Tumor-Immune System Interaction

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Tumor-Immune System Interaction

Tumor

Co-I Ligand

GM-CSF, VEGF, IL-1β

TAM M2

TGFβ

IDO, IL-10

T cell

T cell

Co-inhibitory

MDSC

ARG1

iNOS

Treg

Treg

CTLA-4

IDO

CD80

DC

IL-4

IL-13

IL-4

IL-10

IL-10

T cell

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Potential Immunotherapy Combinations

• Enhancing the Immune effector
  • VACCINE

• Inhibition of immune suppression effect
Immunotherapy Strategy Platform

Induction of immune response → Tumor Eradication → Inhibition of suppression

Idea adopted from Bhardwaj N, 2007
PD-1/PD-L1 Background

Vaccine/Anti-PD-1 Combination

Monitoring of tumor growth and survival

Groups:

1. Non-treated
2. aPD-1
3. E7
4. E7 + aPD-1

Day 7 after implantation of 50,000 TC-1 cell
Vaccine/Anti-PD-1 Combination

Tumor Volume, cm³

Days after tumor implantation

Percent Survival

Days after tumor implantation

- Non-immunized
- E7
- E7+aPD-1
- aPD-1

Vaccine/Anti-PD-1 Combination

E7

E7+aPD-1

aPD-1

Non-treated

n=10

n=9

n=10

n=8
Tumor-Immune Interaction
Tumor-Immune Interaction

- Tumor
  - PD-L1
  - TAM M2
  - MDSC
  - DC
  - IDO
  - IL-10
  - GM-CSF, VEGF, IL-1β
  - IL-4, IL-13
  - TGFβ
  - ARG1, iNOS

- T cell
  - PD-1
  - CD80
  - Treg
  - IL-4, IL-13
  - IL-10
Potential Immunotherapy Combinations

- Enhancing the Immune effector
  - VACCINE

- Inhibition of immune suppression effect
  - Anti PD1
  - CPM
Treg Cell Inhibitor-Cyclophosphamide (CPM)

Monitoring of tumor growth and survival

Days 0 7 8 15 22

TC-1 CPM E7+aPD-1 E7+aPD-1 E7+aPD-1
Tumor Growth

Days after tumor implantation

Tumor Volume, cm³

Non-immunized

E7

aPD-1

CPM

E7+CPM

E7+aPD-1

E7+CPM+aPD-1
Vaccine/Anti-PD-1/CPM Combination Promotes Tumor Rejection

- Non-treated (n=15)
- E7 (n=14)
- aPD-1 (n=15)
- CPM (n=15)
- E7 + aPD-1 (n=15)
- E7 + CPM (n=14)
- aPD-1 + CPM (n=15)
- E7 + aPD-1 + CPM (n=20)

Percent Survival vs Days after tumor implantation
Anti-PD-1/CPM Synergize to Decrease and Maintain Low Level of Tregs in Periphery

% of Tregs within CD4+ T cell population

Tumor (days) 7 8 11 14 18 21 26
CPM (days) 0 +1 +4 +7 +11 +14 +19

NT  CPM  aPD-1  aPD-1+CPM

NS  **  *  **  **  *
Both Vaccine/aPD-1/CPM and Vaccine/PD-L2-Ig/CPM Combinations Induce Potent Antigen-Specific Immune Responses in Tumor Bearing Mice

Anti-PD-1 mAb Synergizes with Vaccine/CPM to Increase CD8 T Cell Infiltration Into the Tumor, While PD-L2-Ig Does Not Affect CD8 T Cell Numbers

Anti-PD-1 mAb

CD8+ cells

*P<0.05, ***P<0.001

Anti-PD-1 Ab and CPM Synergize to Decrease the Level Splenic and Tumor Infiltrated Treg Cells

Day 21

Treg - % of CD4+ cells

0  5  10  15  20  25  30  35  40

E7  E7  E7  E7  aPD-1  NT
+aPD-1 +CPM +aPD-1 +CPM

*P<0.05 and **P<0.01

Absolute numbers per 10e6 tumor cells

0.0E+00  5.0E+00  1.0E+01  1.5E+01  2.0E+01  2.5E+01

E7  E7  E7  E7+aPD-1 +CPM  aPD-1 +CPM
+CPM

Treg cells

*  **

E7  E7  E7  E7+aPD-1 +CPM  aPD-1 +CPM
+CPM
Vaccine/anti-PD-1/CPM Combination Increases the CD8/Treg Ratio in Tumor Microenvironment

CD8/ Treg ratio

*P<0.05, ***P<0.001
A Phase II Clinical Trial of Anti-PD1 and CPM with Provenge® in Prostate Cancer Patients

A Phase II Clinical Trial of Anti-PD1 and Chemotherapy in Pancreatic Cancer Patients as an Adjuvant Therapy

Provenge® is a registered trademark of Dendreon Corporation
PD-1/PD-L1 Engagement Suppresses Effector T Cells

- Tumor cell
- MDSC
- Treg
- APC
- Suppressed/Apoptotic T cell
  - PD-L1
  - PD-1
  - CD28
  - B7
  - TCR
  - MHC/peptide
PD-1/PD-L1 Engagement Suppresses Effector T Cells

**Tumor cell**

**Activated T cell**
- PD-1
- PD-L1
- TCR
- CD28
- B7
- MHC/peptide

**Treg**

**APC**

**MDSC**

**Anti-PD-1**
Combinational Listeria-Based Immunotherapy

Despite all the advantages of Listeria-based vaccines, it has been demonstrated that infection with listeria lead to up-regulation of PD-Ls on immune cells (Rowe JH et al. 2008 J Immunol).

