Immune Checkpoint Blockade in Acute Myeloid Leukemia

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Hematology Fellow’s Conference

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Overview

- Overview of immune checkpoints
- Immune checkpoints as mechanism of immune evasion by tumors
- Blockade of PD-1/PD-L1 as promising treatment strategy in certain solid tumors
- Evidence for role of PD-1 pathway in AML
- Blockade of PD-1/PD-L1 in AML
Cancer and the Immune System

- All tumors have genetic, epigenetic changes
- Express diverse antigens, rendering them susceptible to recognition by immune system
- Survival depends on evasion of immune system
- Exploitation of immune checkpoint receptors important mechanism of resistance
What Are Immune Checkpoints?

- Co-stimulatory, co-inhibitory signals
- Regulate amplitude/quality of T cell response
- Maintain self-tolerance
- Limit collateral damage

Pardoll DM., Nat Rev 2012
Immune Checkpoints

- IL-12, IL-15, IL-6, IL-10, TGF-β, IL-1, IFN-γ

- Cytokines

- CD40

- CD70

- CD40L

- CD27

- LAG3

- TCR

- KIR

- BTLA

- HVEM

- B7-1, B7-2, B7-3, B7R1

- ICOS

- CTLA4

- CD80/CD86

- PD1

- PD-L1

- PD-L2

- Antigen-presenting cell

- T cell

- Peptide

- MHC class I or II

- Adenosine

- CD94

- Gal9

- A2AR

- TIM3

- ADAR

- Signal 1

- Signal 2
CD28
• Constitutively expressed on naïve T cells
• Binds CD80/86 upon TCR activation
• Provides strong stimulatory signal

CD80/86 (ligands): Expressed on T, B, DCs, macrophages

CTLA-4
• Expressed on activated T cells
• Sequestered in intracellular vesicles, transported to cell surface when TCR encounters antigen
• Affinity for CD80/86 >> CD28
• Regulates early stages of T cell activation
• CTLA-4 knockout mice develop fatal lymphoproliferative disorder

PD-1 Pathway

PD-1
- Expressed on **activated** T cells (and B, NK cells)
- Inflammatory signals induce ligand expression
- Limits activity of effector T cells in **peripheral tissues**

PD-L1
- Expressed on activated T, B, DCs, macrophages, monocytes, non-lymphoid tissues

PD-L2
- Expressed on DCs, monocytes
## CTLA-4 vs. PD-1

<table>
<thead>
<tr>
<th></th>
<th>CTLA-4</th>
<th>PD-1</th>
</tr>
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<tbody>
<tr>
<td><strong>Expression pattern</strong></td>
<td>Activated T cells</td>
<td>Activated T, B, NK, monos, myeloid cells</td>
</tr>
<tr>
<td><strong>Ligands</strong></td>
<td>CD80, CD86 (T, B, DCs, macros)</td>
<td>PD-L1 (Activated T, B, DCs, macros, monos, nonlymphoid tissues)</td>
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<tr>
<td></td>
<td></td>
<td>PD-L2 (DCs, monos)</td>
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<tr>
<td><strong>Primary function</strong></td>
<td>Downmodulate amplitude of T cell activation</td>
<td>Limit T cell effector function at time of inflammatory response</td>
</tr>
<tr>
<td><strong>Site of action</strong></td>
<td>At site of T cell activation (central)</td>
<td>At site of effector function (peripheral)</td>
</tr>
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</table>
Tumors Exploit PD-1 Immune Checkpoint

TIL express PD-1

Tumors express PD-1 ligands

Inhibition of tumor-specific T cells

Tumor survival

Sznol M., CCR 2013
Variety of Tumors Express PD-L1

- Malignant brain tumors
- Cervical cancer
- Pancreatic cancer
- Urothelial cancer
- Gastric cancer
- Esophageal cancer
- RCC
- NSCLC
- Glioma
- HCC

- Melanoma
- HNSCC
- Leukemia (42-57%)
- Ovarian cancer
- Prostate cancer
- Multiple myeloma
- Breast cancer
- Bladder cancer

1Sznol M., CCR 2013
2Chen X., Cancer Biol Ther 2008
3Salih H., Exp Hematol 2006
Mechanisms by which Tumors Regulate PD-L1 Expression

