Clinical Trials: Provoking Immunity in the Tumor Microenvironment

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Disclosure Information
Antoni Ribas

I have the following financial relationships to disclose:
• Consultant for: Kite Pharma
• Speaker’s Bureau for: None
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• Stockholder in: Kite Pharma
• Honoraria from: Amgen, Celgene, Genentech-Roche, GSK, Millennium, Novartis, Prometheus
• Employee of: None

-and-
• I will discuss the following off label use and/or investigational use in my presentation: tremelimumab, nivolumab
Monitoring Tumor Immunotherapy

*In vitro*

- Molecules
  - T Cell Receptor

*In vivo*

- Cells
  - T lymphocyte
  - Dendritic cell
  - Proliferation
  - Differentiation
  - Effector T cell
  - B lymphocyte

- Whole-body imaging
Durable responses with anti-CTLA4 in approximately 10-15% of patients

Improved Survival with Ipilimumab in Patients with Metastatic Melanoma

F. Stephen Hodi, M.D., Steven J. O'Day, M.D., David F. McDermott, M.D., Robert W. Weber, M.D., Jeffrey A. Sosman, M.D., John B. Haanen, M.D., Rene Gonzalez, M.D., Caroline Robert, M.D., Ph.D., Dirk Schadendorf, M.D., Jessica C. Hassel, M.D., Wallace Akerley, M.D., Alfons J.M. van den Eertwegh, M.D., Ph.D., Jose Lutzky, M.D., Paul Lorigan, M.D., Julia M. Vaubel, M.D., Gerald P. Linette, M.D., Ph.D., David Hogg, M.D., Christian H. Ottensmeier, M.D., Ph.D., Celeste Lebè, M.D., Christian Peschel, M.D., Ian Quirt, M.D., Joseph I. Clark, M.D., Jedd D. Wolchok, M.D., Ph.D., Jeffrey S. Weber, M.D., Ph.D., Jason Tian, Ph.D., Michael J. Yellin, M.D., Geoffrey M. Nichol, M.D., Axel Hoos, M.D., Ph.D., and Walter J. Urba, M.D., Ph.D.
How can CTLA4 blockade therapy be studied in humans?

- **Anti-CTLA4 antibody**
  - PK: Antibody levels in blood
- **Immune monitoring**:
  - Levels of blood immune cells
- **Tumor biopsies**:
  - Immune cells killing cancer cells
Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in Patients with metastatic melanoma

Autoimmunity Correlates With Tumor Regression in Patients With Metastatic Melanoma Treated With Anti-Cytotoxic T-Lymphocyte Antigen-4

Analysis of the Cellular Mechanisms of Autoimmune Responses in Patients Treated With CTLA4-Blockade

CTL A4 blockade enhances multifunctional NY-ESO-1 specific T cell responses in metastatic melanoma patients with clinical benefit

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

Intratumoral immune cell infiltrates, FoxP3, and indoleamine 2,3-dioxygenase in patients with melanoma undergoing CTLA4 blockade

CTLA-4 blockade confers lymphocyte resistance to regulatory T cells in advanced melanoma: surrogate marker of efficacy of tremelimumab

Anti-CTLA-4 therapy results in higher CD4+CD25+ T cell frequency and IFN-γ levels in both nonmalignant and malignant prostate tissues

Preoperative CTLA-4 blockade: Tolerability and immune monitoring in the setting of a presurgical clinical trial

Cancer Therapy: Clinical
Studying where it counts: The Tumor

Tumor cells

DC
B7
CTLA4
MHC
T cell
IL-2
CD28
CTL
Treg
IDO

T cell

CTL

iDC

DC

MHC

CTLA4

CD28

IL-2

T cell

Tumor cells

iDC

IDO

Treg

CTL
Anti-CTLA4 Antibodies Induce Dense Intratumoral Infiltrated by CD8+ CTLs in Regressing Tumors

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 PD: FoxP3 by IHC or ICS in TIL

