SITC Advances In Cancer Immunotherapy Series:

*Immunotherapy on the Horizon*

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The Angeles Clinic & Research Institute
Disclosures

* Speaker’s bureau for Bristol Myers Squibb
* ASCO Government Relations Committee Member
* Non-FDA approved therapies will be discussed
Immunotherapy’s Great Promise

CTLA-4 Pathway

- Ipilimumab
- Tremelimumab

PD-1 Pathway

- Atezolizumab
- Durvalumab

MHC-TCR Complex

CTLA-4 & CD80/86 Complex

APC

T cell

PD-L1/PD-L2 & PD-1 Complex

T cell, B cell, or NK cell

CD 28

Nivolumab

Pembrolizumab
Improving Long-Term OS

Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. JCO 2015
Association of Pembrolizumab With Tumor Response and Survival Among Patients With Advanced Melanoma. JAMA. 2016;315(15):1600-1609.
## Improving Toxicity*

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Nivolumab</th>
<th>Ipilimumab</th>
<th>*Nivolumab and Ipilimumab</th>
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<tbody>
<tr>
<td></td>
<td>All grade</td>
<td>Grade 3 and 4</td>
<td>All grade</td>
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<tr>
<td>Diarrhea</td>
<td>19.2</td>
<td>2.2</td>
<td>33.1</td>
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<td>Fatigue</td>
<td>34.2</td>
<td>1.3</td>
<td>28</td>
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<tr>
<td>Rash</td>
<td>25.9</td>
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<td>32.8</td>
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<tr>
<td>Increased ALT</td>
<td>3.8</td>
<td>1.3</td>
<td>3.9</td>
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<tr>
<td>Increased AST</td>
<td>3.8</td>
<td>1.0</td>
<td>3.5</td>
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<td>4.2</td>
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<td>1.3</td>
<td>0.6</td>
<td>11.6</td>
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<tr>
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<td>0</td>
<td>6.1</td>
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<tr>
<td>Dyspnea</td>
<td>4.5</td>
<td>0.3</td>
<td>4.2</td>
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</table>

Simple... Right?

CD8+ T cell

Tumor

Immune Priming

CD8+ T cell

CD4+ T cell

Checkpoint Inhibition
Prediction & Prognosis
Critical “Checkpoints”

Host, Tumor Characteristics Have a Major Role in Outcomes
A Theatre of Mighty Designs

Eight Bells, Winslow Homer, 1886
A Theatre of Mighty Designs

Legend:
- Stimulatory receptor
- Inhibitory receptor
- Peptide
- Agonist mAb
- Antagonist mAb

Cognate ligand binding induces secretion of immunosuppressive cytokines, e.g. TGF-β
Host and Tumor Profiling

- **Mutation Burden & Neoantigens**
- **Multispectral Imaging**
  (microenvironment)
- **Tumor IHC, CTC DNA**
  (serology)

- **T Cell Priming**
  (TILs, PDL1, microenvironment)
- **Multivariate Analysis**
  (Immunoscore)
- **Immune Competence**
  (CyTOF)

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- **Match Patient to Therapy**
- **Immunomonitoring**
  (Predictive Biomarkers, Host Response)
Learning Objectives

* Describe current state of biomarker analysis
  * Patient (tissue & liquid biopsies)
  * Tumor (tissue & liquid biopsies)
* Discuss technologies for identifying signatures of immune function
  * Pre- and post- therapeutic intervention
* Applying the science to everyday practice
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Host Profiling

- **T Cell Priming**
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  - (CyTOF)

- Mutation Burden & Neoantigens

- Multispectral Imaging
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**Match Patient to Therapy**

**Immunomonitoring**
- (Predictive Biomarkers, Host Response)
Intratumoral lymphoid infiltrates, PDL1 expression have both been shown to impact outcomes

Becoming as critical as TNM for staging in cancer

Intraepithelial CD8⁺ TILs and a high CD8⁺/Treg ratio are associated with favorable prognosis in ovarian cancer.

