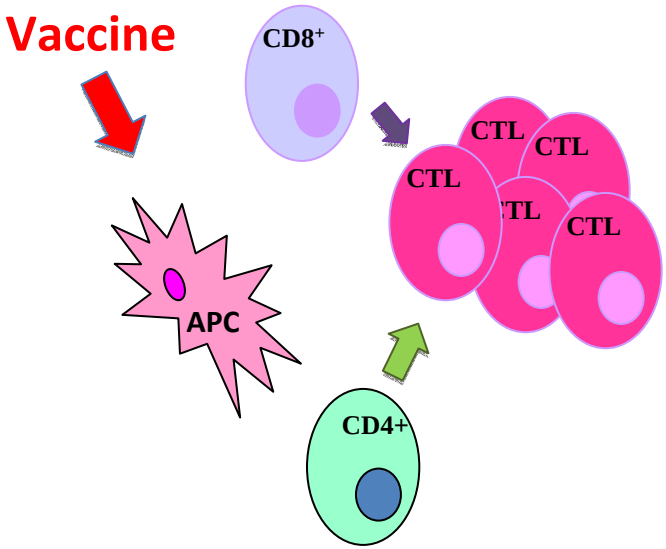
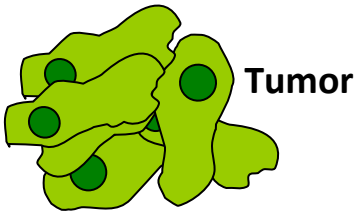
Combination Immunotherapy and Microenvironment Immunogenetcis Biomarkers

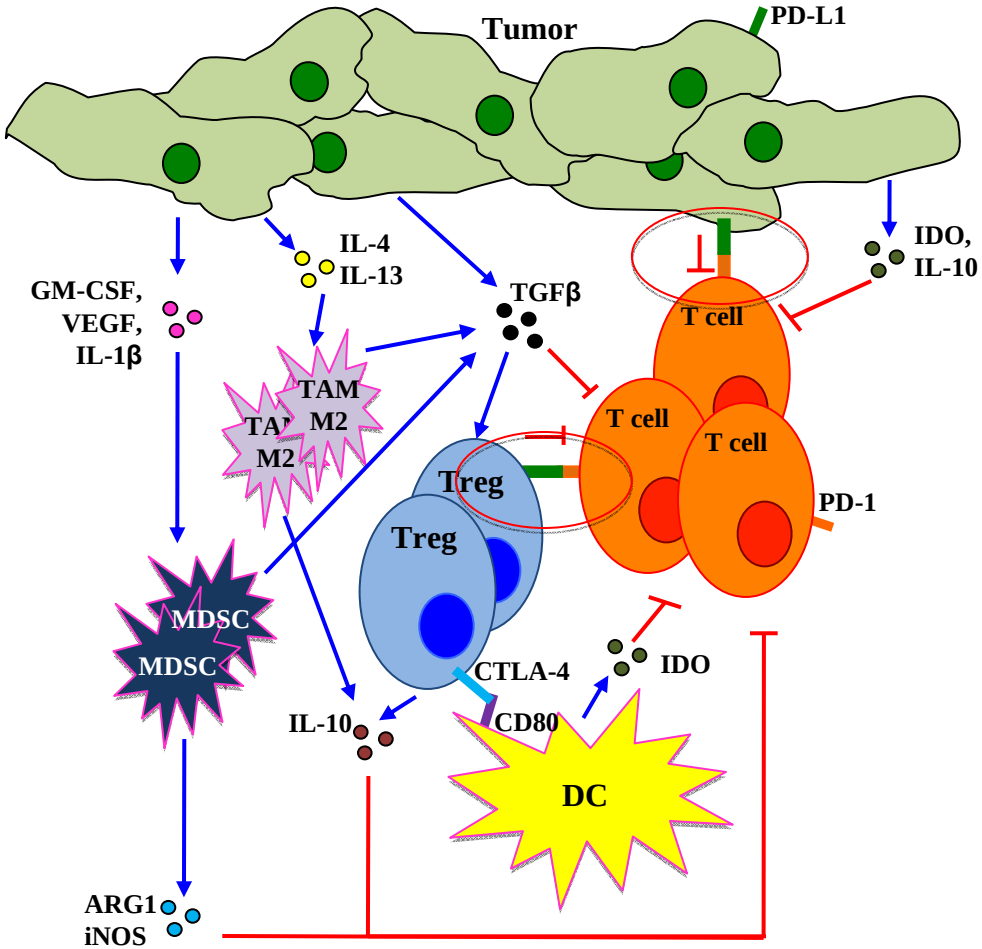
Samir N. Khleif
Georgia Health Sciences University Cancer Center

Cancer Vaccine



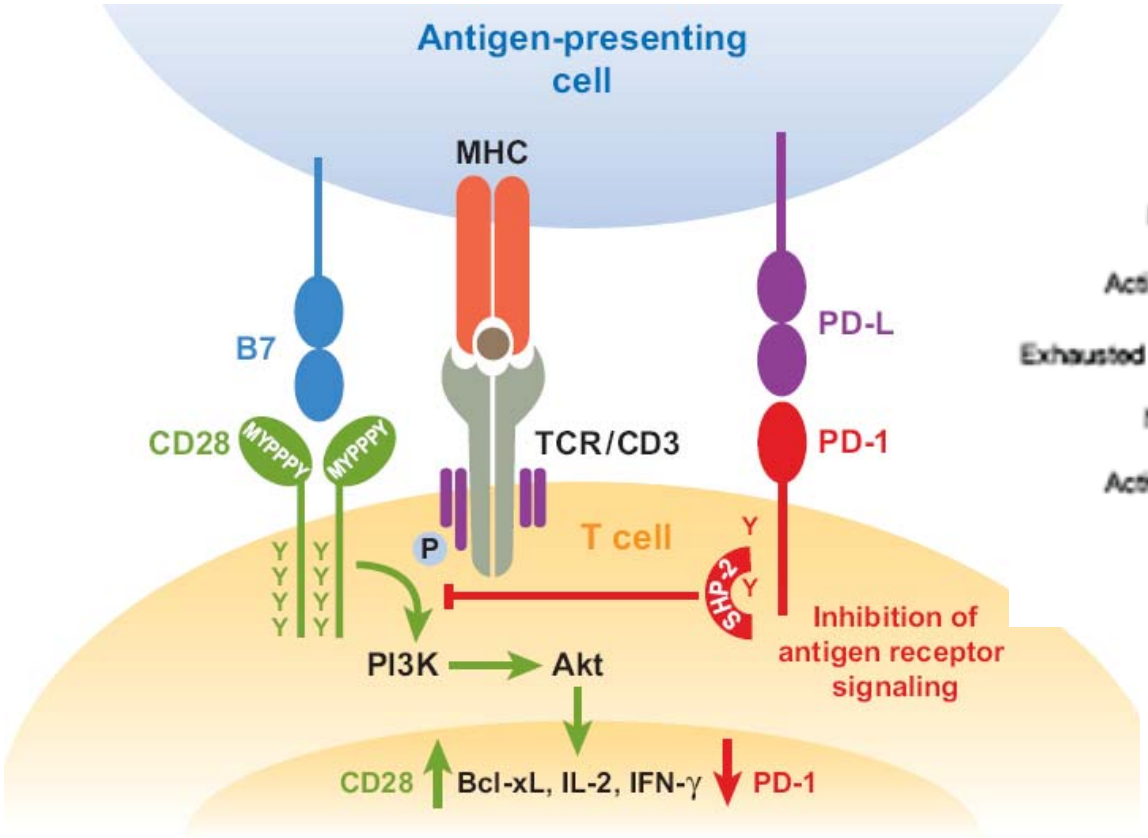


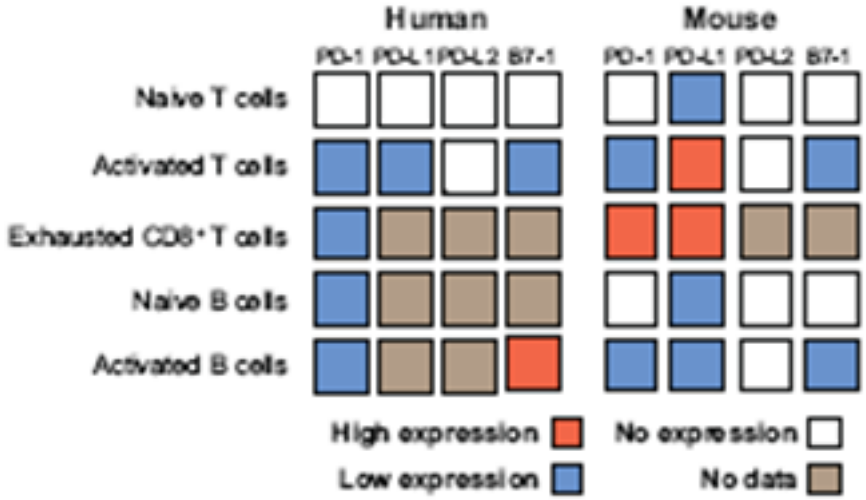
Tumor-Immune Modulating Network



Immune Corrective Therapy

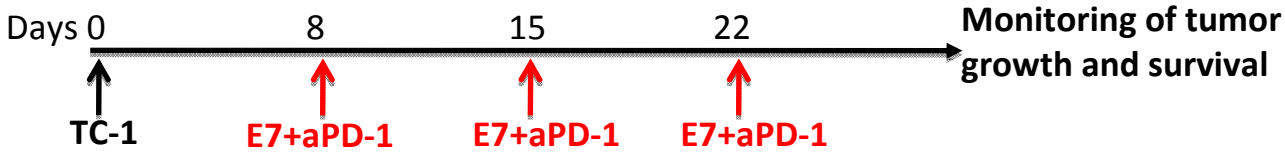
PD-1/PD-L1 Background





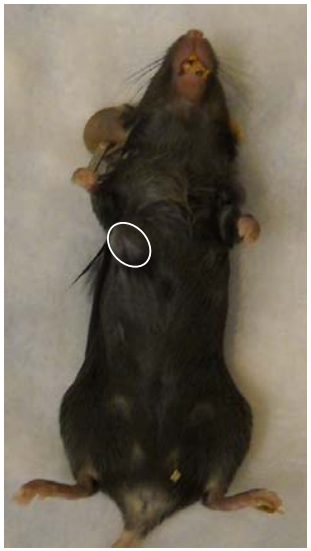
Keir et al, Annu Rev Immunol, 2008

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



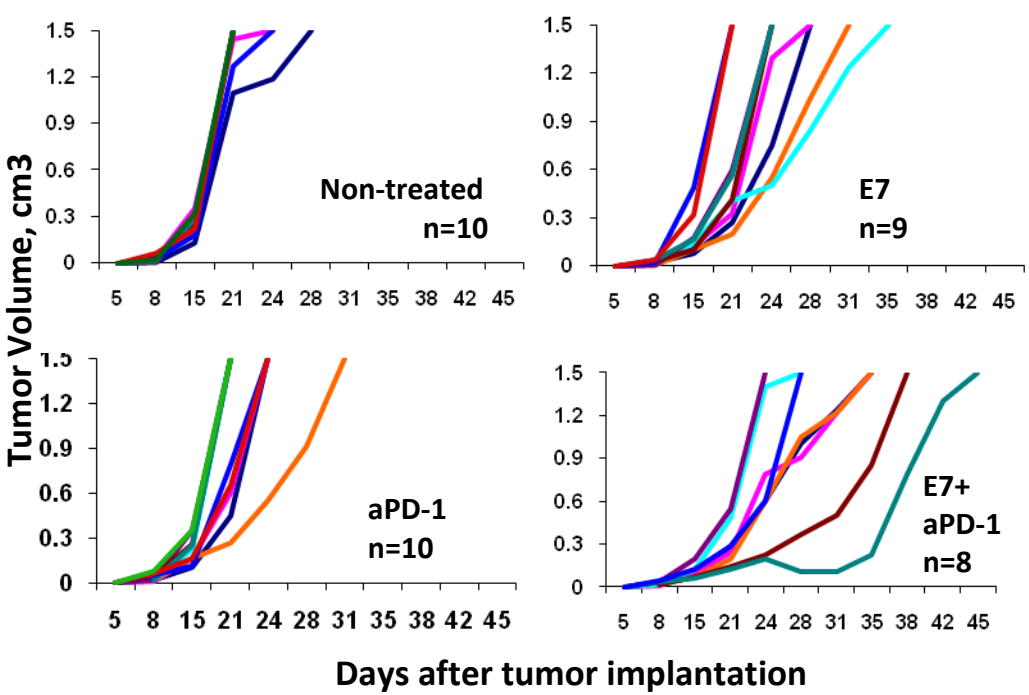
Groups:

