Immune Checkpoint Blockade in Cancer Therapy: New Insights and Opportunities

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Inventor of intellectual property held by the University of California, Berkeley, licensed to Bristol Meyers-Squibb and Pfizer

Consultant for Bristol Meyers-Squibb
Dynamic Integration of TCR and Costimulatory Signals

- **No Proliferation**
  - Anergy?

- **Activation, Initiation**
  - IL-2
  - p27kip
  - pRb
  - Cdk6
  - Cdk4
  - Cyclin D1
  - Cyclin D2
  - Bcl-xL,γ
  - S phase

- **Inhibition**
  - IL-2
  - pRb
  - Cdk6
  - Cdk4
  - Cyclin D1
  - Cyclin D2
  - Bcl-xL,γ

- **Restricted Proliferation**

**Antigen Presenting Cell**

- TCR
- CD28
- CTLA-4
- Peptide/MHC
- B7-1,2

**Gross, Harding, Krummel, Chambers, Brunner, Egen, Kuhns**
Localization of CD28 and CTLA-4 to the T Cell-APC Interface

CD28

CTLA-4

~ 5 minutes
CTLA-4 Blockade Enhances Tumor-Specific Immune Responses

Attenuated or Terminated Proliferation

Unrestrained Proliferation

Tumor

Necrotic Death
Vaccines
Chemotherapy
Irradiation
Hormone therapy
Anti-angiogenesis
Antibodies
“Targeted” Therapies

CTLA-4
Peptide/MHC
B7-1,2

TCR

CD28
Anti-CTLA-4 Induces Regression of Transplantable Colon Carcinoma

Average Tumor Size (mm²)

Days After Tumor Injection

Anti-CD28
No Rx
Anti-CTLA-4

Leach
Anti-CTLA-4 and GM-CSF Tumor Cell Vaccine Synergize to Eradicate Established B16 Melanoma

van Elsas, Hurwitz
αCTLA4/Gvax Induces Activation of the Tumor Vasculature and T Cell Infiltration

Quezada
αCTLA4/Gvax Induces Activation of the Tumor Vasculature and T Cell Infiltration

Quezada
aCTLA-4/GVax Increases Number Of Tumor Infiltrating T Cells

No treatment

Quezada
aCTLA-4/GVax Increases Number Of Tumor Infiltrating T Cells

No treatment

CTLA-4/GVax

Quezada
\( \alpha \text{CTLA-4/GVax Increases Teff/Treg Ratio} \) in Tumor

CD4/Foxp3

CD8/Foxp3

Quezada
Effects of anti-CTLA-4 on Gene Expression in TRAMP C2 Tumors

The graph shows the relative change in gene expression for various genes in response to the treatment conditions. The x-axis represents the gene names (CD3, CD4, CD8, IFNγ, Perforin, FoxP3, IL-10, TGFβ, IL-17, IL-6, HPRT), and the y-axis represents the relative change. The graph compares the expression levels under the No Treatment condition (red bars) and the Combo condition (purple bars).

Key genes indicated in the graph are CD3, CD4, CD8, IFNγ, Perforin, FoxP3, IL-10, TGFβ, IL-17, IL-6, and HPRT.
Mechanisms of anti-tumor effects of CTLA-4 blockade in mouse models

- Activation of vasculature in tumor
- Extravasation/proliferation of T cells
- Increase in Teff/Treg ratio, especially CD8 T cells
- Increase in ratio of IFN\(\gamma\)/IL-10
Who is being targeted by CTLA-4 blockade—

$T_{\text{reg}}$ or $T_{\text{eff}}$?
Chimeric Murine CTLA-4 (Human exon 2) Transgene

Regulated normally in transgenic mice:
- Expressed only after activation, not in naïve T cells

Rescues phenotype in CTLA-4 -/- mice:
- No polyclonal T cell activation
- Normal life span
Uni-compartmental CTLA-4 blockade:

Wild type CTLA-4:

Human tg CTLA-4:

Peggs & Quezada
Uni-compartmental CTLA-4 blockade during anti-tumor responses

B16 melanoma $d_0$

RAG$^{-/}$ reconstituted lymphocyte chimaeras

αCTLA-4 mAb 100μg

Tumor growth and survival

GVax

Peggs & Quezada
Blockade of CTLA-4 on both compartments is necessary for optimal anti-tumor activity

Peggs & Quezada
Dissecting the CD4+ T cell response

Class II restricted, Trp1-TCR Transgene in TRP1/RAG Double Knockout (Restifo NCI)

WT B6  aCTLA-4 mAb 100ug  Conversion

Foxp3- cells

1 2 3

GVax

CD4 expansion Treg expansion Cytokine profiles
TRP1-specific CD4 T cells acquire Foxp3 in response to self antigen \textit{in vivo}

NO TUMOR or VAX CHALLENGE

Day 4 after transfer

Day 7 after transfer

Quezada
CTLA-4 blockade Blocks Conversion/Expansion and Alters Function of Tumor/Self Reactive CD4 cells

Day 0
200K B16BL6

+/- Gvax/aCTLA4

Day 3

+/- Foxp3- Trp1 specific CD4+ cells

Quezada
CTLA-4-blockade Decreases Frequency of Foxp3+ T cells (day 7)

Lymph node:

untreated | Gvax | Gvax+aCTLA4
---|---|---
18% | 9% | 3%

Vaccination site:

Gvax | Gvax+aCTLA4
---|---
26% | 3%

CD4
CTLA-4 Blockade prevents in vivo T cell conversion and reduces the frequency of endogenous Treg at the vaccination site (day7)

Lymph Nodes

Vaccine site

Conversion to Fox

Endogenous Fox

Quezada
αCTLA-4 Increases IFN\(\gamma\) and Reduces IL10 Production by CD4+ TRP1 cells

Lymph node:

Vaccination site:

Stim: B16-pulsed APC

Quezada
Can CTLA-4 Blockade increase in the frequency of tumor reactive CD4 T cells result in rejection of large fully vascularized tumors?
Model to Study the Function of Tumor-reactive CD4+ T cells

