Changes in Intratumoral Immune Cell Infiltrates, FoxP3, and Indoleamine 2, 3-Dioxygenase (IDO) Expression with the CTLA4 Blocking mAb CP-675,206


University of California Los Angeles, CA, and Pfizer Global Research and Development, New London, CT.
Rationale for CTLA-4 Blockade to Induce Antitumor Immune Responses

Additional Potential Mechanisms of Action of CTLA4 Blocking Monoclonal Antibodies

Background

• Role of immune cell subsets in melanoma responses to anti-CTLA4 antibodies:
  – CD4 and/or CD8 cells:
  – Treg:
    • Involved: Reuben et al. Cancer 06.
    • Not involved: Maker et al. J Immunol 06, Comin-Anduix et al. iSBTc 06
  – IDO pDC: No reports to date.

• Where should immune activation be studied?
  – Peripheral blood: Maker et al. J Immunol 06, Comin-Anduix et al. iSBTc 06
  – Lymph nodes: No reports to date
  – Tumor: Anecdotal reports to date
Materials and Methods

• 89 patients have received dosing with CP-675,206 at UCLA, and a small subset of patients underwent tumor biopsies.

• Samples collected for:
  – Diagnostic or therapeutic need (more likely on progressing patients)
  – Research purposes under UCLA IRB# 02-08-067 (more likely on responding patients)

• IHC staining for:
  – Melanoma markers: S-100, HMB45, MART-1, tyrosinase
  – Immune cell subset markers: CD1a, CD3, CD4, CD8, CD20
  – Treg marker: FoxP3
  – Immune suppressive DC marker: IDO

• IHC scoring by Dr. Alistair Cochran:
  – Frequency (0-3+) of reactive cells
  – Distribution (diffuse or patchy) of reactive cells
# Patient Characteristics and Timing of Biopsies

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Sex</th>
<th>CP-675,206 Regimen</th>
<th>Response</th>
<th>Toxicity</th>
<th>Timing of Biopsy (first dose/last dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>M</td>
<td>15 mg/kg q3mo</td>
<td>PR</td>
<td>-</td>
<td>Pre</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Post (3 mo/3mo)</td>
</tr>
<tr>
<td>2</td>
<td>78</td>
<td>M</td>
<td>10 mg/kg qmo</td>
<td>PR</td>
<td>G2 arthritis</td>
<td>Pre</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Post (2 mo/1 mo)</td>
</tr>
<tr>
<td>3</td>
<td>64</td>
<td>M</td>
<td>10 mg/kg qmo</td>
<td>pPR</td>
<td>G2 asthenia</td>
<td>Pre</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Post (9 mo/1 mo)</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>M</td>
<td>10 mg/kg qmo</td>
<td>PR</td>
<td>-</td>
<td>Post Progressing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Post Stable</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Post Responding (8 mo/1 mo)</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>F</td>
<td>10 mg/kg qmo</td>
<td>PD</td>
<td>-</td>
<td>Post (4 mo/1 mo)</td>
</tr>
<tr>
<td>6</td>
<td>62</td>
<td>M</td>
<td>10 mg/kg qmo</td>
<td>PD</td>
<td>G3 diarrhea</td>
<td>Post (8 mo/6 mo)</td>
</tr>
</tbody>
</table>
Patient 1: PR (26+ mo) to skin, adrenal, liver and lung metastasis

6/04 Pre-Dose

10/04 Post-Dose
Patient 2: PR (11+ mo) to in-transit metastasis

Pre: 11/05

Post: 1/06
Phenotype CD8⁺: HLA-DR⁺CD45RO+++CD27++CCR7⁻
(T early memory)
Patient 3: PET and Pathological PR (pPR)

Pre

3/05

H&E

4x

CD4

CD8

Post

7/05

1/06

H&E

90% regressed melanoma

10% viable melanoma

H&E

CD4

CD8
Patient 4: PR (19+ mo) to bulky in-transit metastasis

- Negative Stain
- HMB45 Melanoma
- CD20 B Cells
- CD3 T Cells
- CD4 T helper
- CD8 CTL

