Frequency of Strong Antibody Responses Following Combination Immunotherapy Correlates with Increased PSA-Doubling Time in Men with Androgen-independent Prostate Cancer

SACHIN PURI, PhD

Lab of Molecular and Tumor Immunology
Earle A. Chiles Research Institute
Providence Cancer Center
Portland OR
SACHIN PURI

No Relationships to Disclose
Development of an Immune Response to the allogeneic prostate GVAX™ vaccine

QUESTION:

- How to monitor the development of a T cell response following immunotherapy with a “Complex” vaccine?

- Do not have access to autologous tumor cell lines
B cells also respond to vaccination.

Modified from
Hypothesis:

Identification of a new or increased IgG antibody response following immunotherapy will provide a surrogate for generation of an anti-tumor T cell response.
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Identification of a new or increased IgG antibody response following immunotherapy will provide a surrogate for generation of an anti-tumor T cell response.

Rationale supported by:
Reports in both human and murine systems

Integrated NY-ESO-1 antibody and CD8⁺ T-cell responses correlate with clinical benefit in advanced melanoma patients treated with ipilimumab

Jianda Yuan⁴, Matthew Adamow⁴, Brian A. Ginsberg⁴, Teresa S. Rasalan⁴, Erika Ritter⁵, Humilidad F. Gallardo⁴, Yinyan Xu⁴, Evelina Pogoriler⁵, Stephanie L. Terzulli⁴,⁵, Deborah Kul⁵, Katherine S. Panageas⁶, Gerd Ritter⁵, Mario Sznol⁶, Ruth Halaban⁶, Achim A. Jungbluth⁵, James P. Allison⁴,⁵, f, Lloyd J. Old⁵,⁶,¹, Jedd D. Wolchok⁴,⁵,⁶,¹,², and Sacha Gnajtéc⁵,¹,²

⁴Ludwig Center for Cancer Immunotherapy, Immunology Program, Sloan-Kettering Institute, New York, NY 10065; ⁵Ludwig Institute for Cancer Research, New York Branch, New York, NY 10065; ¹Department of Medicine, ¹Howard Hughes Medical Institute, and ²Department of Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY 10065; and ³Department of Medicine, Yale University, New Haven, CT 06520

Contributed by Lloyd J. Old, July 5, 2011 (sent for review June 10, 2011)
Patients who respond to immunotherapy may have a coordinated immune response (T and B cells).

Identification of the target of the B cell response may provide the target for the T cell response.
Immunological Monitoring Strategy: 
Protein Arrays to Identify the targets of Antibody Response

Pretreatment Apheresis  
Vaccines  
Week 11 Apheresis

Protein Array  
Pretreatment serum  
(n=11)

Protein Array  
Posttreatment serum

Compare: 
Determine post vaccine Ab response
Comparison of IgG levels in (1, 2, 3) Pre and Post Treatment Serum
Identification of Antibody Responses to Specific Antigens

Influenza A
Identification of Antibody Responses to Specific Antigens

Influenza A

Galectin 8 / PCTA
Identification of Antibody Responses to Specific Antigens

Influenza A

Galectin 8 / PCTA

FAM136A
Question:
Did the patient’s tumor cells express the genes identified by the antibodies?

Problem:
How to evaluate tumor gene expression when no biopsies available and patients have expired?
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Circulating Tumor Cells (CTC)
Schema for sorting CTCs and T cells from cryopreserved PBMC (aphereses)

- **CD8⁺ T cells (CD45⁺, CD8⁺, EpCAM⁻)**
- **CTC (CD45⁻, CD8⁻, CD4⁻, EpCAM⁺)**
Schema for sorting CTCs and T cells from cryopreserved PBMC (aphereses)

Schematic of Sorting and Linear Amplification

Day 1
Sorted Cells
RNA Extraction < pg yield

Day 2-4
In vitro Amplification of RNA

ug aRNA

Evaluate
1) EpCAM
2) Antibody targets
3) Gene expression
Analysis of aRNA from sorted CTC and T cells

Sorted CTC express EpCAM
T cells do not express EpCAM

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<td>B-Actin</td>
<td>234 bp</td>
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<td>EpCAM</td>
<td>173 bp</td>
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- **B-Actin 234 bp**: Sorted CTC samples show a band at 234 bp, while T cells do not. EpCAM samples show no band.
- **EpCAM 173 bp**: Sorted CTC samples show a band at 173 bp, while T cells do not.
Analysis of aRNA from sorted CTC and T cells

Sorted CTC express PCTA (4/4) and FAM136A (3/4)

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<th>FAM 136 A</th>
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Gene expression profiling - Affymetrix Human Gene 1.0 ST microarrays.

Preliminary results: Gene expression of isolated CTCs was more similar to prostate cancer cell lines (LNCaP and PC3) than to T cells.
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Gene expression profiling - Affymetrix Human Gene 1.0 ST microarrays.

**Preliminary results**: Gene expression of isolated CTCs was more similar to prostate cancer cell lines (LNCaP and PC3) than to T cells

**Suggests that strategy for isolating CTC is working**
Phase I/II study of allogeneic prostate GVAX™ in advanced prostate cancer patients.
DAMD 17-03-1-0097

- No Objective Clinical Responses
- Three of 10 evaluable men had a 3 fold or greater increase in PSA doubling time - “Responders”
- Seven men had stable or decreased PSA-DT - “Non Responders”
Phase I/II study of allogeneic prostate GVAX™ in advanced prostate cancer patients. DAMD 17-03-1-0097

- No Objective Clinical Responses
- Three of 10 evaluable men had a 3 fold or greater increase in PSA doubling time - “Responders”
- Seven men had stable or decreased PSA-DT - “Non Responders”

Hypothesis:
“Responder” patients would develop strong antibody responses against multiple antigens
Significant (p<0.043) correlation between strong vaccine-induced antibody responses (>15 fold) and increase in PSA-DT (3 fold)
Protein arrays are capable of detecting increased antibody responses but are expensive

Option:

Create multiplex beads with a panel of antigens that were identified as common from our protein array studies. Use these as a High Throughput Screen (HTS).
Schema for Multiplex Bead Array

Beads coupled with individual proteins

Serum sample + Detection Antibody (PE)

Analyze samples by Luminex
Preliminary Multiplex Assay results for 5 protein beads.
Summary:

1. Protein arrays can be used to identify antibody responses following immunotherapy.

2. CTC can be isolated from cryopreserved aphereses products using FACS and used to identify whether targets of the antibody are expressed by a patient’s tumor cells (CTC).

3. An increased number of strong antibody responses correlated with “Response” to Therapy /increase in PSA-DT.

4. Current efforts are directed at characterizing the T cell response against targets of the antibody response and developing a HTS method to assess antibody responses to common protein targets identified by protein arrays.
### Earle A. Chiles Res. Inst.

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Thank You