Trp1-Tan TCR Tg

Generates CD4+ T cells specific for a peptide derived from TRP1 (gp75) and presented on MHC Class II (I-A^b)

1. Track CD4 responses to tumors (effectors and regulators)
2. Test the ability of tumor reactive CD4+ T cells to boost anti-tumor immunity

Muranski P, Boni A, Antony PA, et al
Blood. 2008 Jul 15;112(2):362-73
CD4-ACT protocol
in vivo priming of a small number of tumor/self reactive T cells

- Host conditioning by radiotherapy
- In vivo priming/differentiation of a small number of tumor/self reactive T cells
- Elimination of inhibitory signals through CTLA4

Quezada & Simpson
Rejection of Large, Established Tumors after CD4-ACT and CTLA4-blockade

Quezada & Simpson
Rejection of Large, Established Tumors after CD4-ACT and CTLA4-blockade

Quezada & Simpson
Rejection of Large, Established Tumors after CD4-ACT and CTLA4-blockade

Quezada & Simpson
CTLA-4-blockade Mediates Maximal trp1 Expansion while Decreasing Foxp3 Accumulation in the Blood

Trp1 expansion

Trp1+Foxp3+ Treg expansion

All mice receive 5G total body radiation therapy

Quezada & Simpson
IFNγ transfer and CTLA4-blockade induce high levels of IFNg and TNFα in the serum of treated mice

- Also detected and same trend: MCP-1, IL6
- Not detected or not consistent: IL2, IL4 and IL10

Quezada & Simpson
RT and CTLA-4-blockade increase the number of tumor reactive CD4^+Trp1^+ T cells while preventing Treg accumulation in the tumor

TILs

<table>
<thead>
<tr>
<th>RT</th>
<th>Trp1</th>
<th>αCTLA4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

CD4^+Trp1^+ Teff

CD4^+Trp1^+ Foxp3^+

Quezada & Simpson
RT induces in vivo differentiation of CD4^+Trp1^+ T cells into multi-cytokine producer effector cells

Quezada & Simpson
Tumor rejection is IFN$\gamma$ dependent, TNF$\alpha$ independent

Quezada & Simpson
Tumor rejection is independent of endogenous B, T and NK cells

- Tumor rejection is independent of endogenous B and T cells
- Also independent of endogenous Perforin: CD8+CTL and NK independent

Quezada & Simpson
Although rejection is tumor specific, the inflammatory milieu could be driving bystander rejection of a non-related tumor injected in the same site.
CD4Trp1 cells specifically reject B16-Luc in tumor mix

EL4 and B16-Luciferase mixed and co-injected on same flank

Quezada & Simpson
Tumor rejection by ATC of trp1-specific CD4 cells is:

Independent of endogenous T, B, NK IFN$\gamma$ dependent and acting directly on tumor

Is rejection DIRECTLY mediated by CD4 T cells?
CD4^+Trp1^+ T cells differentiate into GzmB^+CD4^+ cells in vivo and accumulate in tumors

CD4^+Trp1^+ produce GrzmB...so they COULD kill

Quezada & Simpson
CD4⁺Trp1⁺ T cells develop cytotoxic activity in vivo

In vivo cytotoxicity assay: B cell targets

Quezada & Simpson
CD4\(^+\)Trp1\(^+\) in vitro cytotoxic activity is class II and granzyme B dependent
Can CD4+ Trp1+ CTLs directly mediate tumor rejection in vivo?
Class II-dependent killing would require class II expression on tumors

- Melanoma up-regulates CII in vivo when in presence of activated CD4+Trp1+
- IFNγ neutralization ablates CII expression as well as tumor rejection.
- Clear correlation between Trp1, IFNγ, and tumor rejection

Quezada & Simpson
Is it direct MHC II dependent recognition and rejection?
Can CD4\(^+\) Trp1\(^+\) CTLs directly mediate tumor rejection?

(Can’t use CII KO mouse since it is required for CD4 priming)

——— In vivo prime ———

| Day 10: 5 Gy | +aCTLA4 | In vitro expansion DCs+Trp1 peptide |
| WT B6 | 50K TRP1 CD4 TCR Tg |

——— Effector phase ———

| CIIKO B6 |
| Only CII is on tumors |

Rejection?

Quezada & Simpson
Can CD4+ Trp1+ CTLs directly mediate tumor rejection?

(Can’t use CII KO mouse since it is required for CD4 priming)

- Rejection of tumors on CII KO
- Rejection is blocked by anti CII mAbs

Hence tumor rejection is mediated by Trp-1 specific CD4 CTLs directly interacting with tumors via MHC II

Quezada & Simpson
Effective Combinations using anti-CTLA-4 Against Poorly Immunogenic Tumors

**Immunotherapies**
- Gvax: B16 melanoma, TRAMP CaP
- Peptide-pulsed (mugp100) DCs: B16 melanoma
- DNA Vaccine (huTRP2): B16 Melanoma
- Prior depletion of CD25+ cells + vaccine: B16 melanoma
- Adoptive T cell Transfer: B16 melanoma

**Conventional therapies**
- Chemotherapy (cisplatin): Mammary carcinoma
- Local Irradiation: Mammary carcinoma
- Androgen deprivation: TRAMP CaP
- Surgical reduction: TRAMP CaP
- Cryoablation: TRAMP CaP

*Most Anything that kills tumor cells or primes T cells*
Chimeric Murine CTLA-4 (Human exon 2) Transgene

Regulated normally in transgenic mice:
- Expressed only after activation, not in naïve T cells

Rescues phenotype in CTLA-4 -/- mice:
- No polyclonal T cell activation
- Normal life span

17 Kb mouse genomic fragment with human exon 2 (ectodomain)

Chambers & Korman
Effect of Anti-CTLA-4 on MC38 Tumor Growth In Mice expressing Human CTLA-4

Mean Tumor Volume [mm³]

Day

- Anti-Mouse CTLA-4 (9H10)
- Anti-Human CTLA-4 (10D1)
- Anti-Human CTLA-4 (147)
Ipilimumab (MDX-010)