1). Innate immune resistance: PD-L1 expression driven by oncogenic signaling

Mechanisms by which Tumors Regulate PD-L1 Expression

2). Adaptive immune resistance: PD-L1 expression induced by inflammatory milieu

PD-L1 expression often restricted to regions of tumor with TILs

Therapeutic Blockade of PD-1

Sznol M., CCR 2013
# PD-1-Targeting Monoclonal Antibodies in Development

<table>
<thead>
<tr>
<th>Therapeutic</th>
<th>Lead Company</th>
<th>Antibody Type</th>
<th>Affinity/K_d</th>
<th>Interaction Inhibited</th>
<th>Development</th>
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<td>Anti-PD-L1</td>
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<tr>
<td>MPDL3280A</td>
<td>Herbst et al., 2013.</td>
<td>Genentech/Roche</td>
<td>0.4 nM</td>
<td>PD-L1:PD-1 PD-L1:B7.1</td>
<td>Broad (lung pivotal)</td>
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<tr>
<td></td>
<td></td>
<td>Engineered IgG1</td>
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<td></td>
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<td>(no ADCC)</td>
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<tr>
<td></td>
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<td>Modified IgG1</td>
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<tr>
<td></td>
<td></td>
<td>(no ADCC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-PD-1</td>
<td></td>
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<tr>
<td>Nivolumab</td>
<td>Brahmer et al., 2010.</td>
<td>Bristol-Myers Squibb</td>
<td>2.6 nM</td>
<td>PD-L1:PD-1 PD-L2:PD-1</td>
<td>Broad (lung, melanoma, RCC pivotal)</td>
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<tr>
<td></td>
<td>Squibb</td>
<td>IgG4</td>
<td></td>
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<tr>
<td>Lambrolizumab</td>
<td>Patnaik et al., 2012.</td>
<td>Merck &amp; Co</td>
<td>29 pM</td>
<td>PD-L1:PD-1 PD-L2:PD-1</td>
<td>Broad (melanoma pivotal)</td>
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<tr>
<td></td>
<td></td>
<td>IgG4 (humanized)</td>
<td></td>
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<td>Pidilizumab</td>
<td>Rotem-Yehudar et al., 2009; Westin et al., 2012.</td>
<td>CureTech</td>
<td>Not available</td>
<td>PD-L1:PD-1 PD-L2:PD-1</td>
<td>Broad</td>
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<tr>
<td></td>
<td></td>
<td>IgG1 (humanized)</td>
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<td></td>
<td>PD-L2 IgG1 Fc fusion</td>
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</tbody>
</table>

Chen DS., Immunity 2013
PD-1 Blockade and Solid Tumors

- Multicenter, Phase 1 trial
- N=207 patients with advanced cancer who had failed ≥1 prior therapy
Anti-PD-L1 Therapy in Advanced Cancer Patients

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Response rate</th>
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<tbody>
<tr>
<td>Melanoma</td>
<td>9/52 (17%)</td>
</tr>
<tr>
<td>RCC</td>
<td>2/17 (12%)</td>
</tr>
<tr>
<td>NSCLC</td>
<td>3/49 (10%)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>1/17 (6%)</td>
</tr>
</tbody>
</table>

Disease burden over time in melanoma pts

Brahmer JR., NEJM 2012
Anti-PD-L1 Therapy

NSCLC patient

Brahmer JR., NEJM 2012
Increasing evidence that the PD-1 pathway is important for certain solid tumors.

But, what about its role in AML?
PD-1 Pathway and AML: Pre-clinical data
AML and the Immune System

• Majority of adults with AML achieve complete remission with standard chemotherapy
• However, only 20-40% with DFS >5y
• Immune system as treatment modality has proven efficacy (e.g. alloSCT, DLI)
• PD-L1 expression by AML blasts associated with poor prognosis

¹Chen X., Cancer Biol Ther 2008
• Used PD-1 wild-type (WT) and knockout (KO) mice injected with AML cell line transduced to expression green fluorescent protein (C1498.GTP) to explore hypothesis that PD-1/PD-L1 interactions restrain anti-tumor immunity
PD-L1 Expression by AML Cells

• Measured PD-L1 expression on leukemia cells at baseline, *ex vivo*
  – Low levels of PD-L1 expression at baseline
  – High levels after 12d in mouse model
• Suggests leukemia “microenvironment” capable of inducing PD-L1 expression on tumor cells

Zhang L., Blood 2009
KO mice had significantly reduced disease burden (left), improved survival (right).
Improved Survival Associated with Stronger Immune Response?