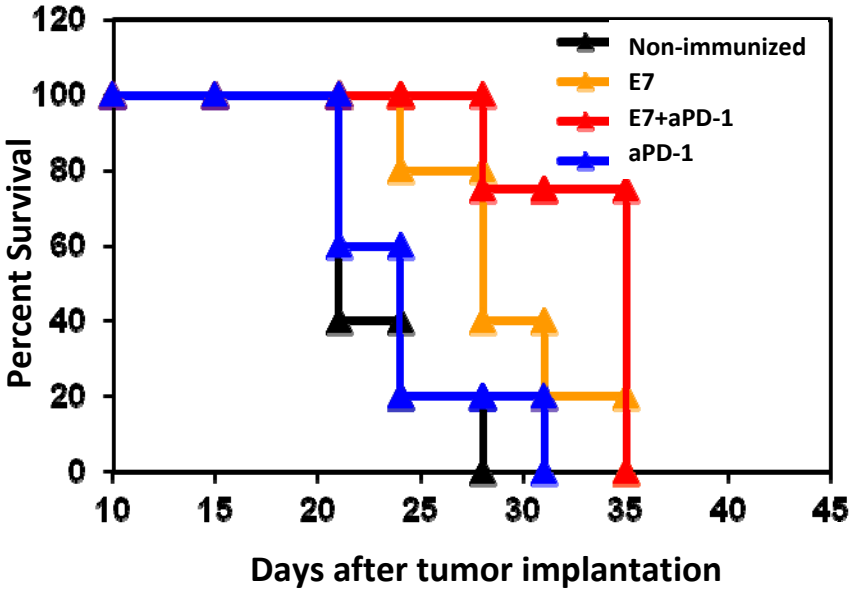
1. Non-treated
2. aPD-1
3. E7
4. E7 + aPD-1



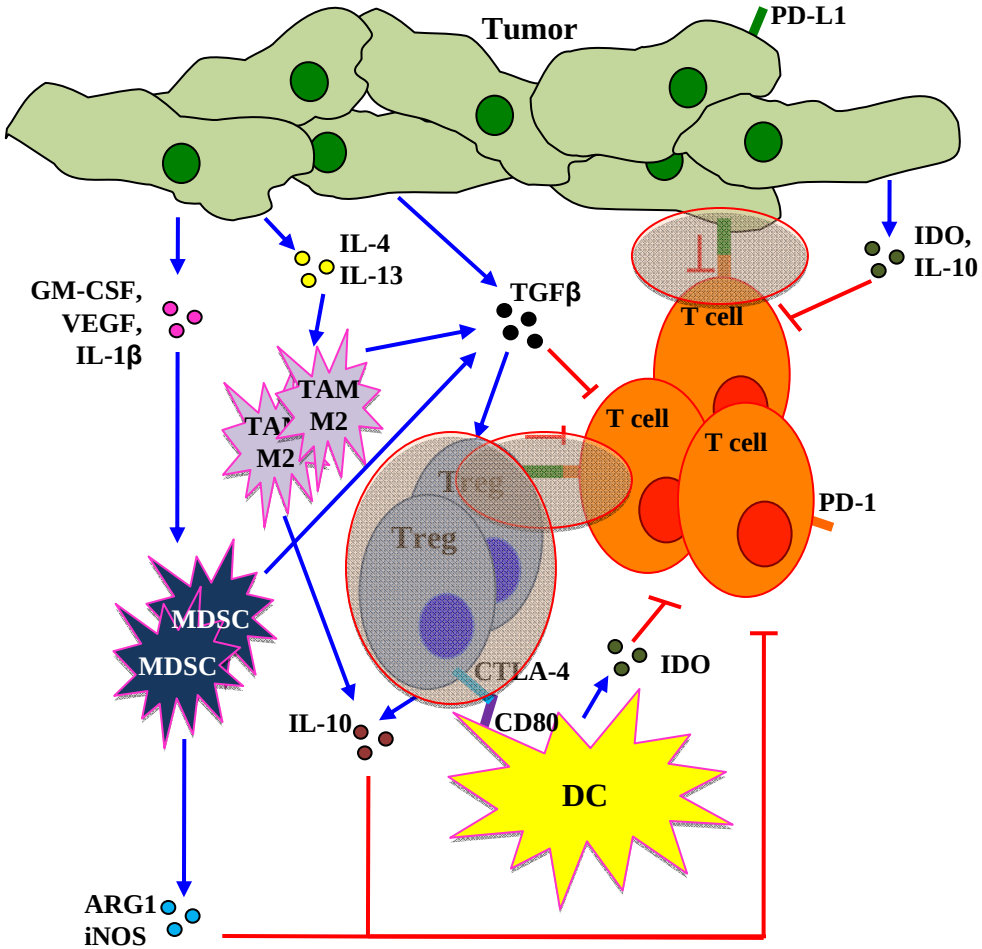
Day 7
after implantation of
50,000 TC-1 cell

