Immune Checkpoints

John Haanen
Overview

- CTLA4
- PD1/PD-L1
- Other checkpoints
T cell signaling

Signal 1: Antigen recognition

Signal 2: Co-stimulation

There are positive and negative second signals
Cancer Immunotherapy: CTLA4

- CTLA-4 suppresses T cell activation and inhibits T cell function
- CTLA-4 regulates T cell tolerance
  -- CTLA-4 KO mice develop lethal lympho-proliferative syndrome
- CTLA-4 (Ipilimumab) first drug in this class approved for tumor immunotherapy
CTLA-4 inhibition

T-cell activation

T cell

TCR

CD28

B7

APC

MHC

CTLA-4

T-cell inhibition

T cell

TCR

CD28

B7

APC

MHC

CTLA-4

T-cell potentiation

T cell

TCR

CD28

B7

APC

MHC

CTLA-4 inhibitor

Adapted from Lebbé et al. ESMO 2008
CTLA-4 blockade (ipilimumab) can induce long-term survival
(pooled overall survival analysis including Expanded Access Program data from 4846 patients)

Median OS (95% CI): 9.5 months (9.0–10.0)

3-year OS rate (95% CI): 21% (20%–22%)

Patients at Risk
Ipilimumab 4846 1786 612 392 200 170 120 26 15 5 0

Schadendorf D, et al. ECCO/ESMO 2013: oral presentation 24LBA
Treatment with anti-CTLA-4 mAb

Maker et al., Ann Surg Oncol 2005
Auto-immune uveitis after anti-CTLA-4 treatment
Immune related adverse events upon anti-CTLA-4 mAb treatment

colitis

hypophysitis
How does CTLA4 blockade lead to anti-tumor immune responses?

Two not mutually exclusive mechanisms have been proposed:

1. Priming of tumor-reactive T cells
   - Against shared tumor associated antigens
   - Against mutated (neo) antigens

2. Depletion of regulatory FoxP3+ T cells from the tumor microenvironment
How does CTLA4 blockade lead to anti-tumor immune responses?

Two not mutually exclusive mechanisms have been proposed:

1. Priming of tumor-reactive T cells
   – Against shared tumor associated antigens
   – Against mutated (neo) antigens

2. Depletion of regulatory FoxP3+ T cells from the tumor microenvironment
CTLA4 plays a role during T cell priming
What could tumor-specific cytotoxic T cells detect on human cancer?

1. Self antigens (to which tolerance is incomplete)
   *Shared between patients*

2. ‘Neo-antigens’, epitopes that arise as a consequence of tumor-specific mutations
   *In large part patient-specific, hence generally ignored*
Analysis of PBMC from 40 ipilimumab treated melanoma patients for 75 tumor associated antigens

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Kvistborg et al., Science Transl Med 2014
Analysis performed by flow cytometry

Peptide 1  Peptide 2  Peptide 3 ....  .... Peptide 26

Assemble fluorochrome conjugated MHC multimers

Generate differentially encoded multimer combinations

Combine two color coded multimers for 26 epitopes and use for staining

Toebes et al., Nat Med 2006; Anderson et al. Nat Protoc 2012
Flow results...

Pretherapy

MART-$1_{ELA}$

MAGE-C2$_{ALK}$

GNT-V$_{VLP}$

Posttherapy

0.027

0.002

0.019

0.017

0.006

0.011

Kvistborg et al., Science Transl Med 2014
Ipilimumab treatment leads to broadening of the anti cancer IR

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Kvistborg et al., Science Transl Med 2014
What could tumor-specific cytotoxic T cells detect on human cancer?

1. Self antigens (to which tolerance is incomplete)  
   *Shared between patients*

2. ‘Neo-antigens’, epitopes that arise as a consequence of tumor-specific mutations 
   *In large part patient-specific, hence generally ignored*
Analyzing the neo-antigen-specific T cell repertoire in human cancer?

