Monitoring of Immune Response during Immunotherapy

Michael Kalos, Ph.D

ESMO
November 16, 2013
Geneva CH
I have the following relevant financial relationships to disclose:

• **Patents and potential royalties from Novartis Pharmaceuticals-CAR technology**
  
  Conflict of Interest managed in accordance with University of Pennsylvania policy and oversight

• **Scientific Advisory Board member for Adaptive Biotechnologies (ex)**

• **Research funding from Adaptimmune Corporation (ex)**

• **Eli Lilly and Company - Employment (current)**
Topics for today

- Biomarkers in Translational and Clinical Research
  - Basic concepts and platforms
Biomarkers in translational research
Biomarkers Drive The Translational and Clinical Research Engine

Cell therapy-specific
- Potency
- Identity
- Characterization
- Phenotype and functionality
- Biological Effects in models
- Patient characteristics
- Clinical readouts
- Biological effects in patients
- Surrogate endpoints?

Product Manufacture

R&D

Clinical trial

Biomarker and correlative studies
Critical issues in biomarker research

A path to success:
- Comprehensiveness
- Objective Quality
- Integrated and Systematic meta-analysis

Comprehensiveness
Multiparameter bead array (Luminex)

- **Advantages:**
  - High on the comprehensiveness scale
  - Minimal sample volumes required
  - Robust platform
  - Quantitative
  - Very cost effective when evaluating multiple cytokines

- **Applications**
  - Soluble factors
  - Phosphoproteins
  - Nucleic acid
Multiplex cytokine analysis of clinical samples reveals unpredicted patterns of systemic cytokine modulation
Seromics- Invitrogen Protoarray

- Over 10,000 full-length human proteins displayed on array chip
- Proteins expressed by baculovirus expression system as GST fusions
- Proteins are purified under non-denaturing conditions and printed to preserve native protein structure
- Arrays probed with sera from patients to identify autoantibodies that develop during treatment
Protoarray analysis provides evidence for epitope spreading following CART therapy

Seromics. Protoarray analysis of serum samples from pancreatic cancer patient 21211-101

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Immune cell diversity profiling - Deep sequencing

- Illumina HySEQ compatible sequencing using multiplex PCR with primers to all known V and J segments
- Amplification of rearranged TcRβ CDR3 and IgH sequences
- Custom software to verify, align, catalogue, and quantify individual sequences
- Compatible with genomic DNA
- Provides integrated and quantitative snapshot of T and B cell diversity and abundance
Deep sequencing reveals a diverse population of persisting gene-modified T cells post immunotherapy

6 month post infusion sample, sorted on CAR19+ and negative CD8+ cells

CD8+ CART19 cells

9 clonotypes >1%
c.a. 20 clonotypes >0.1%

Adaptive Biotechnologies, Seattle, WA
Assay Quality
Principles of quality

• Biomarker studies guide the development of candidate therapeutics from the earliest stages of development all the way through late stage clinical studies and the establishment of surrogate endpoints.

• Go/no-go decisions are made throughout the product development process based on the results of biomarker studies

• Accordingly, in biomarker studies, assays need to:
  – Measure what they claim to measure
  – Be quantitative and reproducible
  – Produce results that are statistically meaningful
Assay integration
Assay Harmonization improves assay and laboratory performance

Objectives:

1. Decrease variability by implementing Harmonization guidelines
2. Refine harmonization guidelines

van der Burg, SH, Kalos, M et al SciTM 2011
Data reporting and repositories
Establishment of infrastructure for meta-mining of data

- MIBBI (Minimum Information for Biological and Biomedical Investigations) ([www.mibbi.org](http://www.mibbi.org))

- What to capture
- How to capture
- Where to capture

Sets the stage to allow for systematic prospective, retrospective, and integrative biomarker evaluation
Here we present the reporting framework MIATA (Minimal Information About T cell Assays) that enables an objective and thorough interpretation of published results from T cell assays.

MIATA summarizes in 5 modules the essential information to be included in the Materials and Methods section of publications to provide the transparency sought by peers.

This website provides all tools for MIATA-compliant reporting and lists MIATA-compliant publications in its Hall of Fame linking to the original paper.
## Categories and attributes of T cell biomarkers

<table>
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<th>Category</th>
<th>Platforms</th>
<th>Assay</th>
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<th>Disadvantages</th>
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<td>Surface marker detection</td>
<td>Individual cells detected</td>
<td>Sample intensive</td>
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<td></td>
<td>Low sensitivity</td>
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<td>Specific detection reagent</td>
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<td>PCR</td>
<td>Transgene-specific amplification</td>
<td>High sensitivity</td>
<td>Bulk analysis</td>
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<td>Deep sequencing</td>
<td>Detection of specific TcR clonotypes</td>
<td>Extremely high sensitivity</td>
<td>Technology intensive</td>
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</table>

*Kalos, M. J. Transl. Med. 2011*
Adoptive T cell Therapy of Cancer

Ideally, will recapitulate the end result of a vaccine to induce T cell-immunity

- Large number of potent antigen specific T cells
- Expansion in vivo in response to antigen encounter
- Potent anti tumor activity
- Contraction and long-term persistence
- Ability to respond to challenge
PILOT STUDY OF REDIRECTED AUTOLOGOUS T CELLS ENGINEERED TO CONTAIN ANTI-CD19 ATTACHED TO TCRζ AND 4-1BB SIGNALING DOMAINS IN PATIENTS WITH CHEMOTHERAPY RESISTANT OR REFRACTORY CD19+ LEUKEMIA AND LYMPHOMA

Regulatory Sponsor: Carl June, M.D.

