Combination Immunotherapy and Microenvironment Immunogenetics Biomarkers

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Cancer Vaccine
Tumor-Immune Modulating Network
Immune Corrective Therapy
PD-1/PD-L1 Background

Evaluation of therapeutic efficacy of vaccine/anti-PD-1 combination in TC-1 mouse model

Groups:

1. Non-treated
2. aPD-1
3. E7
4. E7 + aPD-1
Evaluation of therapeutic efficacy of vaccine/aPD-1 combination in TC-1 mouse model

![Graph showing tumor volume and percent survival over days after tumor implantation for different treatment groups: Non-immunized, E7, E7+aPD-1, aPD-1. The graph illustrates the progression of tumor volume and percent survival over time for each group.]
Tumor-Immune Modulating Network

Tumor

PD-L1

Treg

T cell

TGFβ

IDO, IL-10

GM-CSF, VEGF, IL-1β

IL-4, IL-13

TAM

M2

TAM

M2

MDSC

ARG1

iNOS

IL-10

CD80

CTLA-4

DC
Treg cell inhibitor-cyclophosphamide (CPM)

Days after tumor implantation

Tumor Growth

Tumor Volume, cm³

E7+CPM+aPD

Non-immunized

E7

aPD-1

CPM

E7+CPM

aPD-1+CPM

E7+aPD-1

E7+CPM+aPD-1
Vaccine/anti-PD-1/CPM combination promotes tumor rejection
Anti-PD-1 Ab overcomes tumor-induced suppression of stimulated Tconv cell proliferation

*in vitro*

***P<0.001
Vaccine/anti-PD-1/CPM combination induces potent antigen-specific immune responses in tumor bearing mice

Days 0 7 8 15 21
TC-1 tumor CPM E7+aPD-1 TERMINATION

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E7+aPD-1/CPM induces potent antigen-specific immune responses in tumor bearing mice.

Number of IFN-γ spots per 10⁶ splenocytes

**R²=0.8106**

***P<0.001**
Vaccine/anti-PD-1/CPM combination increases the levels of tumor-infiltrated CD8\(^+\) T cells

*P<0.05 and ***P<0.001
Anti-PD-1 Ab and CPM synergize to decrease the level splenic and tumor infiltrated Treg cells.
Anti-PD-1/CPM synergize to decrease and maintain low level of Tregs in periphery
Vaccine/CPM combination increases the levels of tumor-infiltrated CD4+ non-Treg cells

CD4 T cells

*P<0.05, ***P<0.001
Vaccine/anti-PD-1/CPM combination increases the CD8/Treg ratio in tumor microenvironment

**P<0.05, ***P<0.001**
Vaccine/anti-PD-1/CPM: Corrective Immune Therapy

- CD4+
- CD8+
- CTL
- Tumor
- TGFβ
- PD-1
- PD-L1
- Treg
- APC
Biomarkers and combinational treatment

Are there biomarkers that could be identified and used to predict responses to specific immunotherapy?
After treatment with vaccine/anti-PD-1/CPM the cytokine receptors, chemokines and factors related to tumor-infiltrated T cells (mostly Th1 type and CD8 T cells) were significantly increased in responders compared to non-responders.

Responders vs non-responders (POST-treatment biopsies, Day 21)
Responders vs non-responders (POST-treatment biopsies, Day 21)

CD8+ cells

***P<0.001  Vaccine/aPD-1/CPM

Numbers per 10e6 tumor cells
Biomarkers and combinational treatment

Are there biomarkers that could be identified and used to predict responses to specific immunotherapy?

Days 0 7 8 21 ... 75

Tumor implantation → Treatment with Vaccine/anti-PD-1/CPM

- Complete regression: 50% “Responders”
- No regression: 50% “Non-Responders”

Biopsy
Biomarkers and combinational treatment

Tested 28,000 coding transcripts analyzed (Affymetrix Array)
Identified a panel of 8 genes with over 5-fold statistically significant difference that are predictive of response
Responders vs non-responders (PRE-treatment biopsies, Day 7)
Responders vs non-responders (POST-treatment biopsies, Day 21)
Biomarkers and combinational treatment

Day 21 (Post-treatment)
- IFNg
- IL18
- IL2R
- IL21R
- IL7R
- T cell related chemokines

Pre-treatment 8 genes panel changes
- hypoxia and angiogenesis
- expansion of hematopoietic cells
- chemoattraction of activated T cells

CD8+ cells

Vaccine/aPD-1/CPM

***P<0.001
Clinical Trial Translation

A phase II clinical trial of CT-011 and CPM with Provenge in prostate cancer patients
Design

Part 1, Run In phase, up to 12 patients:

CPM 250 mg/m² (Day -1) +
Sipuleucel –T Day 0, 14 and 28 (3-6 patients)