- Harvested spleens D12, restimulated splenocytes in IFN-γ ELISPOT assay
- KO mice mounted stronger T-cell mediated immune response

Mean number of IFN-γ spot-forming cells

Zhang L., Blood 2009

319 vs. 30, p<0.001
Are CD8+ Tumor-specific T cell Responses Augmented in PD-1 KO Mice?

- PD-1 WT, KO mice injected with leukemia cell line expressing model tumor antigen
- Harvested spleens D7, restimulated splenocytes in IFN-γ ELISPOT assay
- KO mice had better priming of tumor-specific T cells

Zhang L, Blood 2009

0.49% vs.
0.25%, p=0.05

Frequency of tumor-specific CD8+ cells in spleen
Are CD8+ Tumor-specific T cell Responses Augmented in PD-1 KO Mice?

- KO mice had better effector function of tumor-specific T cells, improved survival (p=0.002) (not shown)

Zhang L, Blood 2009
Efficacy of PD-L1 Blockade

- PD-1 WT mice injected with leukemia cell line treated with anti-PD-L1 antibody vs. placebo
- Livers harvested D12
- PD-L1 blockade resulted in decreased burden of disease in liver

Zhang L, Blood 2009
Efficacy of PD-L1 Blockade

- Harvested spleens D12, restimulated splenocytes in IFN-γ ELISPOT assay
- Anti-PD-L1 treatment resulted in improved T cell effector function, longer survival (p=0.045) (not shown)

Mean number of IFN-γ spot-forming cells

50.1 vs. 29.1, p=0.001

Zhang L., Blood 2009
Summary

• PD-L1 expression on leukemia cells induced by leukemia “microenvironment”
• When PD-1/PD-L1 axis interrupted, mice had improved disease control, longer survival, stronger anti-tumor T cell responses
• Supports role for PD-1 pathway in AML
AML cells exploit PD-1 pathway to evade immune response early in disease course.

Does PD-1 pathway contribute to tumor dormancy/long-term persistence in AML?
In a model of tumor dormancy, long-term persistent leukemic cells have increased B7-H1 and B7.1 expression and resist CTL-mediated lysis

Aurore Saudemont and Bruno Quesnel
Model of Tumor Dormancy

- Mice vaccinated with leukemia cells transduced with CD154 (CD40L), IL-12\(^1\)
  - Generation of tumor-specific CD8+ T cells
  - Improved survival
- Minimal residual disease (MRD) detected in subset of long-term surviving mice\(^2\)
- Those cells studied (model of tumor dormancy)

\(^1\)Saudemont A., Leukemia 2002
\(^2\)Saudemont A., Blood 2004
MRD at 1 year

- Morphologically identical to original cells
- Still lethal when injected into naïve mice

Saudemont A., Blood 2004
MRD Less Sensitive to Lysis?

- Tested ability of tumor-specific CTLs to kill leukemia cells
- Sensitivity of tumor cells to CTL-mediated lysis decreased with time (similar results w/ CTL/d365)
- Tumor cells ability to stimulate CTLs also decreased with time

Saudemont A., Blood 2004
Analysis of MRD by Flow Cytometry

- MRD-derived cells taken from hosts after various times of persistence (d5, 15, 20, 25, 35...365)
- Found increasing PD-L1 expression, proportional to time in host

Saudemont A., Blood 2004
Efficacy of PD-L1 Blockade Against MRD

• Increased CTL-mediated killing of MRD-derived cells
• Enhanced cytokine production