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



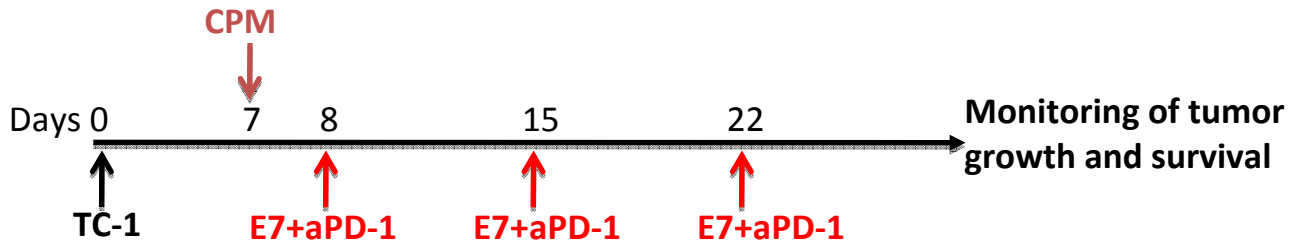


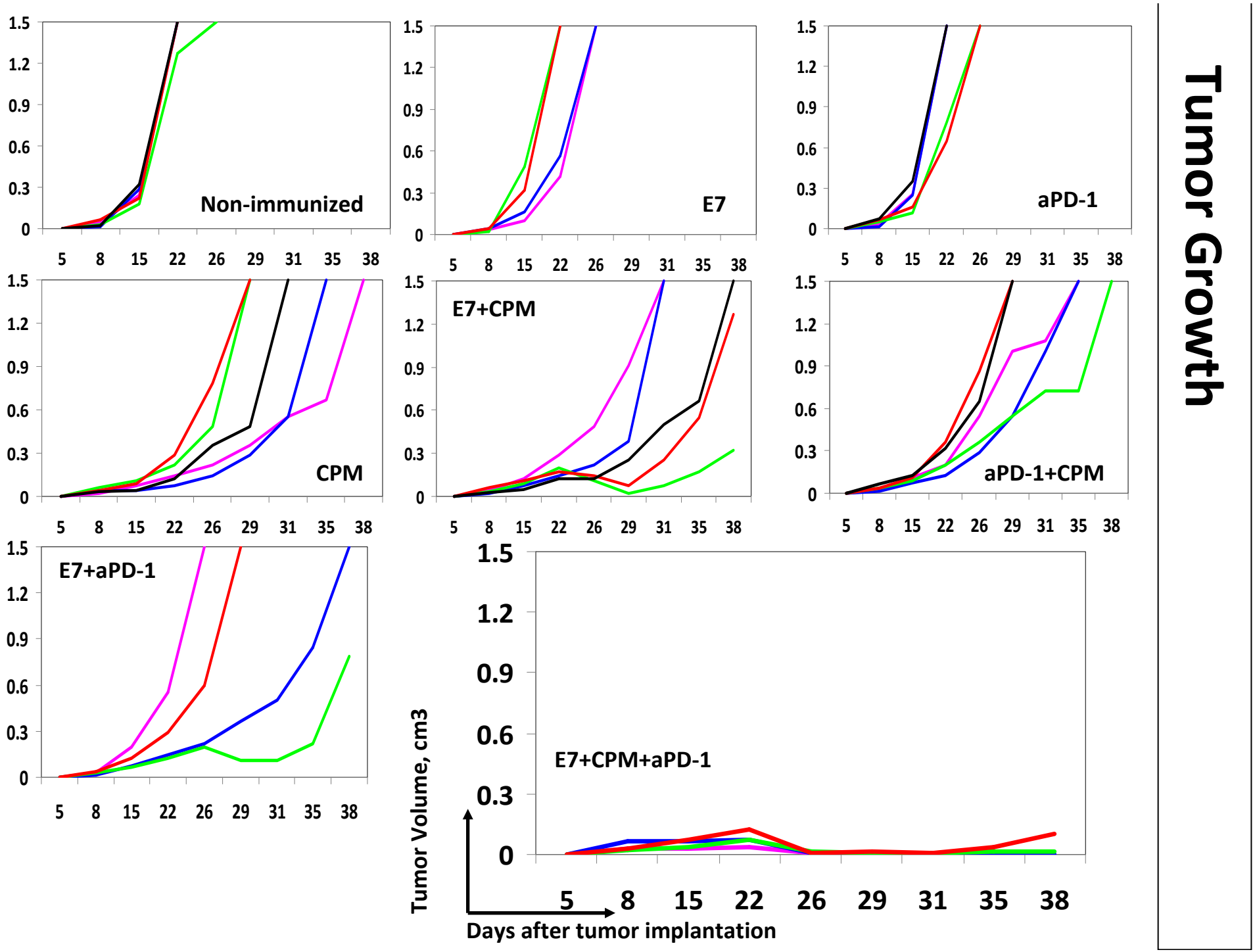
Tumor-Immune Modulating Network



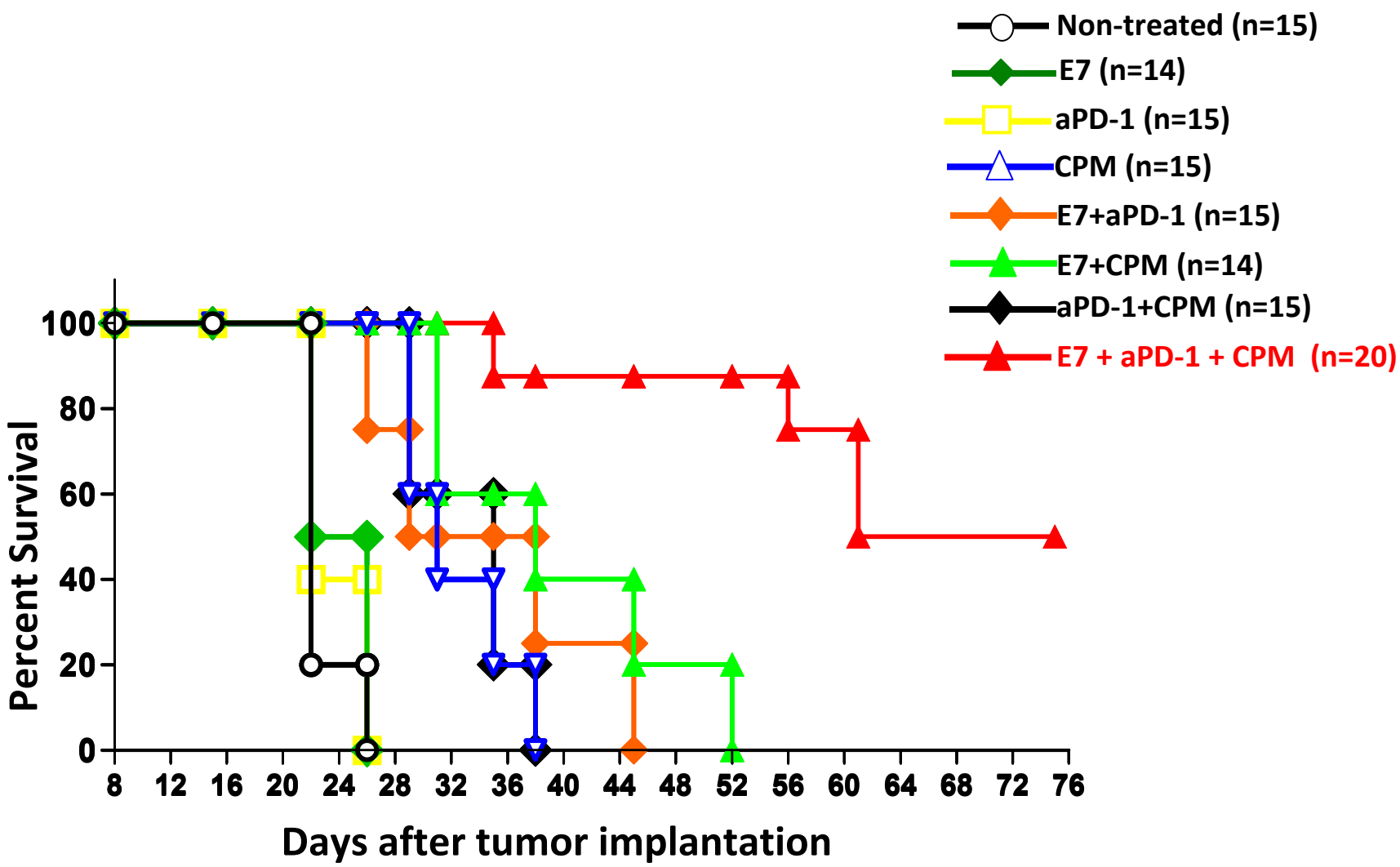
Treg cell inhibitor-cyclophosphamide (CPM)

Low Dose CPM selectively targets Treg cells, leaving other T cell populations intact ***(Lutsiak et al, Blood, 2005; Ikezawa et al, J Dermatol Sci, 2005).***

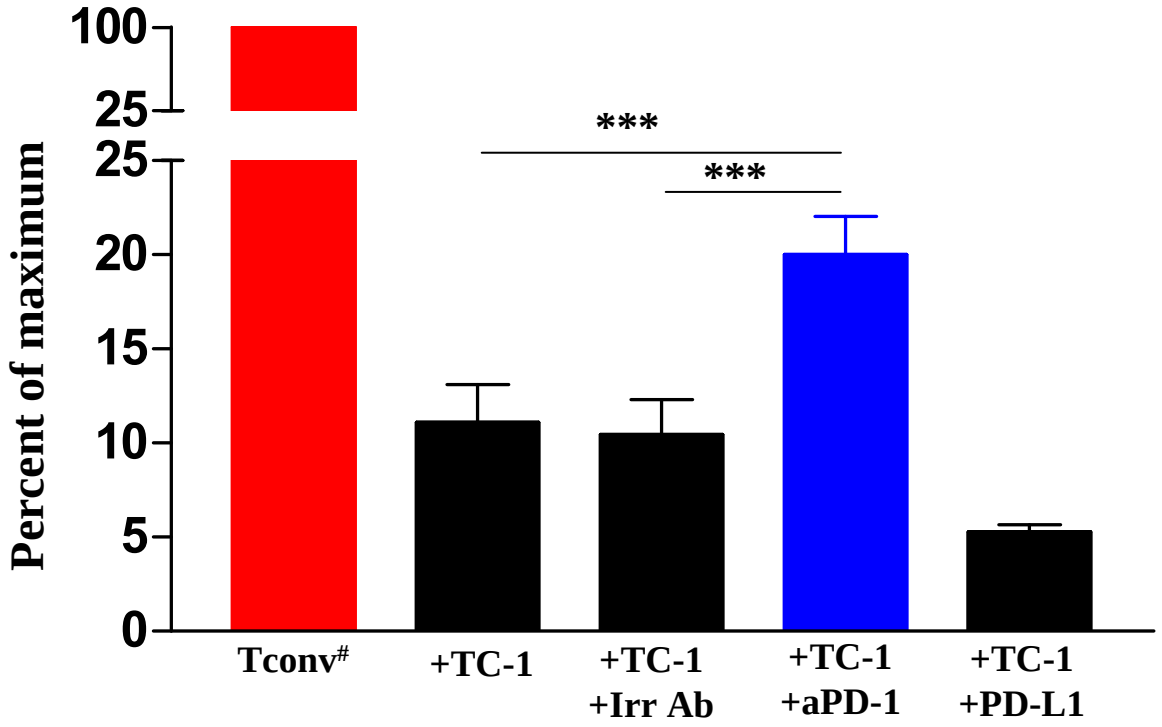



Tumor Growth
Non-immunized
E7
aPD-1
CPM
E7+CPM
aPD-1+CPM
E7+aPD-1
E7+CPM+aPD-1
Tumor Volume, cm3
Days after tumor implantation
1.5
1.2
0.9
0.6
0.3
0
5 8 15 22 26 29 31 35 38

Vaccine/anti-PD-1/CPM combination promotes tumor rejection

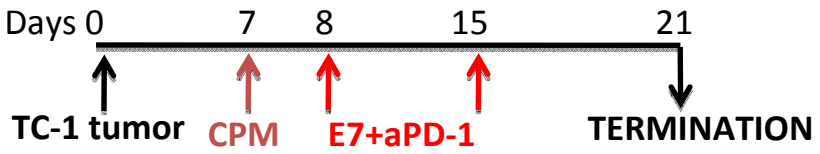


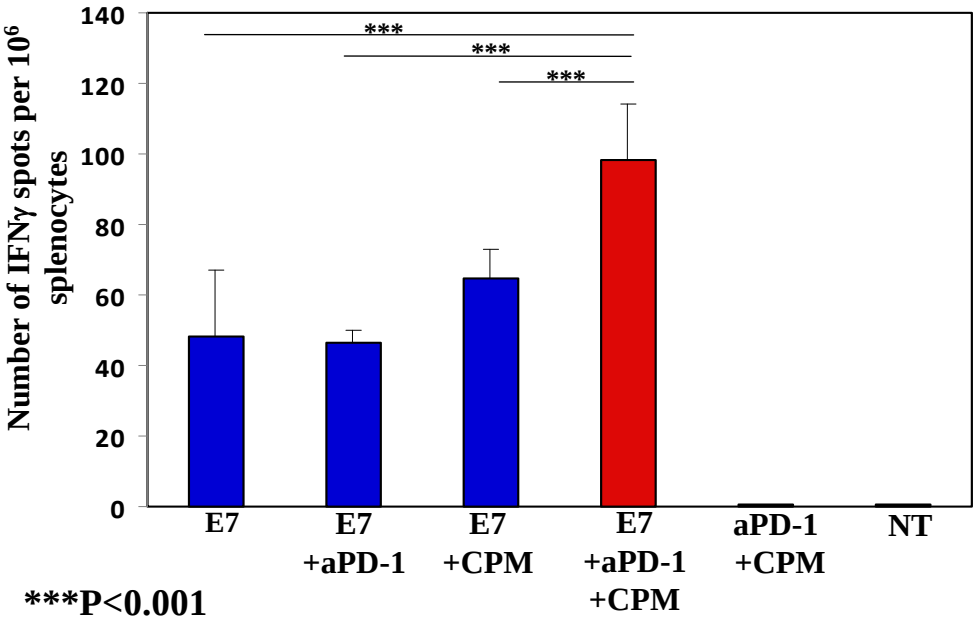
Anti-PD-1 Ab overcomes tumor-induced suppression of stimulated Tconv cell proliferation *in vitro*