- Generate map of tumor-specific mutations (ExomeSeq)
- Determine which mutated genes are expressed (RNASeq)
- Predict epitopes for each mutation/each HLA-allele \textit{in silico}
- Screen for T cell recognition of mutated epitopes
Pt 002: Partial response upon anti-CTLA4 treatment

Van Rooij et al., J Clin Oncol 2013
Analyzing the neo-antigen-specific T cell repertoire in human cancer?

1. Resected tumor material
2. Isolate tumor cells
3. Identify tumor-specific mutations

Mutation spectrum of single base pair substitutions

- $T > A / A > T$
- $T > G / A > C$
- $T > C / A > G$
- $C > A / G > T$
- $C > G / G > C$
- $C > T / G > A$

% of Non-Synonymous mutations

(n=1036)
Analyzing the neo-antigen-specific T cell repertoire in human cancer?

1. Resected tumor material
2. Isolate tumor cells
3. Isolate tumor-infiltrating T cells
4. Screen with MHC multimer technology
5. Identify tumor-specific mutations
6. Predict potential epitopes
Strong T cell response against an ATR$_{S>L}$ neo-epitope within the tumor

- Resected tumor material
- Isolate tumor cells
- Isolate tumor-infiltrating T cells
- Screen with MHC multimer technology
- Identify tumor-specific mutations
- Predict potential epitopes
Increased magnitude of neo-antigen-specific T cell response under anti-CTLA4

van Rooij, van Buuren *J Clin Oncol* 2013
How does CTLA4 blockade lead to anti-tumor immune responses?

Two not mutually exclusive mechanisms have been proposed:

1. Priming of tumor-reactive T cells
   – Against shared tumor associated antigens
   – Against mutated (neo) antigens

2. Depletion of regulatory FoxP3+ T cells from the tumor microenvironment
Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti–CTLA-4 therapy against melanoma

by Tyler R. Simpson, Fubin Li, Welby Montalvo-Ortiz, Manuel A. Sepulveda, Katharina Bergerhoff, Frederick Arce, Claire Roddie, Jake Y. Henry, Hideo Yagita, Jedd D. Wolchok, Karl S. Peggs, Jeffrey V. Ravetch, James P. Allison, and Sergio A. Quezada
Anti-CTLA4 plays a role in T cell priming and Treg depletion

Furness et al., Trends in Immunol 2014
GVAX+α–CTLA-4 combination therapy protects against tumor outgrowth through a CD4+ T cell–dependent mechanism.

α–CTLA-4 therapy does not increase the intratumoral T eff/T reg cell ratio in FcγRIV−/− mice

α–CTLA-4 therapy in FcγRIV−/− mice fails to elicit tumor protection

Does Treg depletion also occur in humans with ipilimumab?

Table 3. Immunomodulatory antibodies in clinical development.

<table>
<thead>
<tr>
<th>Target</th>
<th>Antibody</th>
<th>Species</th>
<th>Isotype</th>
<th>Predicted ADCC</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTLA-4</td>
<td>Ipilimumab</td>
<td>Humanised</td>
<td>IgG1</td>
<td>Yes</td>
<td>Bristol-Myers Squibb</td>
</tr>
<tr>
<td></td>
<td>Tremelimumab</td>
<td>Humanised</td>
<td>IgG2</td>
<td>No</td>
<td>AstraZeneca/Pfizer</td>
</tr>
</tbody>
</table>
Summary CTLA4 blockade

• CTLA4 is an important regulator of peripheral tolerance
• Blockade of CTLA4 can result in anti-tumor immunity and auto-immunity
• CTLA4 plays a role during induction of the immune response
• Blockade results in broadening of the anti-tumor immune response
• CTLA4 is highly expressed by Tregs
• Anti-CTLA4 antibody treatment may deplete Tregs from the tumor
PD1 and PD-L1 checkpoint

Freeman & Sharpe. Nat Immunol 2013
Programmed Death-1 receptor (PD1)