*Note*: Anti-CD80 ~ anti-CTLA-4

Saudemont A., Blood 2004
Efficacy of PD-L1 Blockade Against MRD

Saudemont A., Blood 2004
Summary

• Long-term persistence of leukemia cells observed despite effective anti-tumor immune response
• Persistent cells more resistant to CTL-mediated lysis
• PD-L1 expression on tumor cells increased with time
• Blockade of PD-L1:
  – Enhanced CTL-mediated lysis, production of effector cytokines (in vitro)
  – Prolonged survival of naïve mice injected with persistent leukemic cells (in vivo)
• Suggests persistent leukemia cells may escape anti-tumor immunity via overexpression of PD-L1
PD-1 Pathway is Important in AML

- **Effector T cells**
  - TIM-3 and PD-1 co-expression identified CD8+ T cell “exhaustion” phenotype; partially reversed by PD-1 blockade\(^1\)
  - In presence of activated T cells, leukemia cells upregulated PD-L1/L2, resulted in suppression of T\(_{\text{helper}}\) cell response; suppression abolished by anti-PD-1 antibody\(^2\)

- **Regulatory T cells**
  - T\(_{\text{reg}}\)-mediated immune suppression depends on intact PD-1 axis\(^3\)

\(^1\)Zhou Q., Blood 2011  
\(^2\)Dolen Y., Eur J Immunol 2013  
\(^3\)Zhou Q., Blood 2010
PD-1 Pathway and AML: Clinical data
• N=17 pts (8 pts w/ AML) treated with single infusion of escalating doses of CT-011 (no concomitant anti-cancer treatment)
Results

- 7/8 AML pts with no change in average percentage of blasts in peripheral blood at D21
- 1/8 AML pt with response (50% → 5% blast count in peripheral blood); progressed 61 weeks after receiving CT-011
- “Safe, well-tolerated”
Toxicity Profiles: PD-1 vs. CTLA-4 targeted therapy

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Ipilimumab(^1)</th>
<th>Anti-PD-L1(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any grade No (%)</td>
<td>Grade 3 or 4 No (%)</td>
</tr>
<tr>
<td>Any AE</td>
<td>127 (97)</td>
<td>60 (46)</td>
</tr>
<tr>
<td>Rash</td>
<td>25 (19)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>36 (28)</td>
<td>6 (5)</td>
</tr>
<tr>
<td>Colitis</td>
<td>10 (8)</td>
<td>7 (5)</td>
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<tr>
<td>Hypothyroidism</td>
<td>2 (1.5)</td>
<td>0</td>
</tr>
<tr>
<td>Adrenal insufficiency</td>
<td>2 (1.5)</td>
<td>0</td>
</tr>
<tr>
<td>Increased ALT</td>
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</tbody>
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\(^1\)Hodi FS., NEJM 2010
\(^2\)Brahmer JR., NEJM 2012
Ongoing Trials with PD-1 Pathway-Targeted Immunotherapy

Sznol M., CCR 2013

<table>
<thead>
<tr>
<th>Indication</th>
<th>Compound</th>
<th>NCT#</th>
<th>Phase</th>
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<tbody>
<tr>
<td>Metastatic BRAFV600+ melanoma</td>
<td>MPDL3280A/RG7446 with vemurafenib</td>
<td>NCT01556642</td>
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<td>Resected melanoma</td>
<td>Nivolumab with ipilimumab</td>
<td>NCT01024231</td>
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<tr>
<td>Unresectable melanoma</td>
<td>Nivolumab with vaccine</td>
<td>NCT01176474</td>
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<td>Melanoma</td>
<td>Nivolumab (biomarker identification)</td>
<td>NCT01621490</td>
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<td>Melanoma</td>
<td>CT-011</td>
<td>NCT01435399</td>
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<td>Hematologic malignancies</td>
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<td>NCT01963970</td>
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<td>Multiple myeloma after SCT</td>
<td>CT-011 with DC/IFN vaccine</td>
<td>NCT01067287</td>
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<td>Relapsed follicular lymphoma</td>
<td>CT-011 with rituximab</td>
<td>NCT00904722</td>
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<td>Diffuse large B-cell lymphoma after ASCT</td>
<td>CT-011</td>
<td>NCT00532259</td>
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<td>Acute myelogenous leukemia</td>
<td>CT-011 with DC/ANL vaccine</td>
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<td>RCC</td>
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<td>RCC</td>
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<td>Advanced or mRCC</td>
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<td>Squamous NSCLC</td>
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<td>Nonsquamous NSCLC</td>
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<td>Pancreatic cancer</td>
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<tr>
<td>Metastatic colorectal cancer</td>
<td>CT-011 with FOLFOX</td>
<td>NCT00890305</td>
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CTEP Rapid Communication