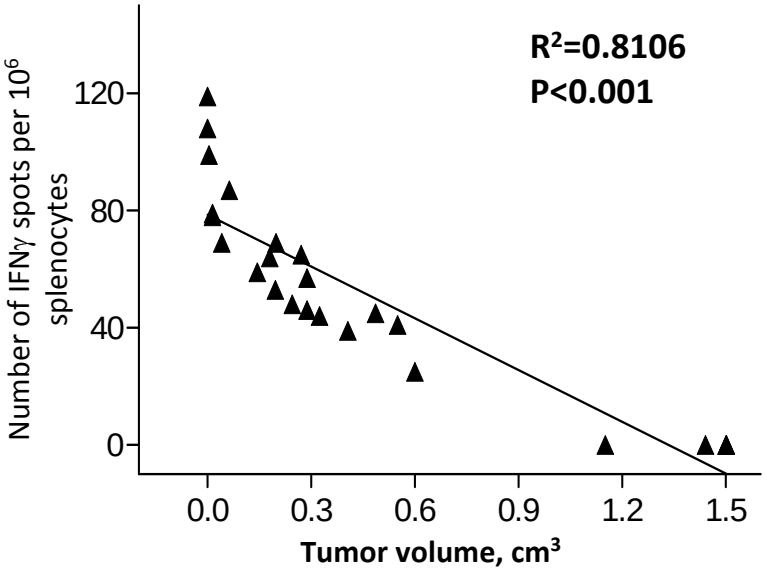


***P<0.001

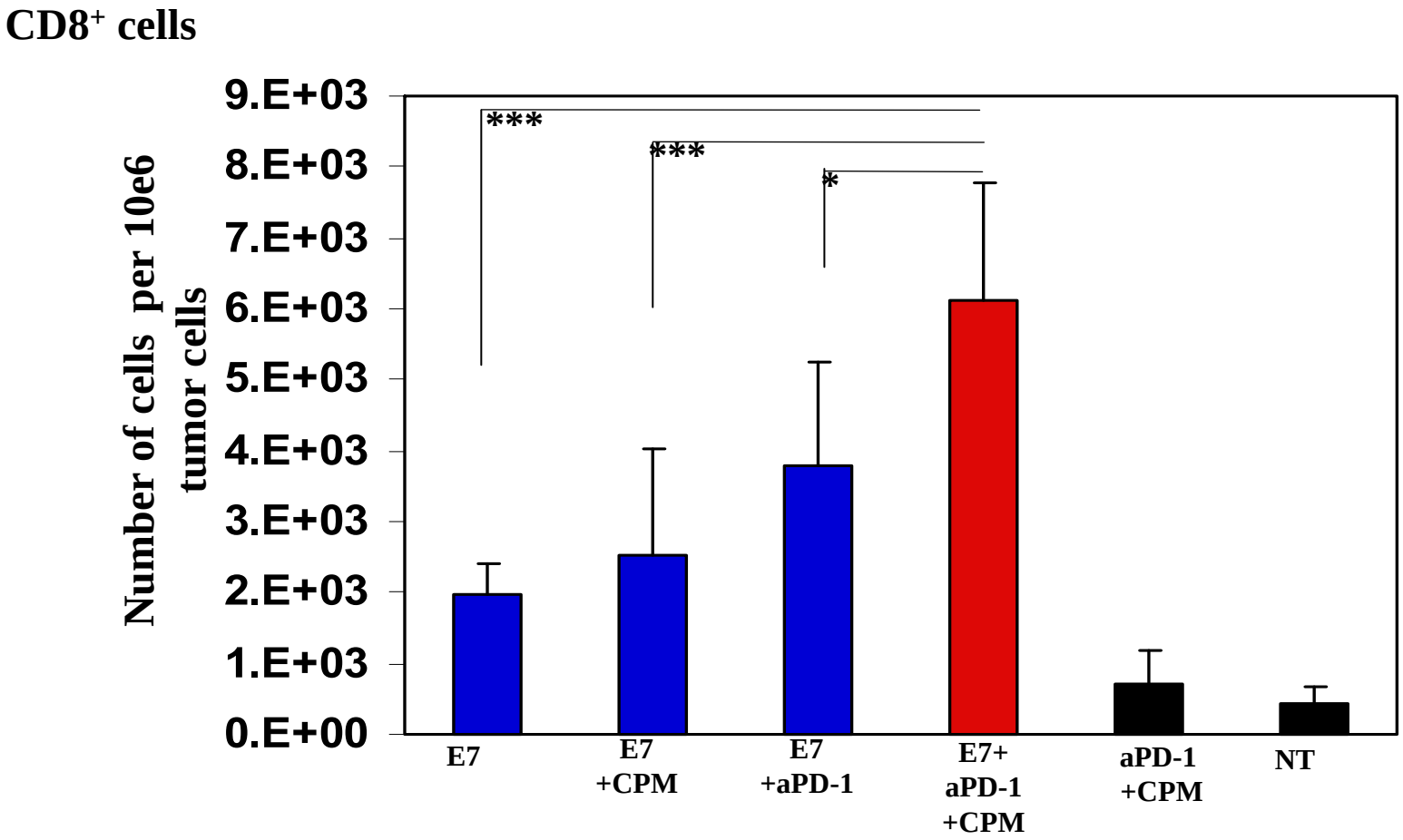
Vaccine/anti-PD-1/CPM combination induces potent antigen-specific immune responses in tumor bearing mice





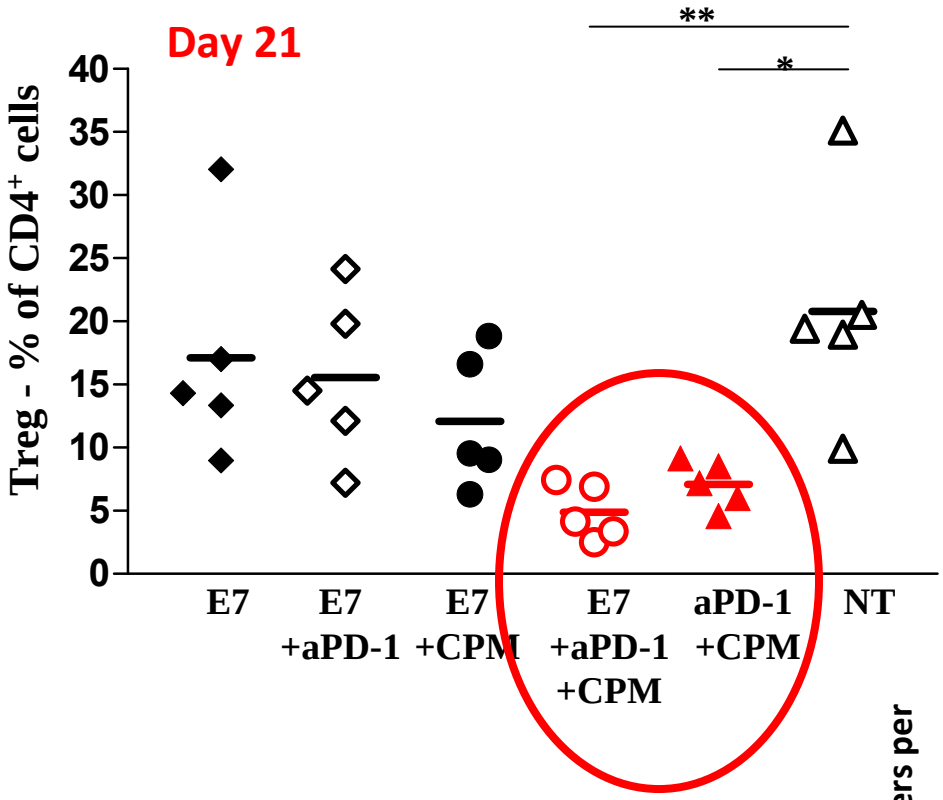


Vaccine/anti-PD-1/CPM combination increases the levels of tumor-infiltrated CD8+ T cells

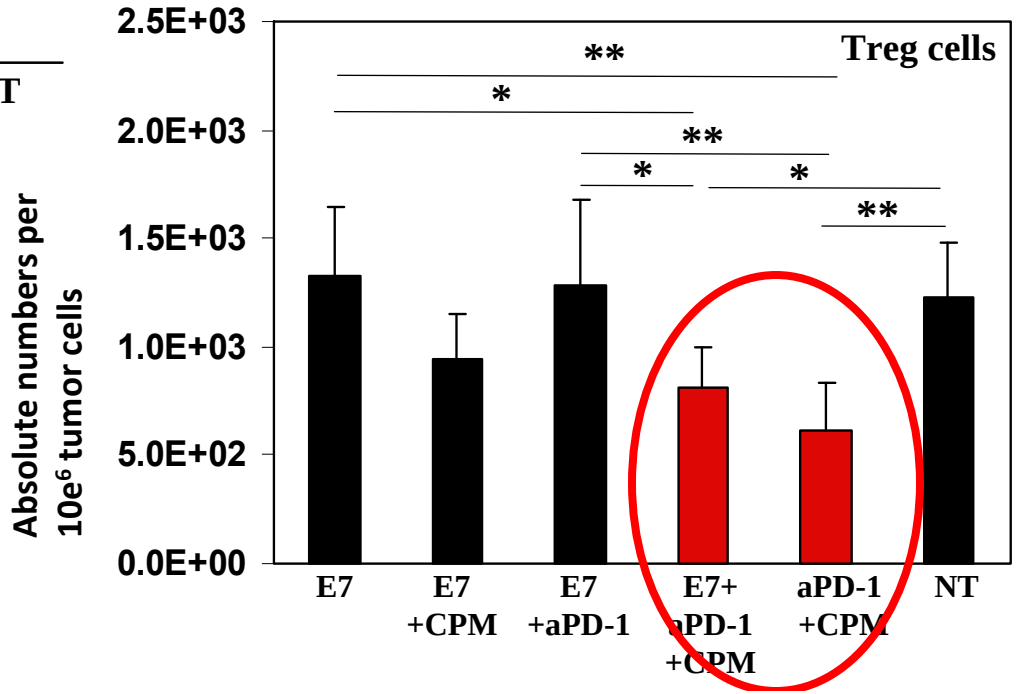


*P<0.05 and ***P<0.001

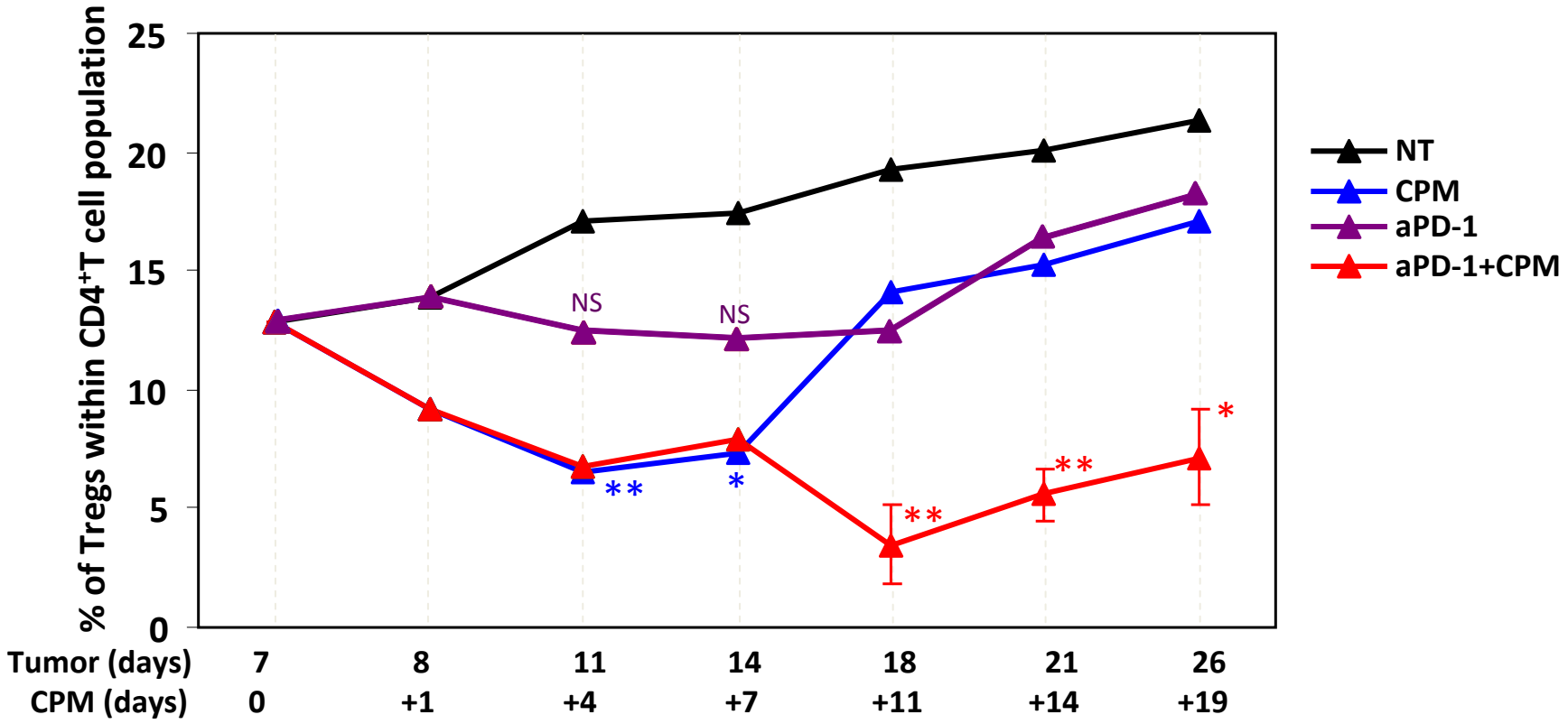
Anti-PD-1 Ab and CPM synergize to decrease the level splenic and tumor infiltrated Treg cells