PDL1: To Be or Not To Be

- May be missed in small Bx specimens
- Can vary over time and by anatomical site
- XRT, chemo, kinase inhibitors may alter expression
- Variability in specimen handling & processing
  - Antibodies used for detection have different affinities and specificities
- Membranous vs cytoplasmic
  - Only membranous PDL1 is functionally relevant
- Expressed by multiple cell types within TME, posing challenges for scoring & interpretation

Inflamed melanomas containing CD8+ T cells have highest expression of immune inhibitory pathways

- **IDO** (indoleamine-2,3-dioxygenase)
  - Tryptophan depletion
- **PD-L1**
  - Engages PD-1 on T cells
- **CD4+CD25+FoxP3+Tregs**
  - Extrinsic suppression
- **T cell anergy** (B7-poor)
  - T cell intrinsic TCR signaling defect
Immunoscore: Type, density, and location of immune cells within human colorectal tumors predict clinical outcome


Adapted from Galon et al. Science 2008;313:1960-4
Liquid Biopsy: CyTOF

- Mass cytometry (CyTOF) enables simultaneous analysis of up to 40 PBMC markers in a single staining panel
  - B-cell subset analysis in pts with allergy vs non-allergy showed distinct B cell population in the allergy group, which correlated with IgE levels
  - Demonstrated heterogeneity in B cell malignancies (DLCBCL, HL)
  - Correlated with immune expansion in pts treated with XRT and PD-1 inhibition

Metastatic breast CA pts randomized to chemo + vaccine

- Predefined analyses of immune subtypes (CD4, CD8, NK, Tregs, MDSC) showed no difference in TTP in chemo groups
- Did show statistically different TTP in pts getting vaccine
- ? surrogate biomarker for vaccine/immunotherapy

Baseline characteristics of melanoma pts prior to ipilimumab

- Low absolute monocyte counts, **MDSC frequencies** and high absolute eosinophil counts + CD4/CD25/FoxP3 **Treg frequencies** were significantly associated with better survival


Tumor Profiling

T Cell Priming (TILs, PDL1, microenvironment)

Multivariat Analysis (Immunoscore)

Immune Competence (CyTOF)

Mutation Burden & Neoantigens

Multispectral Imaging (microenvironment)

Tumor IHC, CTC DNA (serology)

Match Patient to Therapy

Immunomonitoring (Predictive Biomarkers, Host Response)
Mutation Burden & Immunogenicity

NSCLC: Mutational load and clinical outcome to antiPD-1 therapy (MSKCC)

Discovery cohort

Validation cohort

# non-synonymous mutations/tumor

Durable clinical benefit N=7 Non-durable clinical benefit N=9

Durable clinical benefit N=9 Non-durable clinical benefit N=9

p = 0.02

p = 0.04

Rizvi et al, Science 2015

Haanen J. Use of MHC multimers to identify tumor reactive T cells. SITC Nov 2014
**Neoantigens:**
Personalizing Immune Tx

- Inconsistencies in mutational burden and response to immunotherapy
  - Mutated genes in individual tumors may be immunogenic
- Whole exome sequencing identifies neoantigen-specific CD8+
- Clinically applicable for TIL or personalized vaccine therapy + checkpoint inhibition

Tools for high-throughput analysis of neo-antigen specific CD8 T cell responses

Toebes et al. Nat. Med. 2006
Bakker et al. PNAS 2008

Hadrup Nat Methods 2009
Kvistborg Science Transl Med 2014

Allows analysis of T cell responses against 100s-1000s of antigens
Figure 2. Mutant neoantigen-specific peptide vaccines induce therapeutic effects comparable to those of checkpoint blockade therapy. Kaplan-Meier survival curves of tumor-bearing mice therapeutically vaccinated with a vaccine comprising poly I:C plus either ALG8 plus LAMA4 SLP, control SLP (HPV peptide), or buffer (A) or therapeutically treated with mAbs to CTLA-4 and/or PD-1 immune checkpoints (B). Adapted with permission from Nature (ref. 77; Figure 1A and Figure 2, D and E).
Analyzing the neo-antigen-specific T cell repertoire in human cancer?