CPM 125 mg/m²

CPM = Low Dose Cyclophosphamide
Apheresis 2-3 days prior to each dose of Sipuleucel-T for cell generation
Design

Part 2, Randomized, total 45 patients

A. Sipuleucel-T: Q 2 Wk X 3

B. Sipuleucel-T Day 0 +
   CT-011 (3 mg/kg Day 2) Q 2 Wk X 3

C. CPM (Day -1 only) +
   Sipuleucel-T Day 0 +
   CT-011 (3 mg/kg Day 2) >>> Q 2 Wk X 3

CPM = Low Dose Cyclophosphamide
Apheresis 2-3 days prior to each dose of Sipuleucel-T for cell generation
Inclusion criteria

• CRPC with progression, testosterone < 50 ng/dL.

• PSA over nadir at least 2X, 3 weeks apart.

• Failed or refused chemotherapy.
Trial Endpoints

Primary endpoint

- Feasibility Provenge™+ CPM.
- Immune Response

Secondary endpoint

- Progression-free survival
- Overall survival (OS)
- Toxicity
Clinical Trials

- A Phase I clinical trial combining CT-011 with P53 vaccine

- A phase II clinical trial of CT-011 and chemotherapy in pancreatic cancer patients as an adjuvant therapy.
## Acknowledgment

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- Osama Rahma
- Mikayel Mkritchyan
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- Callie Raulfs
- Geoffray Guittard
- Zhison Chen
- Yana Najjar
- Rasha Abu Eid
- Kevin Friedman

### Collaborators:
- Jay Berzofsky
- Francesco Marincola
- Ena Wang
- Mike Schinkler
- Rinat Rotem
- Mark Frohlich
PD-1, PD-L1, PD-L2 type molecules
Vaccine/B7-DC-Ig/CPM combinations induce potent antigen-specific immune responses in tumor bearing mice

Number of IFNγ spots per 10⁶ splenocytes

Percent of apoptotic TC-1 cells

*P<0.05, ***P<0.001
Vaccine/B7-DC-Ig/CPM combinations promote TC-1 tumor rejection

- Non-treated (n=15)
- AMP-24 (n=15)
- E7 (n=15)
- E7+AMP-24 (n=13)
- AMP-24+CPM (n=15)
- E7+CPM (n=15)
- CPM (n=15)
- E7 + AMP-24 + CPM (n=15)
In contrast to anti-PD-1 Ab, B7-DC-Ig does not require CPM to decrease the level of Treg cells

*T P<0.05, ** P<0.01, *** P<0.001
In contrast to anti-PD-1 Ab, B7-DC-Ig decreases the level of vaccine/CPM-induced of tumor-infiltrated CD4^+FoxP3^- T cells

**Anti-PD-1 Ab**

**B7-DC-Ig**

*P<0.05, ***P<0.001
In contrast to anti-PD-1, B7-DC-Ig-based treatment does not affect CD8 T cell numbers

CD8

**Anti-PD-1 Ab**

**B7-DC-Ig**

*P<0.05, **P<0.001
B7-DC-Ig decreases the level of tumor infiltrated PD-1\textsuperscript{high} CD8\textsuperscript{T} cells
In contrast to anti-PD-1 Ab, B7-DC-Ig doesn’t overcome tumor-induced suppression of stimulated Tconv cell proliferation.

No blocking of PD-1/PD-L1 interaction. Due to low affinity of AMP-224 binding to PD-1.
Differential effect of B7-DC-Ig on proliferation of different T cell subsets

**Percent of Proliferating cells**

### Tconv
- DMSO
- B7-DC 10ug
- B7-DC 20ug
- B7-DC 40ug
- IgG 10ug
- IgG 20ug
- IgG 40ug

### Treg
- DMSO
- B7-DC 10ug
- B7-DC 20ug
- B7-DC 40ug
- IgG 10ug
- IgG 20ug
- IgG 40ug

### CD8
- DMSO
- B7-DC 10ug
- B7-DC 20ug
- B7-DC 40ug
- IgG 10ug
- IgG 20ug
- IgG 40ug

* * **
Surface PD-1 expression on different T cell subsets at non-stimulated stage

- **CD8**
- **Tconv**
- **Treg**

*Note: The red bars represent PD-1 expression NS, and the MFI values are indicated on the y-axis.*
Number of surface PD-1 molecules on different T cell subsets at different time-points after stimulation
Periphery

Naive

Tconv

No direct effect of B7-DC

CD8

No direct effect of B7-DC

Treg

No direct effect of B7-DC

Activated

Maximal depletion or inhibition

Maximal effect

Maximal depletion or inhibition

Tumor-infiltration

B7-DC-Ig effect zone

PD-1 level

>80,000
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