*P<0.05 and **P<0.01

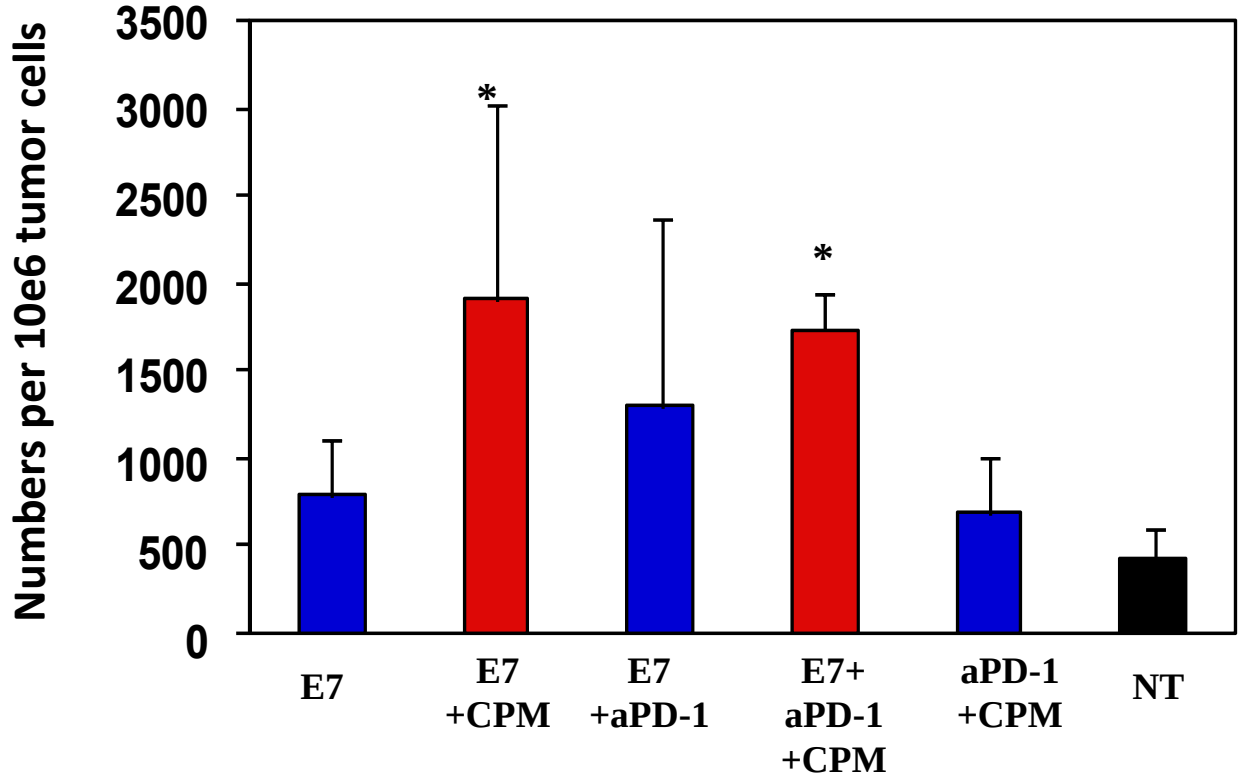


Anti-PD-1/CPM synergize to decrease and maintain low level of Tregs in periphery



Vaccine/CPM combination increases the levels of tumor-infiltrated CD4+ non-Treg cells

CD4 T cells



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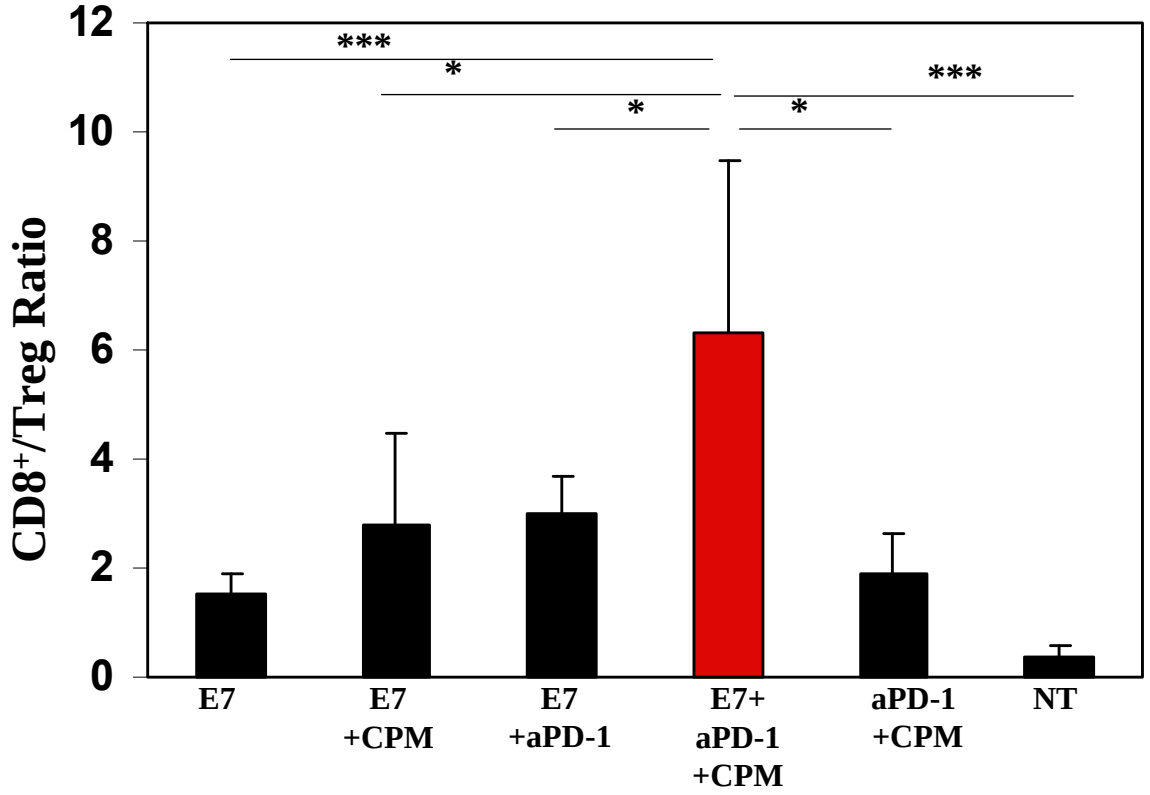
Vaccine/anti-PD-1/CPM combination increases the CD8/Treg ratio in tumor microenvironment

CD8/ Treg ratio



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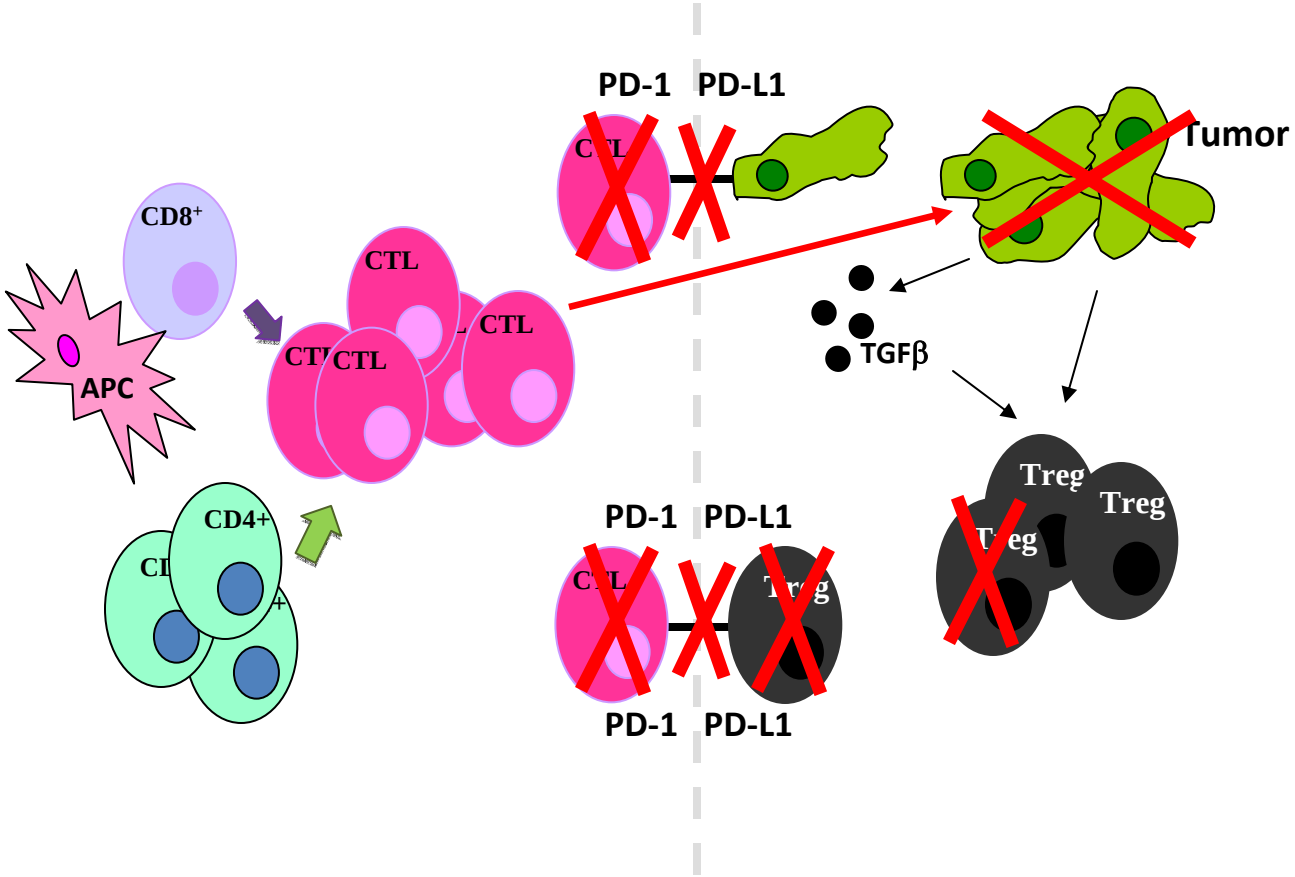
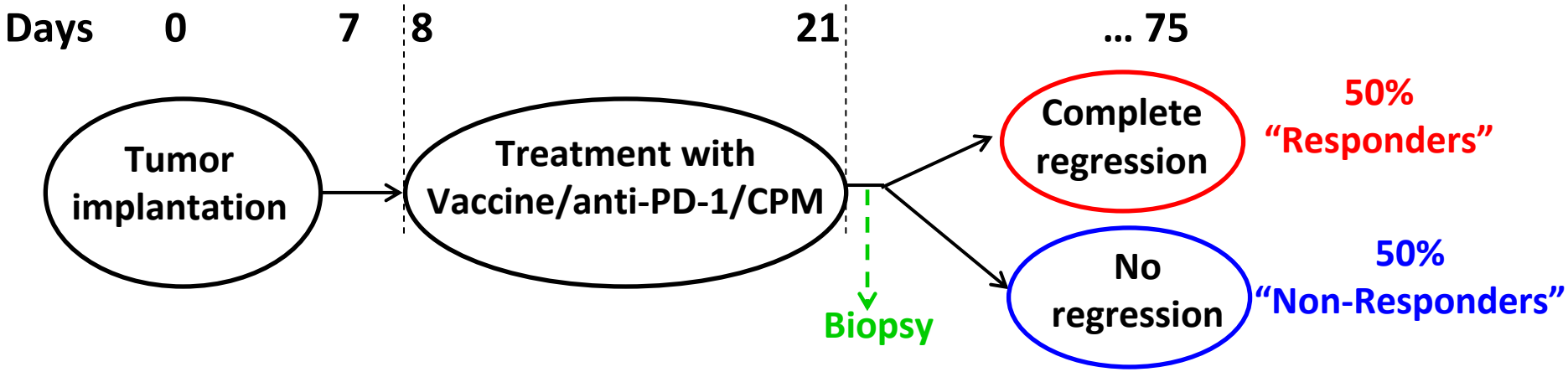
Vaccine/anti-PD-1/CPM: Corrective Immune Therapy