Solicitation for Letters of Intent for Phase 2 Trials in Patients with Sarcoma, Nasopharyngeal Carcinoma, Cervical and Ovarian Cancers, and Acute Myeloid Leukemia

NIVOLUMAB
NSC 748726
Conclusions

• Tumors take advantage of immune checkpoints as mechanism of immune evasion
• Blockade of PD-1 axis has promising activity in certain solid tumor types
• Pre-clinical evidence supports role for PD-1 pathway in AML
• Clinical evaluation of PD-1/PD-L1 blockade in AML patients ongoing
• Given early promising results, CTEP soliciting LOIs for Phase II study of nivolumab in AML
Future Directions

• Immune checkpoint blockade in post-allogeneic stem cell transplant setting
• Targeting other co-inhibitory pathways
• Combining immunotherapeutic strategies
• Many more...
Thank you to Dr. Margolin!
Questions?
Mechanisms of Immune Suppression via PD-1 Pathway in AML

- In murine AML model, TIM-3 and PD-1 CD8+ T cells had reduced ability to produce cytokines in response to ligands; “exhaustion” partially reversed by PD-1 blockade (Zhou 2011, Zhang 2009)
- T\textsubscript{regulatory} cell-mediated immune suppression depends on intact PD-1 axis (Zhou 2010)
- In presence of activated T cells, leukemia cells upregulate PD-L1/L2, which results in suppression of T\textsubscript{helper} cell response via PD-1 pathway (Dolen 2013)
Tumor infiltrating lymphocytes express PD-1

- Ahmadzadeh et al., assessed PD-1 expression on TIL (met melanoma), T cells in normal tissue, T cells in peripheral blood (PB)

PD-1 expression: TIL > normal tissue > PB

PD-1 expression: Tumor-specific CD8 TIL > tumor-specific CD8 T cells in PB

Ahmadzadeh Blood 2009
PD-1+ T cells have exhausted phenotype/impaired effector function

- Tumor microenvironment appears to induce/maintain PD-1 expression on TIL, impairing anti-tumor immune response
PD-1 pathway and chronic infection

- High expression of PD-1 on virus-specific CD8 T cells correlates with increased viremia in patients with chronic HIV, HCV
- These PD-1 positive CD8 T cells have reduced capacity to proliferate and produce effector cytokines (“functionally exhausted”)
- Blocking interaction of PD-1 with its ligand reverses course of infection by restoring effector function in “exhausted” T cells
- As these preclinical studies indicate, PD-1 expression on virus-specific T cells appears to inhibit effectiveness of an immune response against viral infection
- Suggests that continuous exposure to antigen can result in T-cell exhaustion and immune escape
TIL express PD-1

- PD-1 expression:
  - TIL (metastatic melanoma) > normal tissue > peripheral blood (PB)
  - Tumor-specific CD8 TIL > tumor-specific CD8 cells in PB

Ahmadzadeh Blood 2009
**CTLA-4: Mechanisms of Action**

- Limits ligand available to CD28
- Causes removal (via endocytosis) of CD80, CD86 from APC surface
- Triggers intracellular pathways that suppress proliferative and anti-apoptotic signals on effector T cells
- Enhances activity of CD4+ regulatory T cells
- Downmodulates $T_{helper}$ cell activity

See Pardoll refs for all statements
Mouse model of advanced AML

Examine role of PD-1/PD-L1 interaction in T reg-mediated immune suppression
Confirmation that PD-1 pathway important in AML

• “Microenvironment” matters
  – As hepatic disease burden increased, expression of PD-1 by CD8+ T cells in liver increased
  – In spleen, where disease burden much less, PD-1 upregulation not seen

• PD-1+ T cells have reduced effector function
  – IFN-γ production significantly decreased in PD-1(+) vs. PD-1(-) CD8+ T cells

• PD-1 KO mice do better than WT mice
  – Reduced disease burden, increased survival
What about T\textsubscript{regs}? 

