, cytokines profiles
  - Protein arrays
  - Mapping antigenic repertoire
  - “Other ex-vivo assays”
  - ….

One way to systematize this:

The Stanford Human Immune Monitoring Center (Holden Maecker)

Immunology for the people!

One stop shopping-high throughput assays and database

Defining Immunologic Health

Mark M. Davis, PhD – Stanford University School of Medicine

Taskforce Activities since the Oct. 2009 Workshop

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

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

We thank Dr. Raj Puri, (Director, Division of Cellular and Gene Therapies, Office of Cellular, Tissue and Gene Therapies, FDA/Center for Biologics Evaluation and Research), the FDA liaison to iSBTc, for providing critical critique of the manuscript.

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

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

Symposium on Immuno-Oncology Biomarkers, 2010 and Beyond:
Perspectives from the iSBTc Biomarker Task Force
September 30, 2010

Masur Auditorium on the NIH Campus
Building 10, Clinical Center

Topics to include:

•Immunologic Monitoring: Standardization and Validation of Assays
•Correlation of Immunity to Clinical Response and Potency Assays
•Novel Methodologies for Assessing the Immune Landscape: Clinical Utility of Novel Technologies
•Recommendations on Incorporation of Biomarkers into the Clinical Arena

Samir N. Khleif, MD
National Cancer Institute (CCR)

Francesco Marincola, MD
National Institutes of Health (CC, DTM)

Lisa H. Butterfield, PhD
University of Pittsburgh

Mary L. (Nora) Disis, MD
University of Washington

1. Participation of multiple, **overlapping** taskforces, groups, labs and societies.

2. Standardization/Harmonization: critical sample and assay parameters have been identified and standardized protocols are available and cellular products are being better characterized.

3. Cross-presentation & Autoimmunity: clinical, sub-clinical, tumor antigen determinant spreading, normal self antigen.

4. Identifying biomarkers (large studies): genetic biomarker of IFN responsiveness, tumor infiltration and lymph node biomarkers in addition to blood cell biomarkers.

5. iSBTc Biomarkers Resource Document 2010:
References and Websites (Davide Bedognetti, MD, PhD. NIH/Genoa)

Acknowledgements

1. Members of the iSBTc Taskforce (working groups 1&2)
2. Participants in the 2009 Workshop
3. Representatives from the participating International Immunotherapy Societies
4. Participants in the 2010 Biomarkers Symposium
5. Scientists and clinicians performing the studies
6. iSBTc staff