Resected tumor material

Isolate tumor cells

Isolate tumor-infiltrating T cells

Screen with MHC multimer technology

Identify tumor-specific mutations

Predict potential epitopes

Haanen J. Use of MHC multimers to identify tumor reactive T cells. SITC Nov 2014
Multispectral Imaging

- Immune cell phenotypes visualized and quantified simultaneously
- Enables study of spatial distribution and proximity within TME
- Improves understanding of TME immune suppression, TIL harvest potential


Breast CA pts receiving neoadjuvant chemo

- Pts with path CR showed no sig difference in stromal vs intratumoral CD8 (p=.11) or CD4 (p=.75)
- Pts **without pCR** had higher stromal CD8 (p=.03) and CD4 (p=.05) vs intratumoral density
Tumor IHC & Liquid Biopsy

- Converting “cold” to “hot” tumors
- Static vs dynamic sampling
- Detecting immune-resistant clones
- Serum ULBP2 (NK ligand) associated with lower OS (independent predictor of prognosis in Stage I-III melanoma)
- CTCs emerging for baseline & ongoing treatment responses in lung, prostate CA


**Abstract CT306: Radiolabeled anti-PSMA antibody J591 immunotherapy is associated with favorable circulating tumor cell (CTC) count control in men with castration-resistant prostate cancer.” Cancer Research 75.15 Supplement (2015): CT306-CT306.**
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Match Patient to Therapy

Immunomonitoring (Predictive Biomarkers, Host Response)
Challenges of Immune Monitoring

- Is it a “cold” or a “hot” tumor?
  - Should I use single, or combined agent therapy?
- Is my immune therapy working?
  - What should we check? Tissue, or peripheral blood?
  - When should we check, and how often?
- Is the tumor adapting?
  - Can we detect this before tumor grows on imaging?
TCR Sequencing

Adaptive is validating a novel clinical diagnostic to quantify the presence and clonality of tumor infiltrating lymphocytes (TILs) that is a reliable measure of “immunocompetence” to predict and monitor response to immune-modulating cancer therapies.

Preliminary data in variety of tumor types including colorectal cancer, ovarian cancer and glioblastoma suggest that a higher level of clonally expanded TILs may correlate with better patient outcomes. Adaptive is invested in research and development to validate and incorporate into clinical practice this potentially new and meaningful immune molecular diagnostic for better prognostic staging of patients with solid tumors.

The TIL assay is also actively being validated as a potential predictive diagnostic to monitor response to immune-modulating cancer therapeutics. Several human and mouse studies are assessing response to a variety of cancer immunotherapies including anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and anti-programmed cell death-1 (PD-1). Preliminary results suggest that greater TIL clonal expansion and percent tumor infiltration in tumor tissue prior to treatment may be predictive biomarkers of response.

http://www.adaptivebiotech.com/immunoseq
Challenges of Immune Monitoring

Correlation of NY-ESO-1 antibody with clinical course following anti-CTLA-4 treatment with ipilimumab

In collaboration with Jedd Wolchok and Jim Allison MSKCC/Ludwig Center and with Ruth Halaban and Mario Sznol, Yale University - Melanoma sera

Patients with NY-ESO-1 antibodies before CTLA-4 treatment

<table>
<thead>
<tr>
<th>Status at wk 24</th>
<th># patients (%)</th>
<th>NY-ESO-1 SERONEGATIVE # (%)</th>
<th>NY-ESO-1 SEROPOSITIVE # (%)</th>
</tr>
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<tbody>
<tr>
<td>CR</td>
<td>4 (2.9%)</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>PR</td>
<td>14 (10.0%)</td>
<td>10</td>
<td>4</td>
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<tr>
<td>SD</td>
<td>30 (21.4%)</td>
<td>23</td>
<td>7</td>
</tr>
<tr>
<td>Clinical Benefit</td>
<td>48 (34.3%)</td>
<td>36 (30.5%)</td>
<td>12 (54.6%)</td>
</tr>
<tr>
<td>No Clinical Benefit</td>
<td>92 (65.7%)</td>
<td>82 (69.5%)</td>
<td>10 (45.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>140 (100%)</td>
<td>118</td>
<td>22</td>
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</table>