- Fully human IgG1 monoclonal antibody to human CTLA-4 created by Medarex
- Blocks binding of CTLA-4 to CD80 and CD86
- Augments immune responses in primate models
- Co-developed by Medarex and Bristol-Myers Squibb in multiple cancer indications
Ipilimumab:
Summary of Clinical Experience
(Medarex/BMS)

>4,000 patients treated to date

Objective clinical responses in:
  • Melanoma
  • Renal
  • Prostate
  • Ovarian
Ipilimumab: Summary of Clinical Experience

• ~15% (RECIST, mWHO) in melanoma as monotherapy, some are complete responses

• Survival benefit in ~35-40% of patients

• Responses are durable:
  Months to years without retreatment, although maintenance treatment is possible
Ipilimumab:  
Summary of Clinical Experience

Adverse Events:

• Serious, but manageable with frequent (monthly) administration:
  • Colitis, rashes, hypophysitis, hepatitis
  • Resolve with symptomatic treatment and cessation of therapy

• Minor with widely spaced treatment (single or separated by 2 mos or more):
  • Mild skin rashes
Baseline 11/28/06

Wolchok (MSKCC)
Tumorous nodule with melanin pigment (macrophages and lymphocytes; no melanocytes)

Macrophages and lymphocytes are present, but no tumor cells

Wolchok (MSKCC)
CD8-positive T-cells

CD4-positive T-cells (macrophages are also weakly pos for CD4)

Wolchok (MSKCC)
Ipilimumab Pattern of Response
Response after Initial Increase in Total Tumor Burden

Patient treated with 10 mg/kg ipilimumab in study CA184-008

Source: 2008 ASCO Abstract #3008 Hodi.
Ipilimumab Pattern of Response
Response after Initial Increase in Total Tumor Burden

Patient treated with 10 mg/kg ipilimumab in study CA184-008

Source: 2008 ASCO Abstract #3008 Hodi.

Hodi (DFCI)
Ipilimumab Pattern of Response
Response after Initial Increase in Total Tumor Burden

Patient treated with 10 mg/kg ipilimumab in study CA184-008

Screening

Week 12: Initial increase in total tumor burden (mWHO PD)

Week 14: Responding

Source: 2008 ASCO Abstract #3008 Hodi.

Hodi (DFCI)
Ipilimumab Pattern of Response
Response after Initial Increase in Total Tumor Burden

Patient treated with 10 mg/kg ipilimumab in study CA184-008

Screening

Week 12: Initial increase in total tumor burden (mWHO PD)

Week 14: Responding

Week 72: Response

Follow-up ongoing

Source: 2008 ASCO Abstract #3008 Hodi.

Hodi (DFCI)
Ipilimumab Pattern of Response: Responses After the Appearance and Subsequent Disappearance of New Lesions

Pre-treatment

Week 36: Still Regressing

Week 12: Progression

10 mg/kg ipilimumab Q3W X 4

New lesions

Week 20: Regression

Week 36: Still Regressing

Source: 2008 ASCO Abstract #3020 Wolchok

Wolchok (MSKCC)

May, 2007
## Ipilimumab 1 to 4-Year Survival Rate Benchmark Data

<table>
<thead>
<tr>
<th>Study</th>
<th>Disease Setting</th>
<th>1 year</th>
<th>2 years</th>
<th>3 years</th>
<th>4 years</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDX010-08</strong> (3 mg/kg + DTIC) (n=35)</td>
<td>1st line only</td>
<td>~55%</td>
<td>~30%</td>
<td>~25%</td>
<td>10-15%</td>
</tr>
<tr>
<td><strong>MDX010-15</strong> (10 mg/kg) (n=24)</td>
<td>1st and 2nd line pooled</td>
<td>~60%</td>
<td>~35%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>CA184-008</strong> (10 mg/kg) (n=155)</td>
<td>2nd line only</td>
<td>46.7%</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>CA184-022</strong> (10 mg/kg) (n=70)</td>
<td>2nd line only</td>
<td>53.4%</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>CA184-007</strong> (10 mg/kg) (n=57)</td>
<td>1st and 2nd line pooled</td>
<td>59.1%</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Historical DTIC</strong> (Genasense study) (n=305)</td>
<td>1st line only</td>
<td>25-30%</td>
<td>10-15%</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Source for Ipilimumab Data: 2008 ASCO Abstracts #3018 Urba, #9022 Hersh, #9021 O’Day, #9025 Hamid, #9010 Weber and #9055 Thompson.
# Updated Survival Outcomes at 10mg/kg Relative to Historical Data (no brain metastases)

<table>
<thead>
<tr>
<th>Dose</th>
<th>CA184022 (N=217)</th>
<th>CA184008 (N=155)</th>
<th>CA184007 (N=115)</th>
<th>Meta-Analysis (N=705)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival rate at 1 year (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreated 10 mg/kg</td>
<td>48.64 (36.84, 60.36)</td>
<td>47.22 (39.52, 55.11)</td>
<td>50.82 (31.50, 71.11)</td>
<td>71.35 (55.24, 87.19)</td>
</tr>
<tr>
<td>Placebo 10 mg/kg</td>
<td>50.82 (31.50, 71.11)</td>
<td>49.85 (33.33, 66.55)</td>
<td>65.93 (45.02, 85.71)</td>
<td></td>
</tr>
<tr>
<td>Budesonide 10 mg/kg</td>
<td>47.22 (39.52, 55.11)</td>
<td>49.85 (33.33, 66.55)</td>
<td>65.93 (45.02, 85.71)</td>
<td></td>
</tr>
<tr>
<td>Survival rate at 2 years (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreated 10 mg/kg</td>
<td>29.81 (19.13, 41.14)</td>
<td>32.83 (25.37, 40.49)</td>
<td>24.20 (8.00, 42.78)</td>
<td>56.62 (38.35, 74.30)</td>
</tr>
<tr>
<td>Placebo 10 mg/kg</td>
<td>24.20 (8.00, 42.78)</td>
<td>31.58 (16.47, 47.57)</td>
<td>56.51 (30.61, 80.95)</td>
<td></td>
</tr>
<tr>
<td>Budesonide 10 mg/kg</td>
<td>29.81 (19.13, 41.14)</td>
<td>31.58 (16.47, 47.57)</td>
<td>56.51 (30.61, 80.95)</td>
<td></td>
</tr>
<tr>
<td>Overall survival (months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>11.43 (6.90, 16.10)</td>
<td>10.22 (7.59, 16.30)</td>
<td>14.78 (6.64, 20.53)</td>
<td>30.46 (13.96, ---)</td>
</tr>
<tr>
<td>95%CI</td>
<td>11.43 (6.90, 16.10)</td>
<td>10.22 (7.59, 16.30)</td>
<td>14.78 (6.64, 20.53)</td>
<td>30.46 (13.96, ---)</td>
</tr>
</tbody>
</table>