FoxP3 in TIL by IHC

FoxP3 in TIL by ICS

CD4

CD25

CD4=CD25high

0.47%

CD4+/CD25hi

92.66%

#467

FoxP3

FoxP3 in TIL by IHC

FoxP3+ 92.66% #467

FoxP3 ICS

Begonya Comin-Anduix, PhD

Alistair Cochran, MD
Inhibition of IDO by CTLA4 Blocking Monoclonal Antibodies

pPR: IDO Pre and Post CP-675,206

Pre

HMB45

CD8

Post

HMB45

CD8

4x

10x

40x
# Intratumoral FoxP3+ and IDO+ Cells

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>Response</th>
<th>Timing of Biopsy</th>
<th>FoxP3</th>
<th>FoxP3 Change</th>
<th>IDO</th>
<th>IDO Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PR</td>
<td>Pre</td>
<td>0</td>
<td>↑</td>
<td>+ patchy</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post (3 mo/3mo)</td>
<td>+ patchy</td>
<td>↑</td>
<td>+ patchy</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>PR</td>
<td>Pre</td>
<td>0</td>
<td>↑</td>
<td>+ patchy</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post (2 mo/1 mo)</td>
<td>+ patchy</td>
<td>↑</td>
<td>+ patchy</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>pPR</td>
<td>Pre</td>
<td>+ patchy</td>
<td>↑</td>
<td>+ patchy</td>
<td>=</td>
</tr>
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<td>Post (9 mo/1 mo)</td>
<td>++ patchy</td>
<td>↑</td>
<td>+ patchy</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Progr</td>
<td>Pre</td>
<td>+ patchy</td>
<td>=</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post (1 mo/1 mo)</td>
<td>+ patchy</td>
<td>=</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
Phase 2 to Study the Mechanism of Action of Tremelimumab in Patients Using Repeated Outpatient Tumor Biopsies

- 1
  - Leukapheresis #1
  - Tumor Biopsy #1
  - PET CT Scan #1
  - [^{18}F]FDG
  - [^{18}F]FLT

- 30
  - Tremelimumab
  - Leukapheresis #2
  - Tumor Biopsy #2
  - PET CT Scan #2
  - [^{18}F]FDG
  - [^{18}F]FLT

- 60

- 90
  - Standard Re-Staging Exams
  - Tremelimumab
Modulation of Cell Signaling Networks after CTLA4 Blockade in Patients with Metastatic Melanoma

Begoña Comín-Anduix1,2,*, Hooman Sazegar3, Thinla Chodon3, Douglas Matsunaga3, Jason Jalil1, Erika von Euw1, Helena Escuin-Ordinas3, Robert Balderas3, Bartosz Chmielowski3, Jesus Gomez-Navarro5, Richard C. Koya1, Antoni Ribas1,2,3,*

Journal of Translational Medicine

Research
CTLA4 blockade increases Th17 cells in patients with metastatic melanoma
Erika von Euw1, Thinla Chodon3, Narsis Attar2, Jason Jalil1, Richard C Koya1, Begoña Comín-Anduix1 and Antoni Ribas*1,2,3

Cancer Therapy: Clinical

CTLA4 Blockade Induces Frequent Tumor Infiltration by Activated Lymphocytes Regardless of Clinical Responses in Humans

Rong Pong Huang1, Jason Jalil2, James S. Economou3,4, Bartosz Chmielowski3, Richard C. Koya2, Stephen Mok3, Hooman Sazegar2, Elizabeth Seja2, Arturo Villanueva2, Jesus Gomez-Navarro5, John A. Glaspy2,3, Alistair J. Cochran1, and Antoni Ribas2,3,4

Imaging of CTLA4 Blockade–Induced Cell Replication with 18F-FLT PET in Patients with Advanced Melanoma Treated with Tremelimumab

Antoni Ribas1,2,*, Matthias R. Benz2, Martin S. Allen-Auerbach4, Caius Radu2,4, Bartosz Chmielowski3, Elizabeth Seja1, John L. Williams8, Jesus Gomez-Navarro5, Timothy McCarthy2, and Johannes Czernin2,3
Increase in TIL in most patients regardless of tumor response

Intratumoral CD8 Infiltration

Paired t-test p = 0.005
No difference in TIL activation or replication

**HLA-DR+/CD45RO+ Activated T Cells**

<table>
<thead>
<tr>
<th>Pre-Tx</th>
<th>Post-Tx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt w Response</td>
<td>Pt w Response</td>
</tr>
<tr>
<td>Pt w Progression</td>
<td>Pt w Progression</td>
</tr>
</tbody>
</table>

**Ki-67+ Proliferating Cells**

<table>
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<th>Pre-Tx</th>
<th>Post-Tx</th>
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<tbody>
<tr>
<td>Pt w Response</td>
<td>Pt w Response</td>
</tr>
<tr>
<td>Pt w Progression</td>
<td>Pt w Progression</td>
</tr>
</tbody>
</table>
Where in the body is anti-CTLA4 working?