According to Immune-related response criteria:
- **Clinical Benefit**
- CR: Complete Response
- PR: Partial Response
- SD: Stable Disease

Fisher's exact test (two-tailed):
- P value 0.0481
- RR=1.8(1.1-2.9)
Challenges of Immune Monitoring

*ex vivo* Immune Biomarker Monitoring in the Clinic

- The Challenge of Collecting active PBMCs in the Clinic
  - Multi-step processing, variable time intervals & temperature, preservation, transportation
  - Loss of signaling elements (granulocytes, platelets, RBCs)
  - Rejection/Acceptance Criteria?

- Whole Blood Cultures – Standardization and Incorporation into Clinical Trials
  - Novel technology; validation, reproducibility, and suitability for the clinic.
  - Cytokine, Chemokine and gene expression end-points.
  - Monitoring for Safety (Immunotoxicity) and Pharmacodynamics in cancer trials.
Thirteen pts with metastatic BC

Immuno-PET/CT with radiolabeled CEA peptide injected 24-30h before, compared to CT TAP and PET/CT

Immuno-PET sensitivity 93.8% (vs 74.6% and 84.7%)
  
  100% sensitivity for bone, liver, skin, and brain, 94% for LN

Brain lesions only detected by immuno-PET
Anti-CD8 immuno-PET sensitive for changes in systemic and tumor-infiltrating CD8+ in preclinical models using anti–PD-L1 therapy

- Demonstrated tx-induced alterations of a dynamic T-cell population
- May be new opportunity to evaluate antitumor immune responses

An effective immuno-PET imaging method to monitor CD8-dependent responses to immunotherapy.
Cancer research 76.1 (2016): 73-82.
Transcriptomic Signatures of Resistance: IPRES

- IPRES (innate PD-1 resistance)
- Concurrent up-expression of genes involved in mesenchymal transition, cell adhesion, ECM remodeling, angiogenesis, & wound healing
- Identification of IPRES at time of progression may help guide next treatment selection
- IPRES signatures can be induced by MAPKi, which could further account for subsequent poor response to I/O therapy

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Applying the Science

Can these questions be answered by RCTs?

- T Cell Priming
- Immune Competent
- Clonal Selection & Persistence
- Evasion
- Immune Suppressive
- Clonal Selection & Persistence
RCTs: “Ideal” Biomarkers

- Feasible, reproducible
- Validated in prospective studies
- Minimal amount of clinical material (*inexpensive*)
- No *in vitro* manipulation (*rapid*)
- Quantitative, sensitive to a broad range of responses
- Able to be used with preserved/archived material (*convenient*)
- Relates to MOA (*interpretable*)

* IHC is currently only FDA-approved companion diagnostic
* Validation is needed; limitations described earlier
  * Performance, correlates, fresh vs archival tissue
* Validated CLIA test could be used to stratify patients for clinical trials
* Consensus remains: not a guide for treatment selection
Immunomonitoring of pts should be considered an essential component of prospective clinical trials

- Elucidate mechanism(s) of anti-tumor response
- Monitor disease progression
- Evaluate therapeutic effect
- Identify novel targets for immunoTx
- Predictive, prognostic markers for outcome
Regulatory Considerations: Data Sharing

- Multiple forms of data acquisition described here
- Danger of silo formation
- Expensive, dense data sets
- Integration becomes complex
- Fragmented data analysis may undermine “score”
Chasing The Horizon

The known is finite, the unknown infinite; intellectually we stand on an islet in the midst of an illimitable ocean of inexplicability.

Our business in every generation is to reclaim a little more land, to add something to the extent and the solidity of our possessions.

*Thomas Henry Huxley*
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Thank you to SITC and meeting organizers for the opportunity to collaborate & share knowledge!