Hypothesis

Combination of Listeria-based vaccine with PD-1/PD-L interaction blocking antibody will improve the overall anti-tumor efficacy of immunotherapy.
Second Generation of Listeria-Based Immunotherapy Results in Significant Inhibition of TC-1 Tumor Growth and Prolonged Survival

Chen Z et al., CIR 2014
Lm-LLO and Lm-LLO-E7 Infection Upregulates PD-L1 Expression on Mouse DC Surface

Mkrtichyan et al., JITC 2013
Lm-LLO Infection Upregulates PD-L1 Expression on Monocyte-Derived Human DC Surface

Mkrtchyan et al., JITC 2013
Combination of *Lm-LLO-E7* with Anti-PD-1 mAb Significantly Improves Therapeutic Potency of Immunotherapy

Mkrtichyan et al., JITC 2013
Combination of *Lm*-LLO-E7 with Anti-PD-1 mAb Significantly Increases Antigen-Specific Immune Response

**Figure:**

- **Days:** 0, 8, 15, 21
- **Conditions:**
  - TC-1 tumor
  - Lm-LLO-E7 (5x10^6 CFU) + aPD-1 mAb (50 ug)
  - TERMINATION

**Graph:**

- **Y-axis:** Number of IFN\(\gamma\) spots per 10^6 splenocytes
- **X-axis:**
  - Non-treated
  - aPD-1
  - Lm-LLO
  - E7 peptide
  - Lm-LLO-E7
  - Lm-LLO-E7 + aPD-1

**Statistical Significance:**

- \(*P<0.05\)
- **\(*P<0.01\)
- ***\(*P<0.001\)

Mkrtichyan et al., JITC 2013
Treatment with Listeria Significantly Decreases Splenic MDSCs Regardless of E7 or Anti-PD-1 mAb Presence

![Graphs showing MDSC levels in different treatment conditions.]

- **Tumor free**: 2.5%
- **Tumor-bearing Non Treated**: 18.2%
- **Tumor-bearing Lm-LLO**: 6.2%

% of MDSC in spleen

- **No Tumor**: 2.5%
- **Non-treated**: 18.2%
- **E7 peptide**: 6.2%
- **Lm-LLO**: 9%
- **Lm-LLO+E7**: 6.2%
- **Lm-LLO+E7 + aPD-1**: 6.2%

*P<0.05

Mkrtichyan et al., JITC 2013
Treatment with Listeria Significantly Decreases Splenic Treg Cells Regardless of E7 or Anti-PD-1 mAb Presence

% of Treg within CD4 Tcells

*P<0.05

Mkrtichyan et al., JITC 2013
Treatment with Listeria Significantly Decreases Tumor-Infiltrated MDSCs Regardless of E7 or Anti-PD-1 mAb Presence

*P<0.05

Mkrtichyan et al., JITC 2013
Treatment with Listeria Significantly Decreases Tumor-Infiltrated Treg Cells Regardless of E7 or Anti-PD-1 mAb Presence

*P<0.05

Mkrtichyan et al., JITC 2013

CD45-PE
CD4-APC

CD45+CD4+FoxP3+

FoxP3- FITC

Number of Treg cells per 10^6 tumor cells

Non-treated  aPD-1  E7 peptide  Lm-LLO  Lm-LLO-E7 + aPD-1

Mkrtichyan et al., JITC 2013
Combination of *Lm*-LLO-E7 with Anti-PD-1 mAb Significantly Increases Number of Tumor-Infiltrated CD8 T Cells

*Mkrtichyan et al., JITC 2013*

**Graph:**
- X-axis: Number of CD8 T cells per 10^6 tumor cells
- Y-axis: Non-treated, aPD-1, E7 peptide, Lm-LLO, Lm-LLO-E7, Lm-LLO-E7 + aPD-1
- Bar chart showing increased CD8 T cell infiltration with treatment.

**Statistical Notes:**
- *P<0.05, ***P<0.001*
While in Absence of LLO Listeria Treatment by Itself Results in Treg Decrease, *Lm*-LLO Treatment Leads to More Significant Reduction

Chen Z et al., CIR 2014
*Lm* Decreases Treg Frequency by Preferentially Inducing CD4⁺FoxP3⁻ T Cell and CD8 T Cell Expansions

Chen Z et al., CIR 2014
Episomal Expression of a Truncated LLO in LmddA Induces Expansion of CD4⁺FoxP3⁻ T cells and CD8 T Cells to a Higher Level

Chen Z et al., CIR 2014