* Korn et al., *J. Clin. Oncol.* 2008; subset of patients without brain metastases from 42 Phase 2 trials
Beyond RECIST

• ~10% have disease control after progression
  – Correlates with survival comparable to objective responses (CR + PR) by RECIST

• ~40% disease control (CR + PR + SD)
  – 2 year survival +
Ovarian GVAX and Anti-CTLA-4 Ab

VAX

Hodi and Dranoff, DFCI
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?
CANCER-TESTIS (CT) ANTIGENS

- ~100 known family members
- Expressed during germ cell development in immune privileged sites, but not in other normal tissues
- Expressed in many cancer types
- Spontaneous immune responses against these antigens can be detected in some cancer patients
## CT ANTIGENS

<table>
<thead>
<tr>
<th>CT&lt;sup&gt;a&lt;/sup&gt;</th>
<th>System</th>
<th># Genes</th>
<th>Chromosome Location</th>
<th>Detection System&lt;sup&gt;b&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>MAGE</td>
<td>16</td>
<td>Xq28/Xp21</td>
<td>T, Ab</td>
</tr>
<tr>
<td>2</td>
<td>BAGE</td>
<td>2</td>
<td>Unknown</td>
<td>T</td>
</tr>
<tr>
<td>3</td>
<td>GAGE</td>
<td>9</td>
<td>Xp11</td>
<td>T</td>
</tr>
<tr>
<td>4</td>
<td>SSX</td>
<td>&gt;5</td>
<td>Xp11</td>
<td>Ab</td>
</tr>
<tr>
<td>5</td>
<td>NY-ESO-1</td>
<td>2</td>
<td>Xq28</td>
<td>Ab, T, RDA</td>
</tr>
<tr>
<td></td>
<td>LAGE-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>SCP-1</td>
<td>3</td>
<td>1p12-p13</td>
<td>Ab</td>
</tr>
<tr>
<td>7</td>
<td>CT7 / MAGE-C1</td>
<td>1</td>
<td>Xq26</td>
<td>Ab, RDA</td>
</tr>
<tr>
<td>8</td>
<td>CT8</td>
<td>1</td>
<td>Unknown</td>
<td>Ab</td>
</tr>
<tr>
<td>9</td>
<td>CT9</td>
<td>1</td>
<td>1p</td>
<td>Ab</td>
</tr>
<tr>
<td>10</td>
<td>CT10 / MAGE-C2</td>
<td>1</td>
<td>Xq27</td>
<td>RDA, Ab</td>
</tr>
<tr>
<td>11</td>
<td>CTp11</td>
<td>1</td>
<td>Xq26 -Xq27</td>
<td>_&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>SAGE</td>
<td>1</td>
<td>Xq28</td>
<td>RDA</td>
</tr>
<tr>
<td>13</td>
<td>cTAGE-1</td>
<td>1</td>
<td>18p11</td>
<td>Ab</td>
</tr>
<tr>
<td>14</td>
<td>OY-TES-1</td>
<td>2</td>
<td>12p12-p13</td>
<td>Ab</td>
</tr>
</tbody>
</table>
cDNA = 747 bp; coding region = 543 bp
RT-PCR product is 332 bp; Northern blot product is 0.8 kb
Protein is 180 aa
Maps to Xq28
Function unknown
Strongest spontaneous immunogenicity of any CT antigen
Measuring antibody titers by ELISA: Example for NY-ESO-1

- Positive control for NY-ESO-1 (NW29)
- Negative control pool of 5 healthy donor sera
- Test serum from lung cancer patient LU-113

Low volume plates for minimal material usage. Automated washes and reading for high-throughput analyses.
NY-ESO-1 Antibody May Correlate with Clinical Responses in Stage 4 metastatic melanoma patients

[Graph showing clinical responders and non-responders with antibody titers over time.]

Gnjatic & Wolchok
## Correlation of NY-ESO-1 antibody with clinical course following anti-CTLA-4 treatment

Patients with NY-ESO-1 antibodies at any time point during study

<table>
<thead>
<tr>
<th>Response</th>
<th># patients Status at wk24 (%)</th>
<th># NY-ESO-1 SERONEGATIVE Status wk24 (%)</th>
<th># NY-ESO-1 SEROPOSITIVE Status wk24 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>6 (5.1%)</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>PR</td>
<td>14 (12.0%)</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>SD</td>
<td>25 (21.4%)</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>Clinical Benefit</td>
<td>45 (38.5%)</td>
<td>32 (33.7%)</td>
<td>13 (59.1%)</td>
</tr>
<tr>
<td>No Clinical Benefit</td>
<td>72 (61.5%)</td>
<td>63 (66.3%)</td>
<td>9 (40.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>117 (100%)</td>
<td>95</td>
<td>22</td>
</tr>
</tbody>
</table>

According to Immune-related response criteria:
- CR: Complete Response
- PR: Partial Response
- SD: Stable Disease
- POD: Progression of Disease (includes MR: mixed response)
- DOD: Dead of Disease

Fisher's exact test:
- P value 0.0498

Gnjatic & Wolchok, Ludwig Center/MSKCC
Halaban and Sznol, Yale
Correlation of NY-ESO-1 antibody with clinical course following anti-CTLA-4 treatment