02/05
03/05
07/05

Post
Patient 4 (PR): Co-existing responding and progressing lesions

6/05

7/05

8/05

9/05
Patient 4 (PR): Simultaneous Analysis of Regressing and Progressing Lesions

Regressing

H&E MART-1 CD4 CD8

Stable

H&E MART-1 CD4 CD8

Progressing

H&E MART-1 CD4 CD8
Patient 4 (PR): gp100 Tetramer Analysis

**Regressing**

- gp100 Tetramer: 0.24%
- MART-1 Tetramer: 0.01%
- CD8+: 0.23%

**Stable**

- gp100 Tetramer: 0.24%
- MART-1 Tetramer: 0.01%
- CD8+: 0.42%

**Progressing**

- gp100 Tetramer: 0.06%
- MART-1 Tetramer: 0.06%
- CD8+: 0.01%

Peripheral blood:
- gp100 Tetramer: 0.05%
- MART-1 Tetramer: 0.05%
- CD8+: 0.01%

*10/06*
Patient 5 (PD): Progressive Abdominal Mass

Pre

Post

H&E
Neg
S100
HMB45
CD4
CD8 10x

Patient 6 (PD): Progressive Lymph Node Metastasis

Post

H&E
Neg
S100
MART-1
CD4
CD8 10x
Patient 6 (PD): Tetramer Analysis in PBMC and TIL

Peripheral Blood

**MART-1**

- 0.00%

**MART-1**

- 0.03%

**Tyrosinase**

- 0.20%

- 0.03%

TIL:

**MART-1**

- 0.03%

**Tyrosinase**

- 0.03%
### Intratumoral CD8 and CD4 Cells

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Response</th>
<th>Timing of Biopsy (first dose/last dose)</th>
<th>CD8</th>
<th>CD4</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PR</td>
<td>Pre</td>
<td>0</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post (3 mo/3mo)</td>
<td>+++</td>
<td>++ diffuse</td>
<td>↑</td>
</tr>
<tr>
<td>2</td>
<td>PR</td>
<td>Pre</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post (2 mo/1 mo)</td>
<td>++ diffuse</td>
<td>+ diffuse</td>
<td>↑</td>
</tr>
<tr>
<td>3</td>
<td>pPR</td>
<td>Pre</td>
<td>++ patchy</td>
<td>+ patchy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post (9 mo/1 mo)</td>
<td>++ diffuse</td>
<td>+++ diffuse</td>
<td>↑</td>
</tr>
<tr>
<td>4</td>
<td>PR</td>
<td>Post Progressing (8 mo/1 mo)</td>
<td>+ patchy</td>
<td>+++ diffuse</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post Stable (8 mo/1 mo)</td>
<td>+ patchy</td>
<td>+++ diffuse</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post Responding (8 mo/1 mo)</td>
<td>+++ diffuse</td>
<td>++ diffuse</td>
<td>↑ CD8 ↓ CD4</td>
</tr>
<tr>
<td>5</td>
<td>PD</td>
<td>Post (4 mo/1 mo)</td>
<td>+/-</td>
<td>++ diffuse</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>PD</td>
<td>Post (8 mo/6 mo)</td>
<td>+ diffuse</td>
<td>+++ diffuse</td>
<td></td>
</tr>
</tbody>
</table>
Treg Depletion with CTLA4 Blocking Monoclonal Antibodies

- Treg depletion in peripheral blood with anti-CTLA4 mAb:
  - Reuben et al. Cancer 2006

- No Treg depletion in peripheral blood with anti-CTLA4 mAb:
  - Maker et al. J Immunol 2005
  - Comin-Anduix et al. iSBTc 2006
Patient 3 (pPR): FoxP3 Pre and Post CP-675,206