- Discovered in 1992 by Honjo and coworkers
  - Upregulated gene in relation to apoptosis
- Member of the Ig superfamily
- Cytoplasmic domains with ITIM and ITSM
  - Recruits phosphatases
  - Inhibits PI3K and AKT activity
- Inducibly expressed by CD4 and CD8 T cells, NKT cells, B cells, monocytes and subtypes of DC
- Expressed by both effector and regulatory T cells
- PD1/PD-L1 interaction involved in tolerance and chronic inflammation
- PD1/PD-L1 contributes to functional T cell exhaustion during chronic infection and cancer
PD1/PD-L1 play a role at the tumor/effector phase
PD-1 pathway inhibits T cell response directly downstream of the TCR

Freeman PNAS 2008
PD-1/PD-L1 interaction with B7-1
**Blocking PD-1 versus PD-L1**

*PD-1 Blockade*
- Blocks PD-L1 and PD-L2 binding to PD-1
- PD-L1 can still engage B7-1

*PD-L1 Blockade*
- Blocks PD-L1 binding to PD-1 and B7-1
- PD-L2 can still engage PD-1

Arlene Sharpe, ASCO 2013
# Expression of PD1 ligands

<table>
<thead>
<tr>
<th></th>
<th>PD-L1 (B7-H1)</th>
<th>PD-L2 (B7-DC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematopoietic cells</td>
<td>DC, macrophages, B cells, T cells, BM-derived mast cells</td>
<td>DC, macrophages, B cells, Th2 cells, BM-derived mast cells</td>
</tr>
<tr>
<td>Non-hematopoietic cells</td>
<td>Vascular endothelium, epithelia, muscle, liver, pancreatic islets, placenta, eye</td>
<td>Few, airway epithelia</td>
</tr>
<tr>
<td>Stimuli</td>
<td>Interferons ($\alpha$, $\beta$, $\gamma$)</td>
<td>IL-4 + GM-CSG</td>
</tr>
<tr>
<td>Binding partners</td>
<td>PD1, B7.1</td>
<td>PD1</td>
</tr>
<tr>
<td>Expression by tumors</td>
<td>Melanoma, RCC, HNSCC, ovary, NSCLC</td>
<td></td>
</tr>
</tbody>
</table>
PD-L1 on human tumor cells mediates T cell inhibition

Pardoll DM, Nat Rev Cancer 2012
Expression of PD-L1 by melanoma

A: melanocytic nevus
B: primary melanoma
C: magnification of B
D: subcutaneous metastasis

Taube et al. Science Transl Med 2012
PD-L1 NSCLC Sample IHC staining

PD-L1 = 0% positive
Negative

PD-L1 = 2% positive
Weak Positive (1%-49%)

PD-L1 = 100% positive
Strong Positive (50%-100%)

Courtesy of N Rizvi
Expression of PD-L1 co-localizes with TILs

Taube et al. Science Transl Med 2012
PD-1 pathway is a good target for cancer immunotherapy

- PD-1 is highly expressed on tumor-infiltrating T cells and these are functionally exhausted cells.

- Blockade of PD-1 or PD-L1 can reinvigorate exhausted TILs, enhancing their expansion, cytokine production, and cytolytic functions.
### Anti-PD1/anti-PD-L1 mAbs currently in clinical testing

<table>
<thead>
<tr>
<th>Source</th>
<th>Target molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Source</strong></td>
<td><strong>Target molecule</strong></td>
</tr>
<tr>
<td>PD1</td>
<td>PD-L1 (B7-H1)</td>
</tr>
<tr>
<td>Amplimmune Inc./GSK</td>
<td>AMP-224 (PD-L2/IgG1) fusion protein</td>
</tr>
<tr>
<td>Nivolumab (MDX-1106)</td>
<td>MDX-1105/BMS-936559 (human IgG4)</td>
</tr>
<tr>
<td>Pidilizumab (CT-011)</td>
<td></td>
</tr>
<tr>
<td>Pembrolizumab (humanized IgG1)</td>
<td></td>
</tr>
<tr>
<td>Pembrolizumab (humanized IgG4)</td>
<td></td>
</tr>
<tr>
<td>MPDL3280A (engineered human IgG1)</td>
<td></td>
</tr>
<tr>
<td>MEDI-4736 (engineered human IgG1)</td>
<td></td>
</tr>
<tr>
<td>AMP-514</td>
<td></td>
</tr>
</tbody>
</table>
Maximum Percent Change from Baseline in Tumor Size\textsuperscript{a} (Central Review, RECIST v1.1)