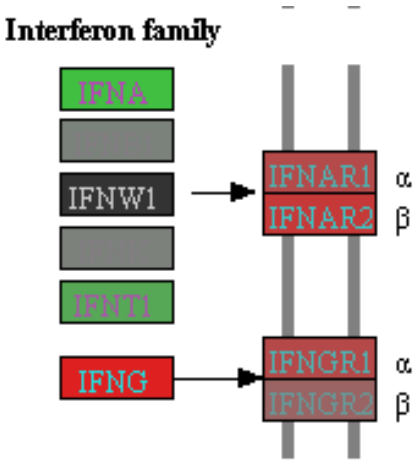
Biomarkers and combinational treatment

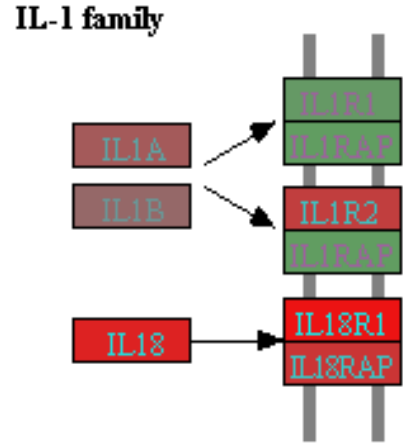
Are there biomarkers that could be identified and used to predict responses to specific immunotherapy?

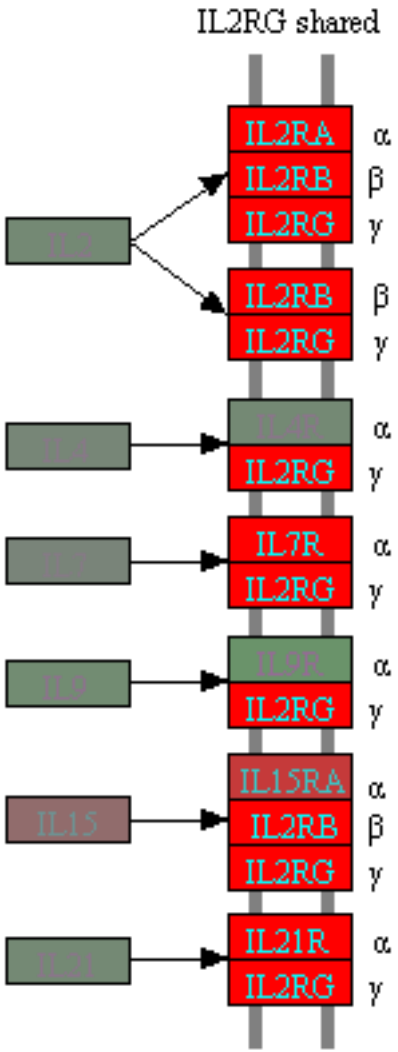


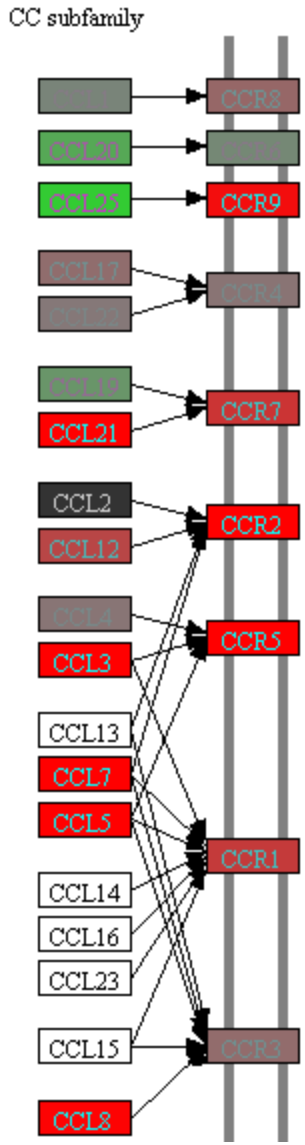
Responders vs non-responders (POST-treatment biopsies, Day 21)

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.

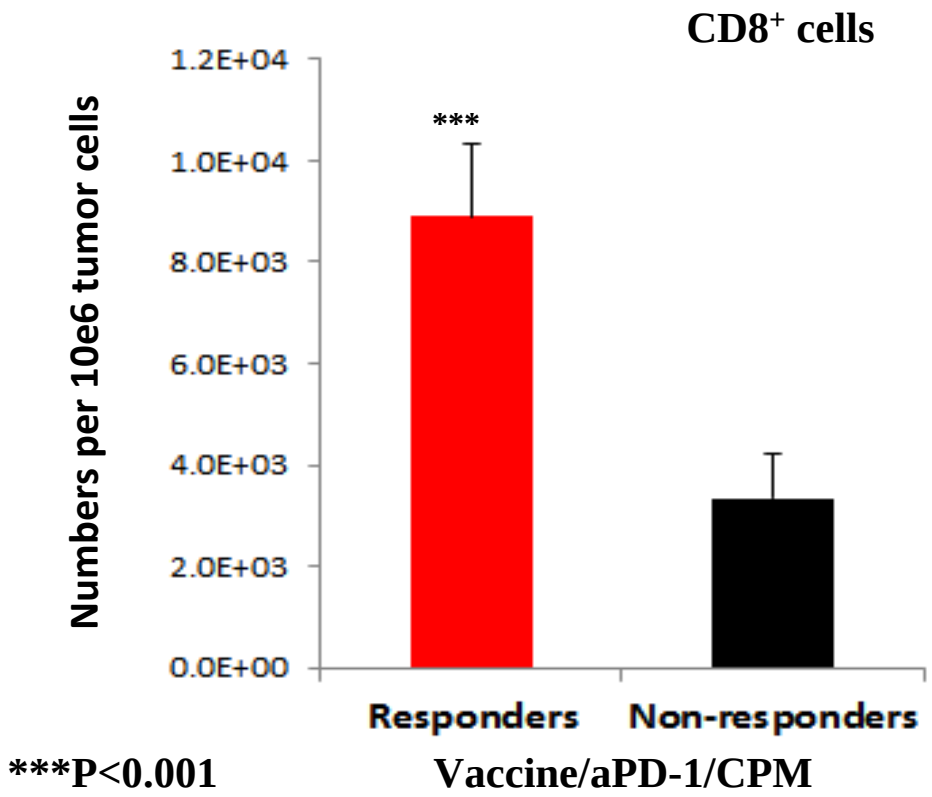








Responders vs non-responders (POST-treatment biopsies, Day 21)



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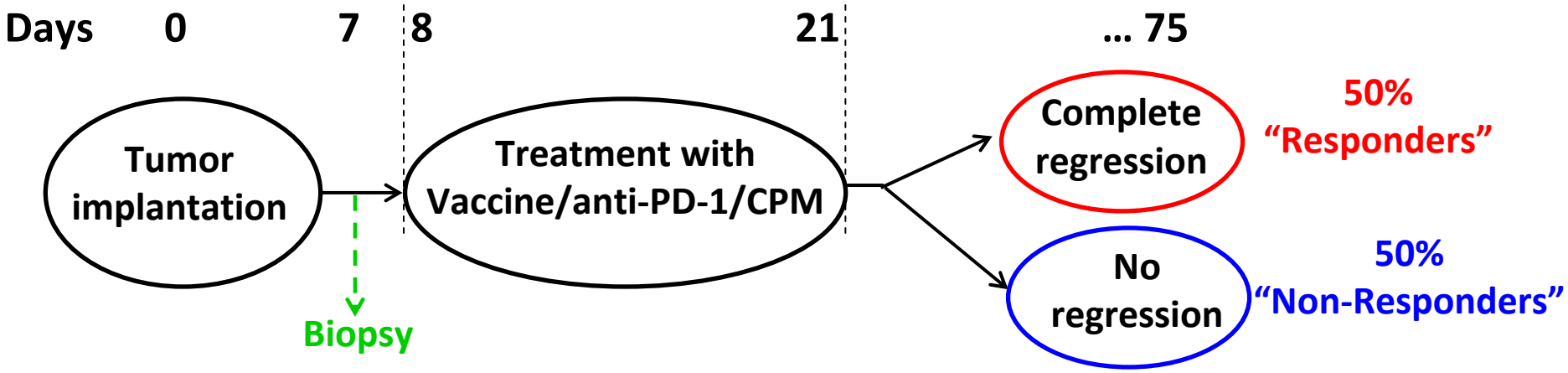
Vaccine/aPD-1/CPM