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

Patients with NY-ESO-1 antibodies at any time point during study

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<thead>
<tr>
<th>Response</th>
<th># patients Status at wk24 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>6 (5.1%)</td>
</tr>
<tr>
<td>PR</td>
<td>14 (12.0%)</td>
</tr>
<tr>
<td>SD</td>
<td>25 (21.4%)</td>
</tr>
<tr>
<td><strong>Clinical Benefit</strong></td>
<td><strong>45 (38.5%)</strong></td>
</tr>
<tr>
<td><strong>No Clinical Benefit</strong></td>
<td><strong>72 (61.5%)</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>117 (100%)</strong></td>
</tr>
</tbody>
</table>

According to Immune-related response criteria:

CR: Complete Response
PR: Partial Response
SD: Stable Disease
POD: Progression of Disease (includes MR: mixed response)
DOD: Dead of Disease
Polyfunctional NY-ESO-1 Specific T cells in Blood Of Melanoma Patients Treated with aCTLA-4

Yuan, Gnjatic, Wolchok
Monitoring NY-ESO-1 immunity during CTLA-4 blockade: Summary

Patients with NY-ESO-1 serum antibodies are almost twice as likely to experience clinical benefit following CTLA-4 blockade.

Frequent increase in antibody titers to NY-ESO-1 during CTLA-4 blockade in patients with preexisting immunity.

Frequent seroconversions to NY-ESO-1 observed during CTLA-4 blockade in baseline seronegative patients.

Presence of NY-ESO-1 serum antibodies is a favorable predictor of clinical outcome to anti-CTLA-4 immunotherapy.

NY-ESO-1 immunity is not sufficient to predict favorable outcome: Other predictive markers must exist.

Efforts to identify more predictive biomarkers that correlate with clinical benefit: Grand serology, Seromics, etc...
Impact of anti-CTLA-4 on Castrate-resistant Metastatic Prostate Cancer
PSA Responses after Anti-CTLA-4 Treatment in Prostate Cancer: Correlation with Anti-NY-ESO-1 Antibodies?

NY-ESO-1 seropositive patient. Expression status of tumor unknown.

* NY-ESO-1 is expressed in ~ 30% of prostate cancers.

Yuan, Gnjatic, Wolchok, Slovin, Scher
NY-EOS-1 Antibody Correlates with Biochemical Responses In Hormone Resistant Metastatic Prostate Cancer

Yuan, Gnjatic, Wolchok
NY-ESO-1 Reactive CD8+ T cells in NY-ESO-1 Seropositive Prostate Cancer (Patient 10) Increase After Irradiation and CTLA-4 blockade

Yuan, Gnjatic, Wolchok
Methodology for Seromic Analysis with Protein Arrays

Array featuring multiple proteins

Incubate with patient serum (1:500)

Reveal antigen-specific serum antibodies with labeled anti-human IgG

Contain >8000 proteins mostly full-length baculovirus-produced GST-fusion proteins randomly selected, both known and predicted sequences
Serological identification of targets in CTLA-4 immunotherapy by Seromics

Sera from 19 patients treated with anti-CTLA-4 were analyzed.

In 11 patients, sera were compared for 2 time points: before and after (week 12 or later) treatment with ipilimumab.

Measure serum antibody response to 8000 antigens by Seromics: Includes normalization procedure and validation.

Identify antigens with increased or reduced immunogenicity following anti-CTLA-4 treatment.

Identify antigens recognized by patient sera before anti-CTLA-4 treatment that can predict better or worse outcome.
Example: Decrease in Ab against self-antigen Ro-52 in patient #18 from prestudy to week 35 of Ipimilumab treatment
Seromics-defined antigens showing frequent significant (>5x) changes in reactivity following CTLA-4 blockade in melanoma patients.
Overview of Results with Seromics

- Profiling serum autoantibodies may be important to define antigens that can be used as predictive biomarkers of clinical response.
- Find antibody responses to limited number of antigens, a majority of them being patient-specific events: limited overlap in antigen specificity.
- Find proteins with increased or decrease immunogenicity following CTLA-4 blockade: candidates targets for tumor immunity or autoimmunity.
- Find candidates immunogenic before treatment that could predict worse or better outcome.
Neoadjuvant anti-CTLA-4 trial in Bladder Cancer

Week - 4 to -1

Ipilimumab Dose

Blood & Tumor

Blood

Blood

Blood & Tumor (if feasible)

Blood & Tumor

Surgery

1

4

7

8

Padmanee Sharma, MDACC
Tissue Analysis

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

Histology sections

IF in frozen sections

IHC in paraffin sections

RNA later

Core biopsy

TIL expansion

Fine needle aspiration and chunks
Bladder:
ICOS expression is higher in tumor tissues from anti-CTLA-4 treated patients

Non-malignant tissues: untreated

Tumor tissues: untreated

Tumor tissues: anti-CTLA-4

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

Sharma, MDACC
Blood:
\( \text{ICOS}^{\text{high}} \text{ CD4 T cells in Blood of Bladder Cancer Patients after anti-CTLA-4 Treatment} \)

Pre-therapy

Post-therapy week 3

Post-therapy week 7

Sharma, MDACC
Extended B7-CD28 Family

- PD-L1
- PD-L2
- B7x
- B7-H3
- B7-1
- B7-2
- B7h
- ICOS
- CD28, CTLA-4
ICOS: Marker of Treg or Teff? Important for Th2 or Th1 immune responses?