- Tregs and CD8 T cells in liver of AML-bearing mice
- In PD-1\textsuperscript{(-/-)} mice, increased number of T regs in naïve and AML-bearing mice

Zhou Q., Blood 2010
Importance of PD-1 expression by CD8 T cells

- WT Tregs suppress proliferation, production of IFN-γ by WT CD8 T cells
- PD-1 KO CD8 T cells more resistant to suppressive effect of WT Tregs

- Treg suppression depends on PD-1 expression by CD8 T cells

Zhou Q., Blood 2010
Importance of PD-1 expression by Tregs

- Proliferation, production of IFN-γ by WT CD8 T cells suppressed by WT Tregs
- PD-1 KO T regs less able to suppress WT CD8 T cells

- Treg suppression depends on PD-1 expression by CD8 T cells AND Tregs

Zhou Q., Blood 2010
Importance of PD-L1 expression by APC

• WT Tregs unable to suppress CD8+ T cell in presence of PD-L1 negative APC

• Treg suppression depends on PD-1 expression by CD8 T cells AND Tregs AND PD-L1 expression by APCs
PD-1 blockade

- Anti-PD-L1 Ab treatment results in increased CD8 T cell proliferation, production of IFN-γ

Zhou Q., Blood 2010
PD-1 blockade plus...

- Anti-PD-L1 Ab +/- T reg depletion +/- adoptively transferred CTL

Zhou Q., Blood 2010
Summary

• Study offers additional mechanism by which PD-1+ CD8+ T cells are suppressed:
  – Activated, PD-1+, CD8+ T cells bind PD-L1/L2 → inhibition of kinases involved in T cell activation
  – Activated, PD-1+, Tregs bind PD-L1/L2 → further suppress CD8+ T cell immune response

• Efficacy of PD-1 blockade may be enhanced by concomitant Treg depletion

Zhou Q., Blood 2010
PD-1

- Expressed on **activated** T cells (and B, NK cells)
- Inflammatory signals induce expression of ligands (PDL-1, PDL-2)
- Limits activity of effector T cells in **peripheral tissues** at time of inflammatory response

**PD-L1**: Expressed on activated T, B, DCs, macrophages, monocytes, non-lymphoid tissues (liver, lung, spleen, bone marrow)

**PD-L2**: Expressed on DCs, monocytes

Mechanisms of Suppression of Immune Response via PD-1 Pathway in AML

• AML cells express co-stimulatory molecules CD86, CD28 (Yao refs)
• Expression of co-stimulatory molecules associated with hyperleukocytosis, worse disease-free and overall survival (Yao refs)
• How do blasts take advantage of these immune stimulatory ligands to create a suppressive environment?
Mechanisms of Immune Suppression via PD-1 Pathway in AML

• In presence of activated T cells, leukemia cells upregulate PD-L1/L2, which results in suppression of $T_{\text{helper cell}}$ response via PD-1 pathway (Dolen)
• In murine AML model, TIM-3 and PD-1 CD8$^+$ T cells had reduced ability to produce cytokines in response to ligands; “exhaustion” partially reversed by PD-1 blockade (Zhou 2011)
• $T_{\text{regulatory cell}}$-mediated immune suppression depends on PD-1 immune checkpoint (Zhou 2010)
Adaptive Resistance in AML

- *In vitro* model system using myeloid leukemia cell line expressing CD86, CD28
- Naïve T cells co-cultured with CD86+, CD28+ leukemia cells
  - Resulted in CD4+ T_{helper} cell proliferation, cytokine production
  - However, continued co-culture of leukemia cells and activated T cells resulted in upregulation of PD-L1, PD-L2
- Naïve T cells co-cultured with conditioned leukemia cells
  - Reduced T cell activation, cytokine production
  - Enhanced proliferation of T_{regulatory} cells
- Suppressive effect of leukemia cells dependent on PD-1 pathway, as evidenced by introduction of antibody against PD-1 ligands
- Upregulation of PD-L1/L2 on leukemia cells suppressed T_{helper} cell response through PD-1 pathway;
Adaptive Resistance: A tumor strategy to evade immune attack

Yao S., Eur J Immunol 2013