Pre

HMB45

CD8

Post

HMB45

CD8

4x

10x

40x
Patient 4 (PR): FoxP3 in Regressing and Non-regressing Lesions

Regressing

Stable

Progressing

FoxP3

Melanoma

Granuloma-like

Melanoma

Granuloma-like
Patient 6 (PD): FoxP3 by IHC or ICS in TIL

**FoxP3 in TIL by IHC**

**FoxP3 in TIL by ICS**

- **CD4**
  - 0.47% CD4=CD25high

- **FoxP3**
  - 92.66% FoxP3 ICS
  - 92%

- **Side Scatter**
  - 1024

- **CD4/CD25hi**
  - 0.47% CD4=CD25high

- **FoxP3 in TIL by IHC**
  - S100
  - CD4
  - FoxP3
  - 10x
  - 40x

- **FoxP3 in TIL by ICS**
  - CD4
  - FoxP3
  - 10x
  - 40x

**Post**
## Intratumoral FoxP3+ Treg

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Response</th>
<th>Timing of Biopsy</th>
<th>FoxP3</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PR</td>
<td>Pre</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post (3 mo/3mo)</td>
<td>+ patchy</td>
<td>↑</td>
</tr>
<tr>
<td>2</td>
<td>PR</td>
<td>Pre</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post (2 mo/1 mo)</td>
<td>+ patchy</td>
<td>↑</td>
</tr>
<tr>
<td>3</td>
<td>pPR</td>
<td>Pre</td>
<td>+ patchy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post (9 mo/1 mo)</td>
<td>++ patchy</td>
<td>↑</td>
</tr>
<tr>
<td>4</td>
<td>PR</td>
<td>Post Progressing</td>
<td>+ patchy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post Stable</td>
<td>+ patchy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post Responding (8 mo/1 mo)</td>
<td>+ patchy</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>PD</td>
<td>Post (4 mo/1 mo)</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>PD</td>
<td>Post (8 mo/6 mo)</td>
<td>+++ diffuse</td>
<td></td>
</tr>
</tbody>
</table>
Inhibition of IDO by CTLA4 Blocking Monoclonal Antibodies

Patient 3 (pPR): IDO Pre and Post CP-675,206

Pre

HMB45

CD8

IDO

Post

HMB45

CD8

IDO

IDO

4x

10x

40x
Patient 4 (PR): IDO in Regressing and Non-regressing Lesions

Regressing

IDO

Progressing

IDO

Melanoma

Granuloma-like

Stable

Melanoma

Granuloma-like
# Intratumoral IDO+ Cells

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Response</th>
<th>Timing of Biopsy</th>
<th>IDO</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PR</td>
<td>Pre</td>
<td>++ diffuse</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post (3 mo/3mo)</td>
<td>+ patchy</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>PR</td>
<td>Pre</td>
<td>+ patchy</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post (2 mo/1 mo)</td>
<td>+ patchy</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>pPR</td>
<td>Pre</td>
<td>+ patchy</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post (9 mo/1 mo)</td>
<td>+ patchy</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>PR</td>
<td>Post Progressing</td>
<td>++ patchy</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post Stable</td>
<td>++ patchy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post Responding</td>
<td>+++ patchy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(8 mo/1 mo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>PD</td>
<td>Post (4 mo/1 mo)</td>
<td>++ patchy</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>PD</td>
<td>Post (8 mo/6 mo)</td>
<td>+ patchy</td>
<td></td>
</tr>
</tbody>
</table>
Conclusions

• Regressing lesions after CTLA4 blockade with CP-675,206 have:
  – Dense intratumoral infiltrates by CD8+ CTL, and variable CD4+ T helper infiltrates
  – No consistent decrease in intratumoral infiltrates by FoxP3+ Tregs and no inhibition of IDO expression

• Contrary to the posed hypothesis, FoxP3 and IDO positive cells may cluster at areas of active immune response against melanoma
Acknowledgements

• UCLA:
  – Begonya Comin-Anduix, PhD
  – Pilar de la Rocha, BS
  – Timothy Donahue, MD
  – John A. Glaspy, MD, MPH
  – James S. Economou, MD, PhD
  – Alistair Cochran, MD

• Pfizer Global Research and Development:
  – Viviana A. Bozon, MD
  – Cecile A. Bulanhagui, MS
  – Margaret Marshall, MD
  – Jesus Gomez-Navarro, MD

• Support:
  – Research funding from Pfizer.
  – AR is supported by: K23 CA93376, the Jonsson Comprehensive Cancer Center and the Melanoma Research Foundation.