• Diverse function of ICOS
  – ICOS⁻/⁻ mice have decreased IL-10 production and defect in antibody class switching (Dong et al., 2001)
  – Marker of follicular Th cells
  – IL-10 producing Tregs are induced by pDCs expressing ICOS-ligand (Ito et al., 2007)
  – ICOS co-stimulation is necessary for IFN-g 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)

• Role of ICOS on T cells seems dependent on other factors, including T cell subset, APCs, and costimulatory molecules
ICOS\textsuperscript{hi} T cells from peripheral blood recognize NY-ESO-1 tumor antigen and produce IFN\textsubscript{\gamma}

NY-ESO-1

\[ \beta\text{-actin} \]

![IFN\textsubscript{\gamma} graph](image)

Sharma, MDACC
ICOS and FOXP3 in Blood of Metastatic Melanoma Patient after anti-CTLA-4 Treatment

Yuan, Wolchok, Sharma
ICOS and FOXP3 in Blood of Metastatic Melanoma Patient after anti-CTLA-4 Treatment

Yuan, Wolchok, Sharma
Sustained Elevation of ICOS$^{\text{high}}$ CD4 T cells Correlates with Clinical Benefit

Yuan, Wolchok
Sustained Elevation of ICOS\textsuperscript{high} CD4 T cells Correlates with Clinical Benefit

Yuan, Wolchok
Sustained Elevation of ICOS$^{\text{high}}$ CD4 T cells Correlates with Clinical Benefit

Yuan, Wolchok
Sustained Elevation of ICOS$^{\text{high}}$CD4 T cells Correlates with Survival

With a median follow-up of 25 weeks, there is also a trend toward improved OS in the “ICOS-high” group (p=0.03).

Yuan, Wolchok
What is the functional significance of ICOS expression after CTLA-4 blockade?
ICOS is up-regulated on TILS after \( \alpha \)CTLA-4/Gvax treatment

ICOS

Simpson & Quezada
Does ICOS expression correlate with control of tumor by anti-CTLA-4 treatment?

Simpson & Quezada
ICOS expression increases on CD4⁺Foxp3⁻ in the blood of mice after anti-CTLA-4 Rx
Delayed tumor outgrowth in 2/10 mice in responses to anti-CTLA-4 monotherapy
ICOS expression increases on Foxp3⁺ CD4 in the blood by anti-CTLA-4
Anti-CTLA-4 treatment increases ICOS on CD4 and CD8 T cells in the blood
Correlation between tumor volume at day 20 and ICOS expression on CD4$^+$ and CD4$^+$Foxp3$^-$ at day 14 in anti-CTLA-4 treated mice

ICOS expression on Treg and CD8 T cells do not correlate with tumor volume

Simpson & Quezada
Tumor volume day 21 vs. $\% \text{ICOS}^{hi}$ Foxp3$^{-}$ CD4 cells day 14 B16-F10
Anti-CTLA-4 treated mice with a higher percentage of ICOS$^{hi}$ CD4 Teff have reduced tumor outgrowth and better survival.

A. Tumor growth  
B. Survival

All mice treated with anti-CTLA-4  
Event is a tumor over 250mm$^3$ or death

Simpson & Quezada
ICOS and ICOS-ligand knockout mice have impaired tumor rejection after anti-CTLA-4 therapy

ICOS and ICOS-ligand knockout mice have impaired tumor rejection after anti-CTLA-4 therapy

**p = 0.189 vs 9H10 treated B6**

**p = 0.011 vs 9H10 treated B6**
ICOSL+ B16 cell vaccine augments anti-tumor effect of CTLA-4 blockade

P < 0.0001
N = 20
Day -923: L anterior thigh melanoma resection and SLNBx: Breslow 2.5cm, negative SLN

Day -810: Adjuvant vaccine (Tyrosinase peptide; GP100 peptide; GMCSF-DNA)

Day -700: FNA Bx: L inguinal recurrence

Day -683: L inguinal node dissection: 1/12 positive

Day -272: VATS Lung Bx: Metastasis

Day -249: Tx: Temazolomide 5/28

Day -228: POD, Tx: CVT

Day -198: Tx: Switch to Carboplatin + VT x 6 cycles

Day -56: LUL and LLL wedge resection for oligometastatic recurrence

Day 0: Begin ipilimumab

Day 102: R inguinal node resection of recurrence
IMF-31 Melanoma

<table>
<thead>
<tr>
<th>Day</th>
<th>Hx</th>
</tr>
</thead>
<tbody>
<tr>
<td>-951</td>
<td>BxL toe-2mm, positive margins; amputation -4.3mm, ulcerated; SLNBx negative--IIC</td>
</tr>
<tr>
<td>-951</td>
<td>2 doses peptide vaccine, Fox Chase</td>
</tr>
<tr>
<td>-744</td>
<td>FNALinguinal node positive</td>
</tr>
<tr>
<td>-737</td>
<td>CLND, 6/10 positive</td>
</tr>
<tr>
<td>-694</td>
<td>CT shows L pelvic mass</td>
</tr>
<tr>
<td>-694</td>
<td>IL-2 x 6 cycles</td>
</tr>
<tr>
<td>-544</td>
<td>8 cycles temodar + sorafenib</td>
</tr>
<tr>
<td>-163</td>
<td>CT shows POD in R inguinal</td>
</tr>
<tr>
<td>-132</td>
<td>Carbo+pacli</td>
</tr>
<tr>
<td>-111</td>
<td>Carbo+pacli</td>
</tr>
<tr>
<td>-97</td>
<td>PET shows POD</td>
</tr>
<tr>
<td>0</td>
<td>begin ipi</td>
</tr>
<tr>
<td>133</td>
<td>begin FOLFIRI/flavo due to POD on ipi</td>
</tr>
<tr>
<td>175</td>
<td>last visit, did not make 2 week followup to discuss new trial</td>
</tr>
</tbody>
</table>
Impact of anti-CTLA-4 on Castrate-resistant Metastatic Prostate Cancer
PSA Responses after Anti-CTLA-4 Treatment in Prostate Cancer: Correlation with Anti-NY-ESO-1 Antibodies?

* NY-ESO-1 seropositive patient. Expression status of tumor unknown. NY-ESO-1 is expressed in ~ 30% of prostate cancers.

Yuan, Gnjatic, Wolchok
NY-EOS-1 Antibody Correlates with Biochemical Responses In Hormone Resistant Metastatic Prostate Cancer

Yuan, Gnjatic, Wolchok
NY-ESO-1 Reactive CD8+ T cells in NY-ESO-1 Seropositive Prostate Cancer (Patient 10) Increase After Irradiation and CTLA-4 blockade

Yuan, Gnjatic, Wolchok
Pt PC-10: 61 year-old man with Gleason 4+5 prostate cancer.