Biomarkers and combinational treatment

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

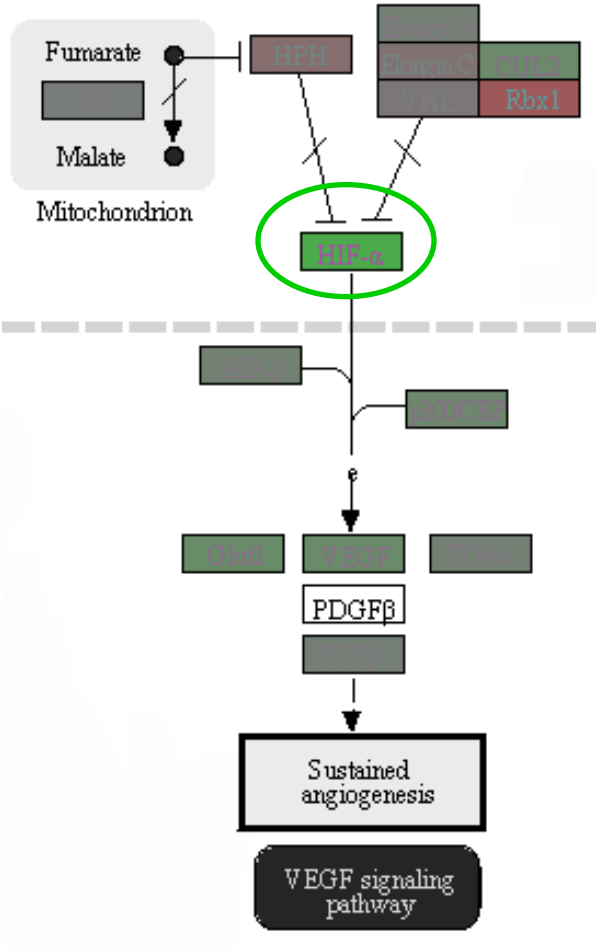


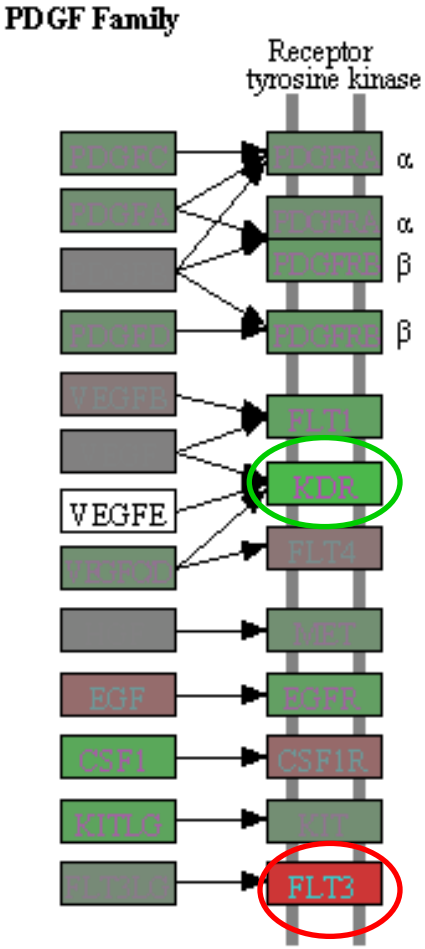
Biomarkers and combinational treatment

Tested 28,000 coding transcripts analyzed (Affymetrix Array)

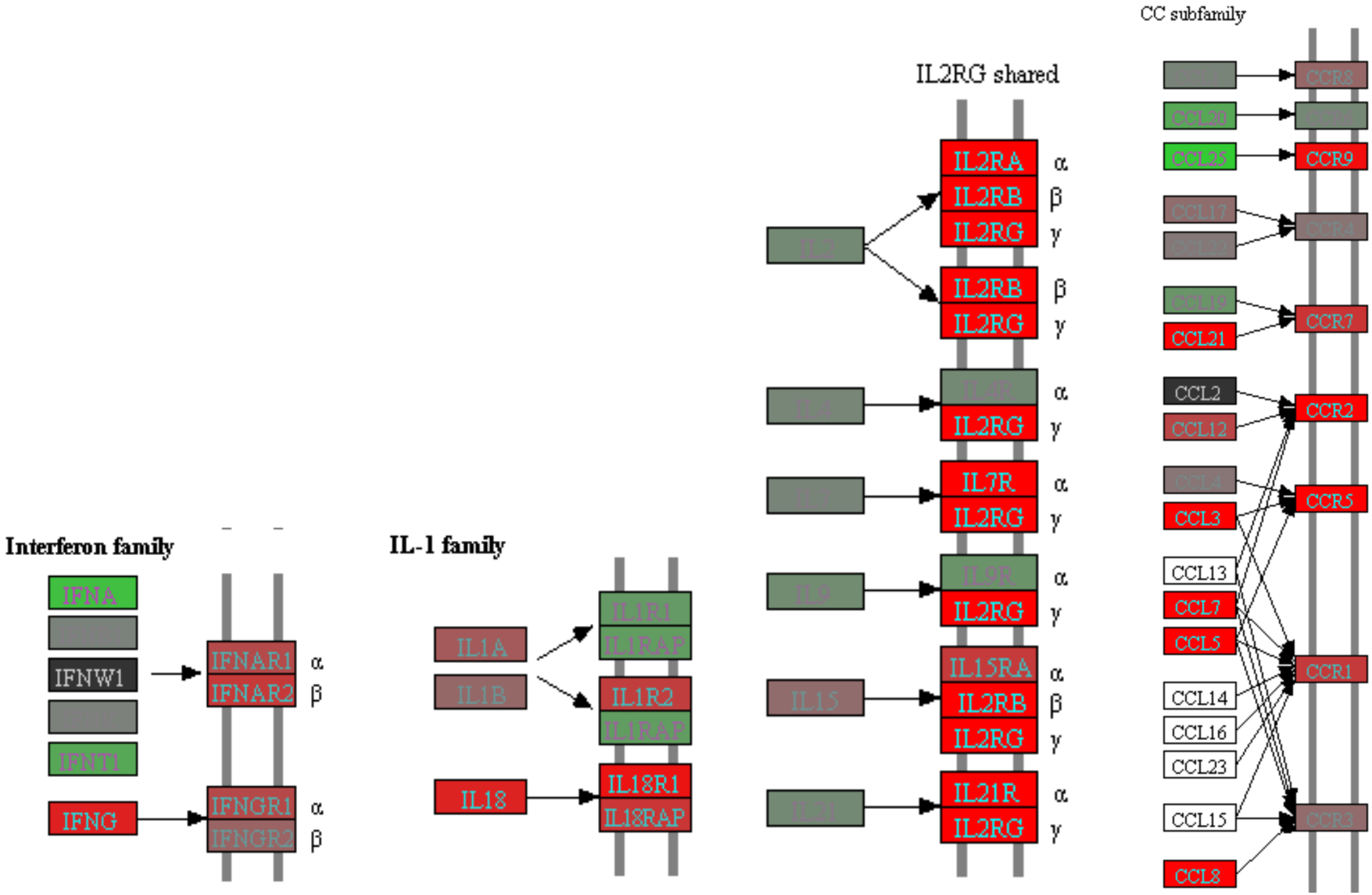
Identified a panel of 8 genes with over 5-fold statistically significant difference that are predictive of response

Responders vs non-responders (PRE-treatment biopsies, Day 7)





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



Biomarkers and combinational treatment

Pre-treatment 8 genes panel changes

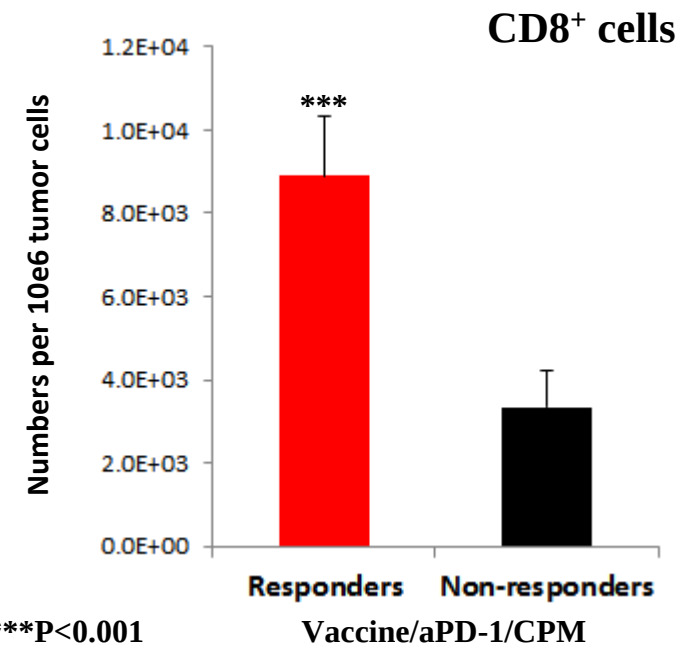
hypoxia and angiogenesis

expansion of hematopoietic cells

chemoattraction of activated T cells

Day 21 (Post-treatment)

- **IFNg**
- **IL18**
- **IL2R**
- **IL21R**
- **IL7R**
- **T cell related chemokines**



***P<0.001

Vaccine/aPD-1/CPM

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/m^2 (Day -1) +
Sipuleucel –T Day 0, 14 and 28 (3-6 patients)

CPM 125 mg/m^2

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

Khleif's Lab

- Osama Rahma
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- Zhison Chen
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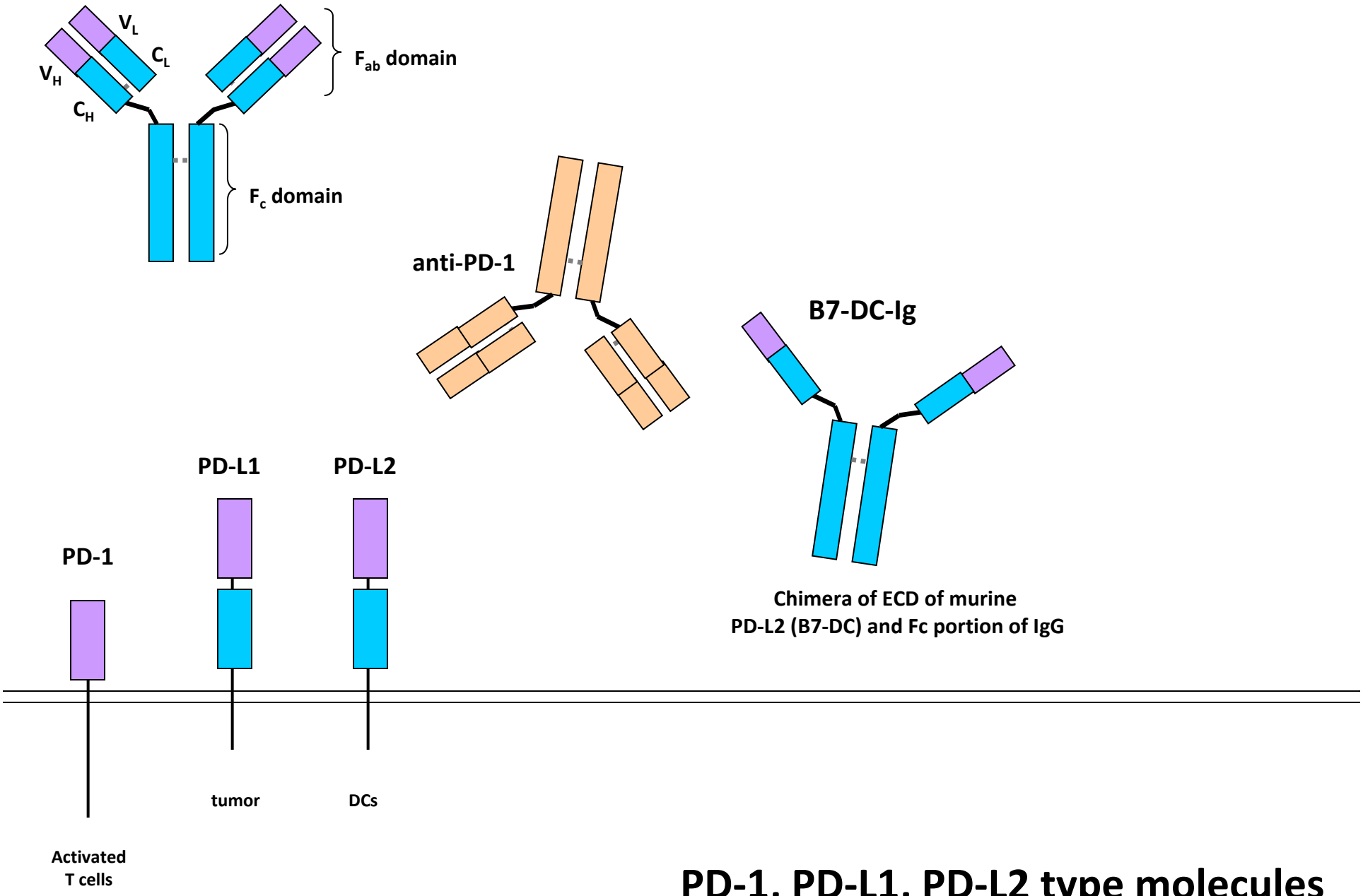
- Kevin Friedman