Where does lymphocyte replication happen?
Whole Body Imaging with PET Probes: $[^{18}\text{F}]$FDG and $[^{18}\text{F}]$FLT

$[^{18}\text{F}]$FDG:
- Positron emitting glucose analog
- Images glucose metabolism

$[^{18}\text{F}]$FLT:
- Positron emitting thymidine nucleoside analog
- Images cell replication

Tse, ... Phelps, Glaspy et al. The application of positron emission tomographic imaging with fluorodeoxyglucose to the evaluation of breast disease. Ann Surg 1992

[\textsuperscript{18}F]FDG and [\textsuperscript{18}F]FLT PET in a Patient with Response to Tremelimumab

**Pre-treatment**

**[\textsuperscript{18}F]FDG**
- Positron emitting glucose analog
- Images glucose metabolism

**[\textsuperscript{18}F]FLT**
- Positron emitting thymidine nucleoside analog
- Images cell replication

**Post-treatment**

**[\textsuperscript{18}F]FDG**
- Positron emitting glucose analog
- Images glucose metabolism

**[\textsuperscript{18}F]FLT**
- Positron emitting thymidine nucleoside analog
- Images cell replication
Molecular imaging with the PET probe $[^{18}\text{F}]\text{FLT}$ (radiolabeled thymidine) allows mapping and non-invasive imaging of cell proliferation in spleen after CTLA4 blockade in patients with metastatic melanoma.

Ribas... Czernin. JNM 2010
Where in the body is anti-CTLA4 working?

Where does lymphocyte replication happen? In lymphoid organs
Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer


Tumor Immunotherapy Directed at PD-1

Antoni Ribas, M.D., Ph.D.

Safety and Activity of Anti–PD-L1 Antibody in Patients with Advanced Cancer

Julie R. Brahmer, M.D., Scott S. Tykodi, M.D., Ph.D., Laura Q. M. Chow, M.D., Wen-Jen Hwu, M.D., Ph.D., Suzanne L. Topalian, M.D., Patrick Hwu, M.D., Charles G. Drake, M.D., Ph.D., Luis H. Camacho, M.D., M.P.H., John Kauh, M.D., Kuni Odunsi, M.D., Ph.D., Henry C. Pitot, M.D., Omid Hamid, M.D., Shailender Bhatia, M.D., Renato Martins, M.D., M.P.H., Keith Eaton, M.D., Ph.D., Shuming Chen, Ph.D., Theresa M. Salay, M.S., Suresh Alaparthi, Ph.D., Joseph F. Grosso, Ph.D., Alan J. Korman, Ph.D., Susan M. Parker, Ph.D., Shruti Agrawal, Ph.D., Stacie M. Goldberg, M.D., Drew M. Pardoll, M.D., Ph.D., Ashok Gupta, M.D., Ph.D., and Jon M. Wigginton, M.D.
Colocalization of Inflammatory Response with B7-H1 Expression in Human Melanocytic Lesions Supports an Adaptive Resistance Mechanism of Immune Escape

Janis M. Taube,1,2* Robert A. Anders,2 Geoffrey D. Young,3,4 Haiying Xu,1 Rajni Sharma,2 Tracee L. McMiller,4 Shuming Chen,4 Alison P. Klein,2,5 Drew M. Pardoll,5 Suzanne L. Topalian,4* Lieping Chen1,5,6*
Conclusions

• The main goal of tumor immunotherapy is to bring activated T cells into tumors:
  – Vaccination with DC can occasionally achieve durable immune responses to cancer
  – CTLA4 blockade induces reproducible but low frequency durable tumor responses to cancer

• T cell infiltration is necessary but not sufficient to result in tumor responses

• FOXP3 and IDO expression in tumors does not seem to be associated with resistance to CTLA4 blockade

• T cell replication upon CTLA4 blockade happens in lymphoid organs and not in tumors
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John Glaspy, MD
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WAM Clinical Trials Team:
Liz Seja, Art Villanueva

Ribas lab