<table>
<thead>
<tr>
<th>Month</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>-56</td>
<td>Diagnosed with metastatic disease to bone</td>
</tr>
<tr>
<td>-56</td>
<td>Hormonal therapy</td>
</tr>
<tr>
<td>-36</td>
<td>Protocol with dutasteride</td>
</tr>
<tr>
<td>-31</td>
<td>Docetaxel</td>
</tr>
<tr>
<td>-28</td>
<td>Vitamin D/selenium</td>
</tr>
<tr>
<td>-20</td>
<td>Dexamethasone/calcitriol</td>
</tr>
<tr>
<td>-15</td>
<td>Mitoxantrone</td>
</tr>
<tr>
<td>-9</td>
<td>Protocol with abiraterone</td>
</tr>
<tr>
<td>0</td>
<td>Began ipilimumab</td>
</tr>
<tr>
<td>+2.3</td>
<td>Therapy held for exanthem, transaminitis; started on oral steroids</td>
</tr>
<tr>
<td>+5.3</td>
<td>Steroids discontinued</td>
</tr>
<tr>
<td>+ 6.5</td>
<td>Steroids restarted for autoimmune nephritis</td>
</tr>
</tbody>
</table>
PD-1 is up-regulated on TILS after αCTLA-4/Gvax Treatment

CD8\(^+\) cells  CD4\(^+\)FoxP3\(^-\) cells  CD4\(^+\)FoxP3\(^+\) cells

Naïve LN cells  TILs- untreated tumors  TILs- Gvax+αCTLA4 treated tumors
Blockade of PD-1 Pathway Synergizes with CTLA-4 Blockade

**Tumor Growth**

- Untreated
- 9H10+ratIgG
- 9H10+PD-L1+PD-L2

**Overall Survival**

- Untreated
- 9H10+ratIgG
- 9H10+PD-L1+PD-L2

Quezada
Extended B7-CD28 Family

- PD-1
- PD-L1
- PD-L2
- B7x
- B7-H3
- B7-1
- B7-2
- B7h
- ICOS
- CD28, CTLA-4
B7-H4 (B7x) and B7H3

- Co-inhibitory *in vitro*
- Expressed at low levels in many tissues
- Not expressed by antigen-presenting cells
- May play a role in tissue defense against autoimmune attack
- Expressed by many tumor cell types
- Soluble form in serum of prostate and renal cancer patients.
Increased Expression of B7x in Pancreatic Islets of RIPB7x Transgenic Mice

A
RipB7x transgenic

B
B7x RQ

B7x
insulin
overlay

C
WT
TG
Overexpression of B7x in Pancreas in BDC2.5 Mice Prevents Diabetes
Figure 3.2. Overexpression of B7x in the pancreata of BDC2.5 mice does not prevent insulitis. Representative hematoxylin and eosin staining of pancreatic sections from mice that are 32 days old. The pancreata were isolated from (A) BDC2.5^{+}\text{RipB7x}^{-} \text{ mice}, (B) BDC2.5^{+}\text{RipB7x}^{+} \text{ mice}, (C) and B6.g7 control mice.
Overexpression of B7x in pancreatic islets inhibits IFN-γ and IL-17 production by BDC2.5 T cells

Intracellular cytokine staining of pancreatic islets

- BDC2.5⁺RipB7x⁻
- BDC2.5⁺RipB7x⁺

p = 0.0272
p = 0.0361
p = 0.0488
p = 0.0291

• BDC2.5⁺RipB7x⁻
• BDC2.5⁺RipB7x⁺
Human prostate cancer

B7-H3

B7-H4 (B7x)

Reuter
Comparison of features by tumor B7-H3 intensity

<table>
<thead>
<tr>
<th>Feature</th>
<th>Tumor B7-H3 Intensity</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None-Moderate n=591</td>
<td>Strong n=212</td>
</tr>
<tr>
<td><strong>Median Preoperative Serum PSA (IQ Range)</strong></td>
<td>5.6 (7.8, 12.7)</td>
<td>5.2 (8.0, 13.9)</td>
</tr>
<tr>
<td><strong>Gleason Score (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-6</td>
<td>215 (36)</td>
<td>64 (30)</td>
</tr>
<tr>
<td>7</td>
<td>317 (54)</td>
<td>127 (60)</td>
</tr>
<tr>
<td>8-10</td>
<td>59 (10)</td>
<td>21 (10)</td>
</tr>
<tr>
<td><strong>Extracapsular Extension (%)</strong></td>
<td>160 (27)</td>
<td>88 (42)</td>
</tr>
<tr>
<td><strong>Seminal Vesicle Invasion (%)</strong></td>
<td>40 (7)</td>
<td>26 (12)</td>
</tr>
<tr>
<td><strong>Positive Surgical Margins (%)</strong></td>
<td>203 (34)</td>
<td>82 (39)</td>
</tr>
<tr>
<td><strong>Lymph Node Involvement (%)</strong></td>
<td>15 (3)</td>
<td>10 (5)</td>
</tr>
<tr>
<td><strong>Non-Organ Confined (%)</strong></td>
<td>177 (30)</td>
<td>96 (45)</td>
</tr>
</tbody>
</table>
Associations with clinical outcome

<table>
<thead>
<tr>
<th></th>
<th>Hazard Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biochemical Recurrence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B7-H3 Intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None-Moderate</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>1.39 (1.01 – 1.92)</td>
<td>0.042</td>
</tr>
<tr>
<td>B7x Intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None-Moderate</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>1.38 (0.94 – 2.02)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Clinical Failure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B7-H3 Intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None-Moderate</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>2.79 (1.74 – 4.46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B7x Intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None-Moderate</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>2.22 (1.27 – 3.87)</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Death from Disease</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B7-H3 Intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None-Moderate</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>3.48 (1.50 – 8.09)</td>
<td>0.004</td>
</tr>
<tr>
<td>B7x Intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None-Moderate</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>2.71 (1.04 – 7.02)</td>
<td>0.040</td>
</tr>
</tbody>
</table>
Extended B7-CD28 Family

