Manipulation of the Tumor Microenvironment by CTLA-4 Blockade

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CTLA-4 blockade enhances tumor-specific immune responses

Attenuated or Terminated Proliferation

Unrestrained Proliferation

Tumor

APC

CTLA-4 blockade enhances tumor-specific immune responses

JP Allison
• CTLA-4 blockade has a consistent anti-tumor response rate of ~10%

• Partial and complete regression of disease observed

• All studies to date (> 4000 patients) conducted in metastatic disease setting (limited access to tumor tissues)

• Identification of biomarker to predict disease outcome or select appropriate patients for therapy is necessary
Critical Questions for Further Clinical Development of anti-CTLA-4

• What are the cellular and molecular mechanisms involved in the anti-tumor effect?

• What distinguishes responders from non-responders?

• What are the best conventional therapies or vaccines to be used combinatorially?
Immune Monitoring

• Cannot rely on solely monitoring of peripheral blood

• Need to identify immunological events that occur in tumor tissues after therapy

• Need to correlate changes in tumor tissues with those that occur in systemic circulation

• Identified markers can then be used for future immune monitoring
Pre-surgical clinical trial: 
Analysis of blood and tumor tissues

<table>
<thead>
<tr>
<th>Study Week</th>
<th>anti-CTLA-4 antibody</th>
<th>Surgery</th>
<th>Post-operative follow-up visits</th>
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Pre-therapy blood*  
After dose #1 blood*  
After dose #2 blood*  
Blood  
Blood

*Blood drawn prior to antibody dose administered and prior to surgery
Tissue Analysis

Core biopsy (~ 1 cm x 1mm x 1mm)

RNA later

Fine needle aspiration and chunks

Core biopsy

TIL expansion

Histology sections

IF in frozen sections

IHC in paraffin sections
BLADDER

ICOS expression is higher in tumor tissues from anti-CTLA-4 treated patients

Liakou et al., *Proc Natl Acad Sci*, 2008
BLADDER

FOXP3 expression is lower in tumor tissues from anti-CTLA-4 treated patients

Non-malignant tissues: untreated

Tumor tissues: untreated

Tumor tissues: anti-CTLA-4 treated

Liakou et al., *Proc Natl Acad Sci*, 2008
PROSTATE
ICOS and FOXP3 expression

Untreated:
Non-malignant prostate

CD4
ICOS
7%

CD4
FOXP3
5%

Untreated:
Prostate cancer

CD4
ICOS
9%

CD4
FOXP3
48%

Anti-CTLA-4 treated:
Prostate cancer

CD4
ICOS
31%

CD4
FOXP3
5%

Chen et al., Proc Natl Acad Sci, 2009
ICOS: Marker of Treg or Teff? Important for Th2 or Th1 immune responses?

• Diverse function of ICOS
  – Marker of follicular helper T cells and plays a role in T:B cell interactions
  – ICOS\(^{-/-}\) mice have decreased IL-10 production and defect in antibody class switching (Dong et al., 2001)
  – IL-10 producing Tregs are induced by pDCs expressing ICOS-ligand (Ito et al., 2007)
  – ICOS co-stimulation is necessary for IFN-\(\gamma\) production and containment of viral infection (Humphreys et al., 2006)
  – ICOS\(^{hi}\), ICOS\(^{med}\), and ICOS\(^{low}\) cells have different cytokine profiles (Lohning et al., 2003)
  – ICOS may promote survival of activated T cells, including Tregs and Teff (Burmeister et al., 2008)

• Impact of ICOS expression on T cell function appears to be dependent on T cell subset and possibly interaction with ICOS-ligand on APCs
Are ICOS-expressing T cells effector cells in the setting of anti-CTLA-4 therapy?
Expression of NY-ESO-1 tumor antigen allowed for functional analyses of TILs

Chen et al., Proc Natl Acad Sci, 2009
Recognition of NY-ESO-1 by TILs

![Bar graph showing IFN-γ production](image)

Chen et al., Proc Natl Acad Sci, 2009
Increased IFN-γ and T-bet mRNA in treated tissues with concomitant decrease in FOXP3 mRNA levels

Liakou et al., *Proc Natl Acad Sci*, 2008
What about immunologic events in the systemic circulation?

Do they correlate with observed changes in tumor tissues?
ICOS expression significantly increases on CD4 T cells in peripheral blood after treatment with anti-CTLA-4 antibody.

Liakou et al., *Proc Natl Acad Sci*, 2008
ICOS$^{hi}$ T cells in peripheral blood from anti-CTLA-4 treated patients produce IFN-$\gamma$

Liakou et al., *Proc Natl Acad Sci*, 2008
ICOS$^{hi}$ T cells from peripheral blood recognize NY-ESO-1 tumor antigen

**Results:**

The figure shows the recognition of NY-ESO-1 by CD4$^+$ ICOS$^{hi}$ T cells from peripheral blood. The graph compares the number of spots per 50,000 T cells between pre- and post-therapy samples for different patients.

- **Pre-therapy:**
  - Pt #2: 40 spots
  - Pt #4: 200 spots
  - Pt #6: 50 spots

- **Post-therapy:**
  - Pt #2: 200 spots
  - Pt #4: 500 spots
  - Pt #6: 300 spots

**Notes:**

- The graph includes controls for APCs pulsed without peptide (no peptide) and APCs pulsed with NY-ESO-1 peptides (all peptides).
- The data shows a significant increase in the number of spots post-therapy for all patients.

**Conclusion:**

The study indicates that ICOS$^{hi}$ T cells can recognize NY-ESO-1 antigen, suggesting potential therapeutic implications for patients with NY-ESO-1 positive tumors.
Next Steps

• Larger cohorts of patients to correlate ICOS-expression and clinical outcomes
  
  Phase III clinical trial in prostate cancer patients

• Combination Strategies: clinical trials with anti-CTLA-4 therapy plus other agents that prime T cell responses

• Murine Studies: 1) determine the role of ICOS-expressing T cells in anti-tumor responses and; 2) identify rational combinations for future clinical trials
Phase III clinical trial with Ipilimumab + XRT vs. Placebo + XRT in CRPC

800 patients
Bone Mets

Randomize

XRT + Placebo
Overall Survival
Immune Monitoring

XRT + anti-CTLA-4 antibody (administered within 2 days of XRT)

No crossovers allowed

Estimate participation of 145 sites
• Sharma Lab Team
  – Derek Ng Tang
  – Hong Chen
  – Chrysoula Liakou
  – Jingjing Sun
  – Tihui Fu
  – Qiuming He
• GU Medical Oncology, Urology, Pathology
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• Bioinformatics and Statistical Collaborators
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• MSKCC and LICR Collaborators
  – Lloyd Old, Achim Jungbluth, Sacha Gnijatic (LICR)
  – Jim Allison, Jedd Wolchok, Jianda Yuan (LCCI, MSKCC)
• BMS Team
  – Rachel Humphrey, Axel Hoos, Ramy Ibrahim