- ORR: I-N 40% (8% CR) I-T 28% (2% CR)
- No dose schedule superior
- FDA approved September 2014

\textsuperscript{a}In patients with measurable disease at baseline by RECIST v1.1 by central review and ≥1 postbaseline assessment (n = 317).
Percentage changes >100% were truncated at 100%.
Analysis cut-off date: October 18, 2013.

Presented by: Antoni Ribas at ASCO 2014
Overall Survival for Patients with Melanoma Treated with Nivolumab

- 1 year OS 63%
- 2 year OS 48%
- 3 year OS 41%

**Died/Treated**
- 64/107

**Median OS, mo (95% CI)**
- 17.3 (12.5, 36.7)

**OS**

<table>
<thead>
<tr>
<th>OS</th>
<th>Pts at risk, n</th>
<th>Rate, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Mo</td>
<td>86</td>
<td>82 (74, 88)</td>
</tr>
<tr>
<td>1 Yr</td>
<td>63</td>
<td>63 (53, 71)</td>
</tr>
<tr>
<td>2 Yr</td>
<td>44</td>
<td>48 (38, 57)</td>
</tr>
<tr>
<td>3 Yr</td>
<td>22</td>
<td>41 (31, 51)</td>
</tr>
</tbody>
</table>

**Presented by:** F. Stephen Hodi at ASCO 2014
Pembrolizumab

Baseline: April 13, 2012

April 9, 2013

72-year-old male with symptomatic progression after bio-chemotherapy, HD IL-2, and ipilimumab

Images courtesy of A. Ribas, UCLA.
Conclusion PD1/PD-L1 blockade

- PD1/PD-L1 are involved in peripheral tolerance
- PD1 is expressed by activated and “exhausted” T cells
- Tumors may express PD-L1 as a result of immune interrogation by T cells or as a result of an oncogenic event
- Blockade of PD1/PD-L1 interaction at the tumor site results in tumor destruction and clinical response
- Tumors expressing PD-L1 have a higher chance of responding to PD1 blockade compared to PD-L1 negative tumors
- PD1/PD-L1 blockade is an active treatment for many tumor types (melanoma, RCC, NSCLC, bladder cancer)
Immune related adverse events

- Hypophysitis
  - Ipilimumab (anti-CTLA-4)
  - All Grades: 4%

- Hyperglycemia
  - BMS-936559 (anti-PD-L1)
    - Grade 3 or 4: 1%
  - MPDL3280A (anti-PD-L1)
    - Grade 3 or 4: 7% (Melanoma)

- Pneumonitis
  - Nivolumab (anti-PD-1)
    - Grade 3 or 4: 2% (Non-small cell lung cancer)

- Liver Damage
  - MPDL3280A (anti-PD-L1)
    - Grade 3 or 4: 7% (Melanoma)
  - MK-3475 (anti-PD-1)
    - All Grades: 9% (Melanoma)
  - Tremelimumab (anti-CTLA-4)
    - Grade 3 or 4: 6% (Gastric)
  - CP-870,893 (anti-CD40)
    - Grade 3 or 4: 7% (Solid tumour)
  - BMS-663513 (anti-CD137)

- Colitis
  - MK-3475 (anti-PD-1)
    - Grade 3 or 4: 1% (Melanoma)
  - Ipilimumab (anti-CTLA-4)
    - All Grades: 7.6%; grade 3: 5.3% (Melanoma)
  - Tremelimumab (anti-CTLA-4)
    - Grade 1: 4.3%; Grade 3 or 4: 9% (Non-small cell lung cancer)