- PD-L1
- PD-L2
- B7-1
- B7-2
- B7h
- ICOS
- B7x
- B7-H3

CD28, CTLA-4
Extended B7-CD28 Family

- **PD-I**
  - Limits Responses

- **PD-L1**
  - B7x
  - B7-H3
  - Inhibits Effector Function

- **PD-L2**
  - B7h
  - ICOS

- **B7-1**
- **B7-2**

- **CD28, CTLA-4**
  - Inhibits Proliferation

- **B7-2B7-1**

- **ICOS**
  - Promotes Survival
Extended B7-CD28 Family

- **PD-I**
  - **PD-L1**
  - **PD-L2**

- **B7x**
- **B7-H3**

- **B7-1**
- **B7-2**

- **ICOS**

**Limits Responses**

- Inhibit Effector Function

**Promotes Survival**

- Inhibits Proliferation
Extended B7-CD28 Family

- **PD-I**
  - Limits Responses
  - Inhibits Proliferation
  - Promotes Survival

- **PD-L1**
- **PD-L2**

- **B7x**
  - Inhibit Effector Function

- **B7-H3**

- **B7h**
  - Limits Responses
  - Inhibits Proliferation
  - Promotes Survival

- **ICOS**
  - Inhibit Effector Function

- **B7-1**
- **B7-2**

- **CD28, CTLA-4**
  - Limits Responses
  - Inhibits Proliferation
Extended B7-CD28 Family

- **PD-I**
  - Limits Responses
  - Inhibits Proliferation
  - Promotes Survival

- **PD-L1**
  - Inhibits Effector Function

- **PD-L2**

- **B7x**
  - Inhibits Effector Function
  - Limits Responses

- **B7-H3**

- **B7-1**

- **B7-2**

- **B7h**

- **ICOS**
  - Promotes Survival
  - Inhibits Proliferation

- **CD28, CTLA-4**
  - Inhibits Proliferation
Future Development of Checkpoint Blockade

• Monotherapy with anti-CTLA-4 effective
  Need biomarkers to predict responders

• Test and understand mechanisms of additional blockers

• Combinations likely to have higher response rates
  Different checkpoint blockers
  Checkpoint blockade with other therapies
  Need to understand impact on immune function, including T cell priming
Cancer Immunotherapy:
Where do we go from here?
Cancer Immunotherapy: Where do we go from here?

- Each tumor has multiple (90?) mutations resulting in coding changes (Vogelstein et al. Science 2006)

- Many of these, depending on MHC type of host, will represent neoantigens
Neoepitopes Generated by Genomic Instability Inherent in Cancer

- Algorithms predict 9/tumor for HLA-0201
- 6 MHC alleles/tumor, so assuming even distribution predicts 54 total neoepitopes/tumor
- Assuming algorithm is wrong 90% of time, would predict 5-6 neoepitopes/tumor

*Effective checkpoint blockade leading to immunotargeting may turn 1 drug/therapy into 6-7, raising the possibility of combinatorial therapies even with single drug*
Implications for Cancer Vaccines

Don’t worry about:

• Variegated expression of antigens that are targeted by vaccines
• Whether vaccine targets are essential to the tumor survival
  • Clonality of transferred T cells

Because death of cells should lead to cross-priming with a battery of other antigens which should be enhanced by checkpoint blockade
Implications for “Immunosupportive” Therapies

Agents that kill tumor cells without causing immunosuppression (chemotherapy, radiation, hormone therapy, antibodies, “targeted” therapies) are vaccines

Use with checkpoint blockade to unleash the immune system to maximize T cell responses to multiple targets
LCCI Immune Monitoring Schema

Specimen

Normal tissue, LN
Tumor
Blood (PBMC, PMN, plasma, sera)

Ex vivo Assays

• White counts…
• Class I & II Tetramer WB
• Phenotype subsets on fresh sample
• Intracellular cytokine assay (ICS)
• Gene chip
• IHC & RT-PCR

In vitro culture

• aAPC: K562/A0201
• Autologous CD4-CD8-
• Autologous T-APC
• Autologous DC

• Single peptide
• Overlapping peptide
• Protein
• mRNA, viral-vector

Recall Assays

• Class I & II Tetramer
• Phenotype tetramer+ T cells (effector memory, central memory…)
• ICS polyfunctional assay
• Sorting, cloning, functional assay

HLA typing

Serology
ELISA
CBA cytokine
Peptide array
Seromics

Cytokines
Immune Monitoring Protocol for Anti-CTLA4 Trials

Blood from (pre-, post-therapy) patients

Whole blood or PBMCs

Phenotyping:
Panel A. CD4+CD25+FOXP3+ T cell staining
Panel B. NK/NK T cells staining
Panel C. T-cell/B cell/monocyte staining
Panel D. CD4, CD8 naïve, effector memory, central memory subpopulation
Panel E. CD4, CD8 recent thymic emigrants

Serum

NY-ESO-1 status

Polymorphonuclear leukocytes (PMN)

NY-ESO-1 sero-positive

B cell NY-ESO-1 epitope

Spectratyping

Antigen specific T-cell response
Panel F. Resting/Activated Memory tetramer+ T-cell staining
Panel G. ICS IFN-gamma+ T-cell staining
Panel H. IFN-gamma+ secreting cells

Sorting, cloning T-cell

Tumor sample

In situ Immune monitoring

HLA typing
Proposed Monitoring Schema for Sipuleucel-T

**Pre-vaccine product monitoring**
- Phenotype:
  - CD54, HLA-DR, CD80, CD86, CD83
  - CD11b, CD14, CD19, CD56
- Function:
  - T cell response to CEF, PAP

**Immunological response**
- Serological monitoring:
  - PSA response
  - PAP specific antibody response (protein, peptide array)
  - Other tumor antigen-specific Ab response
- Cellular monitoring:
  - Absolute lymphocyte counts
  - CD4, CD8, FOXP3, NK, NKT phenotype
  - T cell proliferation assay
  - PAP and other antigen-specific T cell response using overlapping peptides
  - (intracellular cytokine assay, polyfunction, tetramer)
Acknowledgements

Lab members:
Alejandro Sepulveda
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Jocelyn Lu
Joyce Wei
Michael Curran
Peter Savage
Rachel Gottschalk
Rebecca Waitz
Sergio Quezada
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