- Clinical PI:
  - UPCC04409 (CLL, ALL): David Porter, M.D./Noel Frey M.D.
  - CHP959 (Pediatric ALL): Stephan Grupp M.D. Ph.D.
  - UPCC03712 (CLL, dose finding): Noelle Frey, M.D.

- Cell Product Manufacturing: Bruce Levine Ph.D.

- Biomarker Development and Analyses: Michael Kalos, Ph.D.
Secondary endpoints: immunomonitoring (biomarkers)

Mechanistic insights to guide rational clinical development

- Persistence and homing to tumor: Q-PCR, flow cytometry, TcR complexity
- Phenotype/Functionality*: Flow cytometry - immunophenotyping, effector assays
- Bioactivity: Luminex - Cytokine modulation, MRD measure
- Immune responses (anti CART19): HACA, cellular

Aphereses:
- Pre-infusion
- Month 3

Peripheral blood
Marrow

DNA
Mononuclear cells
Serum

D-1

D+1, +2, +3, +10, +12, +14, +21, +28, monthly

infusion
Long-term persistence and ongoing B cell aplasia in CR patients

UPCC04409-09

**Q-PCR analysis**

![Q-PCR graph showing the decline of CAR19+ cells over time](image)

**Flow cytometric analysis**

![Flow cytometry scatter plots showing CD20 and CAR19 expression](image)
Flow cytometric analysis:

B cells (tumor): CD19+/CD5+/kappa or lambda
CART19+: CD3+/CART19+
Proof of mechanism: Delayed onset tumor lysis syndrome co-incident with maximal CAR Expansion

CART-19 persistence and trafficking blood (subject #3):

Hospitalized for tumor lysis syndrome

Porter et al. NEJM, 2011
Potential companion diagnostic - Higher levels of peripheral CTL019 cells detected in complete responders

Q-PCR analysis
CTL019 cells/microgram genomic DNA
B-ALL: Long–term functional persistence of CART-19 cells and persistent B cell aplasia

Grupp, Kalos, et al NEJM 2013
Extending treatment options to CNS disease: 
CTL019 T Cells traffic to CNS in ALL

Blood

Morphology of CARs In Vivo

Blood Day 10

CSF Day 23

Grupp, Kalos, et al NEJM 2013
Long-term safety: Persisting CART19 cells are polyclonal- TcR Deep sequencing

6 month post infusion sample, high purity sorted on CAR19+ and negative CD8+ cells

9 clonotypes >1%
c.a. 20 clonotypes >0.1%
Many clonotypes between 0.01-0.1%
Complete responses are associated with deep molecular remissions: IGH deep sequencing

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<th>Tissue</th>
<th>timepoint</th>
<th>Cell equivalents</th>
<th>total productive reads</th>
<th>Total unique sequences</th>
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The bad news: CTL019 Toxicities

- B cell aplasia
  - Observed in all responding patients to date
  - Managed with replacement therapy
- Tumor lysis syndrome (TLS)
  - May be delayed for 20 to 50 days post infusion
- Cytokine release syndrome (CRS)
- Macrophage activation syndrome (HLH / MAS)
Agnostic cytokine analysis reveals unexpected elevations in IL-6 and drives development of a new treatment paradigm

On-Target Delayed Cytokine Release Syndrome following CART-19 therapy

pALL patient cohort

Grupp, Kalos, et al NEJM 2013
The promise of CART19 therapy

August 28, 2013
Third Grade, First day
Successful development and implementation of biomarker studies requires:

- Quality-supporting infrastructure
- Assays that enable hypothesis generating insights
- Infrastructure to support integrated meta-analysis of data

The application of quality-supported biomarker platforms and studies that enable more comprehensive, hypothesis generating evaluation of patient samples offers the opportunity for functional and mechanistic insights into T cell immunotherapies.
Colleagues and collaborators

TCSL
Simon Lacey, PhD
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Hima Patel
Suzanne Pavluk
Tamara Tripic

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Don O’Rourke
Arati Desai

Study Participants

NOVARTIS

The Leukemia & Lymphoma Society®
Fighting Blood Cancers

ACGT
Perelman School of Medicine
University of Pennsylvania

ALIANCE FOR CANCER GENE THERAPY
National grants for cancer research