- Pruritus
  - BMS-936559 (anti-PD-L1)
    - All Grades: 6%
  - MK-3475 (anti-PD-1)
    - All Grades: 21%; grade 3 or 4: 1% (Melanoma)
  - Tremelimumab (anti-CTLA-4)
    - Grade 1: 8.4% (Colorectal)
    - All Grades: 31%; grade 3 or 4: 1% (Melanoma)

- Vitiligo
  - BMS-936559 (anti-PD-L1)
    - All Grades: 2%
  - MK-3475 (anti-PD-1)
    - All Grades: 9% (Melanoma)
  - Ipilimumab (anti-CTLA-4)
    - All Grades: 2.3% (Melanoma)

- Rash
  - BMS-936559 (anti-PD-L1)
    - All Grades: 7%
  - MK-3475 (anti-PD-1)
    - All Grades: 21%; grade 3 or 4: 2% (Melanoma)
  - Tremelimumab (anti-CTLA-4)
    - Grade 1: 8.9% (Colorectal)
    - All Grades: 33%; grade 3 or 4: 2% (Melanoma)
  - Ipilimumab (anti-CTLA-4)
    - All Grades: 19.1%; grade 2: 0.8% (Melanoma)

- Lymphopenia
  - BMS-936559 (anti-PD-L1)
    - Grade 3 or 4: 1%
  - CP-870,893 (anti-CD40)
    - Grade 3 or 4: 38% (Solid tumour)

- Diarrhoea
  - BMS-936559 (anti-PD-L1)
    - All Grades: 2%
  - MK-3475 (anti-PD-1)
    - All Grades: 20%; grade 3 or 4: 1% (Melanoma)
  - Nivolumab (anti-PD-1)
    - All Grades: 11%; grade 3 or 4: 2% (Melanoma)
  - Ipilimumab (anti-CTLA-4)
    - All Grades: 27.5%; grade 3: 4.6% (Melanoma)
    - Grade 3 or 4: 21% (Renal carcinoma)
  - Tremelimumab (anti-CTLA-4)
    - Grade 2: 6.4%; grade 3: 10.6% (Colorectal)
    - Grade 3 or 4: 6% (Gastric)
    - All Grades: 91%; grade 3 or 4: 18% (Melanoma)

- Cytokine Release Syndrome
  - CP-870,893 (anti-CD40)
  - Dacetuzumab (anti-CD40)
ipilimumab + nivolumab, response at 12 weeks

Prior therapy with HD-IL2, multiple resections, Vemurafenib, and RT; LDH > 2000 at baseline; LDH nearly normal within 3 weeks

Courtesy of R. Kefford
Checkpoint molecules

Peripheral cells or APC

PD-L1 (B7-H1)
PD-L2 (B7-DC)
B7.1/CD80
B7.2/CD86
ICOS-L (B7RP1, B7-H2)
B7-H3
B7-H4
HVEM
Gal9
MHC
CD40
CD137L
CD70
OX40L
GITRL
Adenosine
Cytokines

T cell

PD-1
CD28
CTLA-4
CD160
BTLA
Light
TIM3
LAG3
CD40L
CD137 (4-1BB)
CD27
OX40
GITR
A2aR

Signal 1

Signal 2

Signal 3
Immune Checkpoints

• Blocking of immune checkpoint has revolutionized immunotherapy of cancer
• Many cancer types show reactivity for immune checkpoint inhibition
• No true biomarkers have been found to highly predict response to treatment
• Immune checkpoint inhibitors induce a new class of adverse events
• Immune checkpoint inhibitors can be combined with other treatment
NSCLC: clinical responder to PD-1 blockade (MSKCC)
Induction of neo-antigen specific T cell reactivity upon PD-1 blockade

Day 0: 0.001%
Day 21: 0.043%
Day 44: 0.044%
Day 93: 0.022%
Day 256: 0.003%
Day 297: 0.005%
Day 456: 0.001%