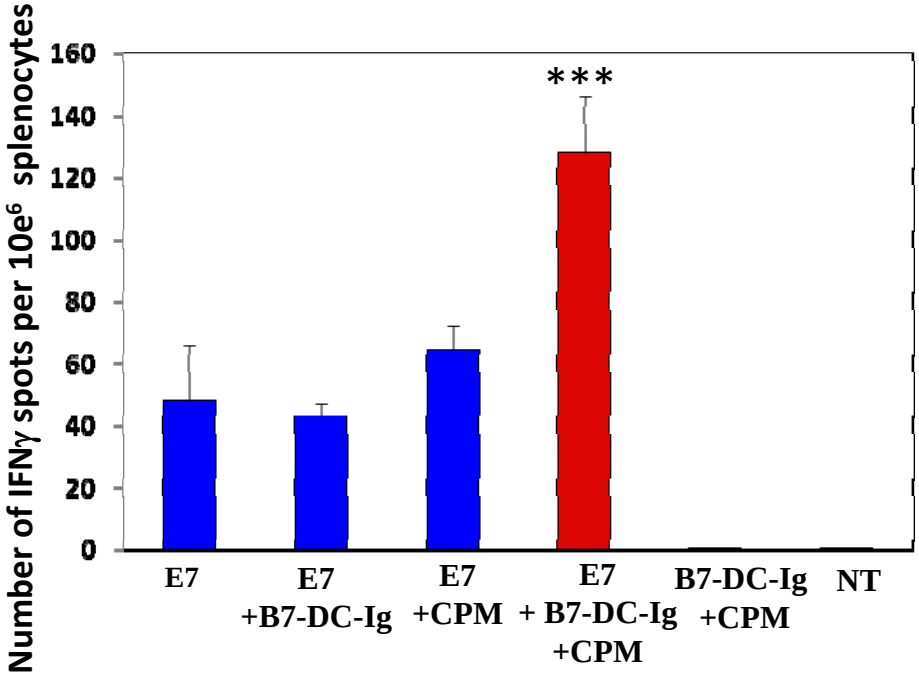
Collaborators:

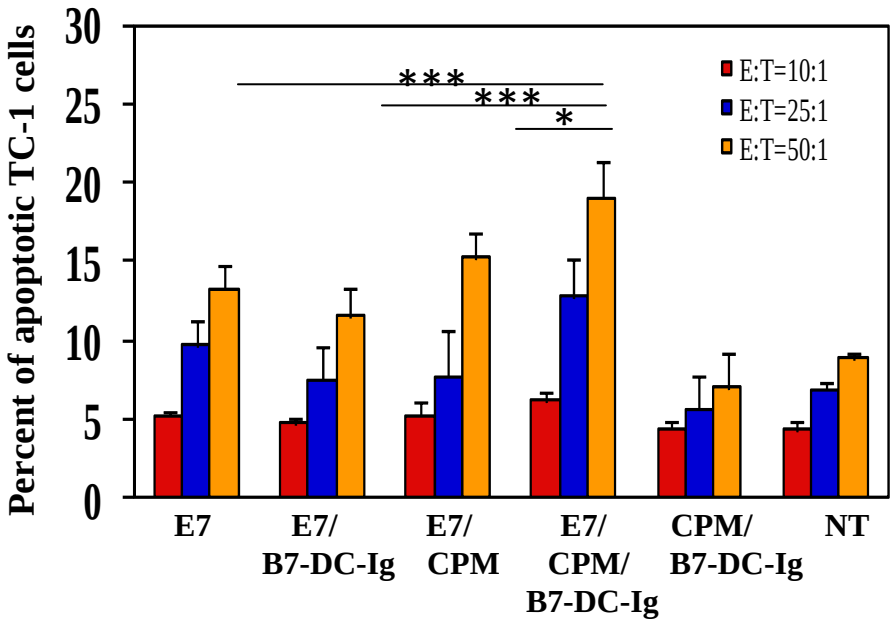
- Jay Berzofsky
- Francesco Marincola
- Ena Wang
- Mike Schinkler
- Rinat Rotem
- Mark Frohlich



PD-1, PD-L1, PD-L2 type molecules

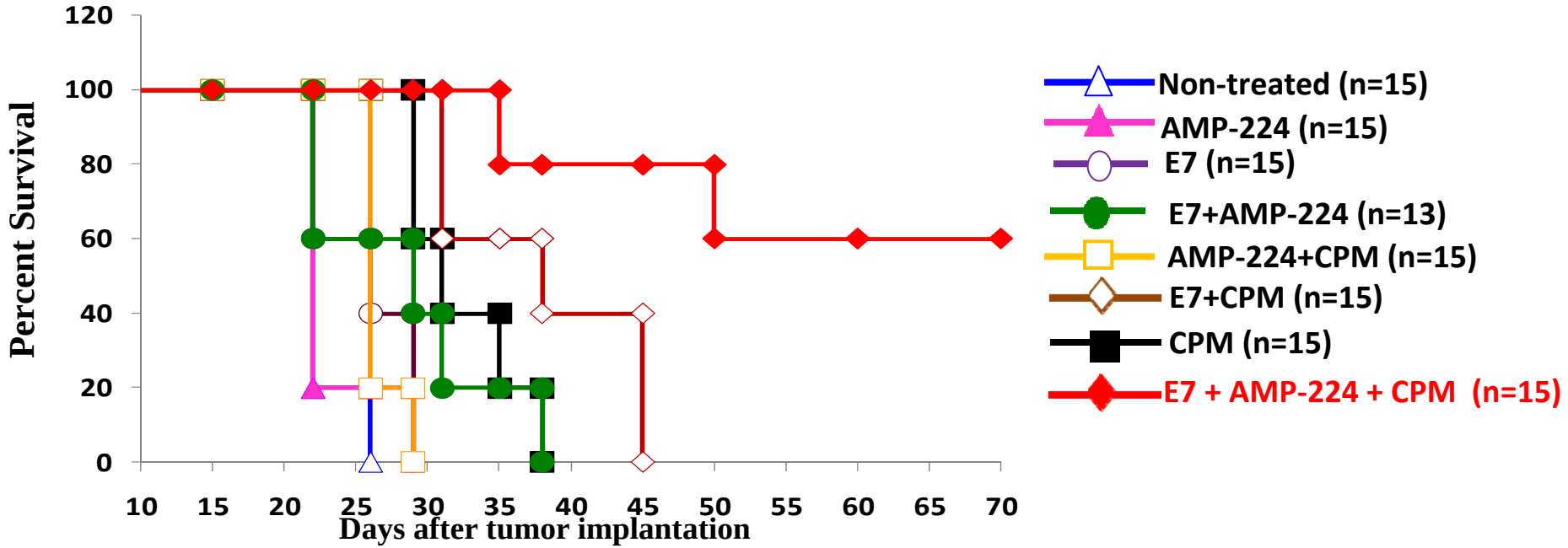
Vaccine/B7-DC-Ig/CPM combinations induce potent antigen-specific immune responses in tumor bearing mice

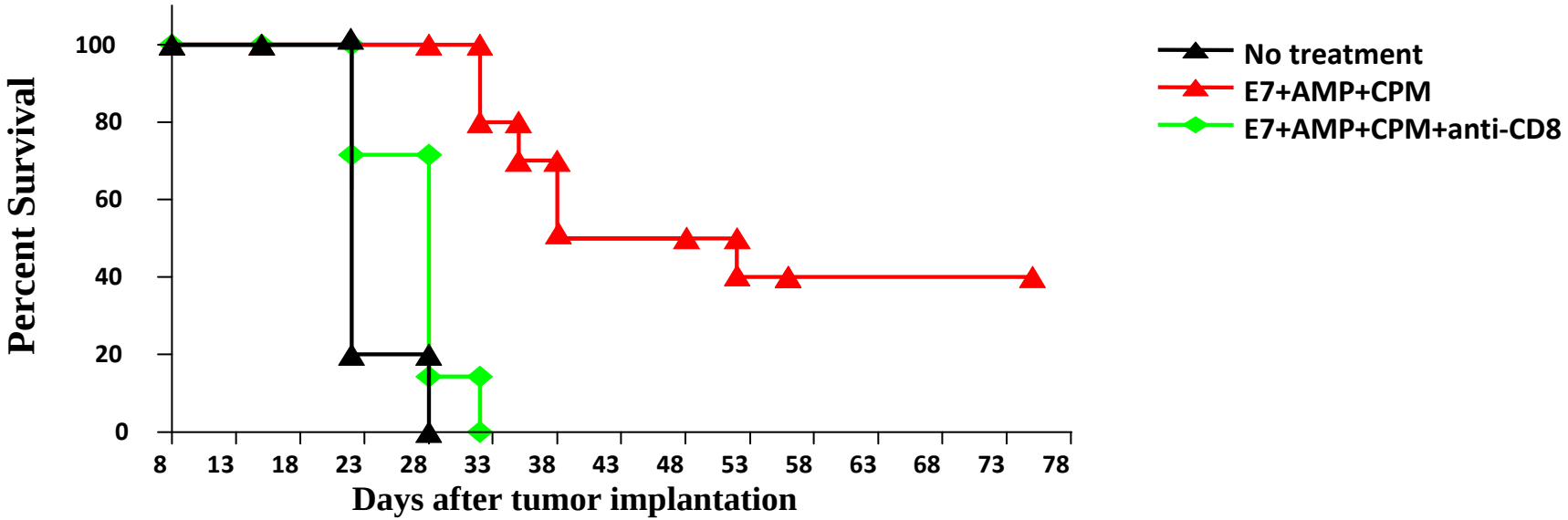




*P<0.05, ***P<0.001

Vaccine/B7-DC-Ig/CPM combinations promote TC-1 tumor rejection

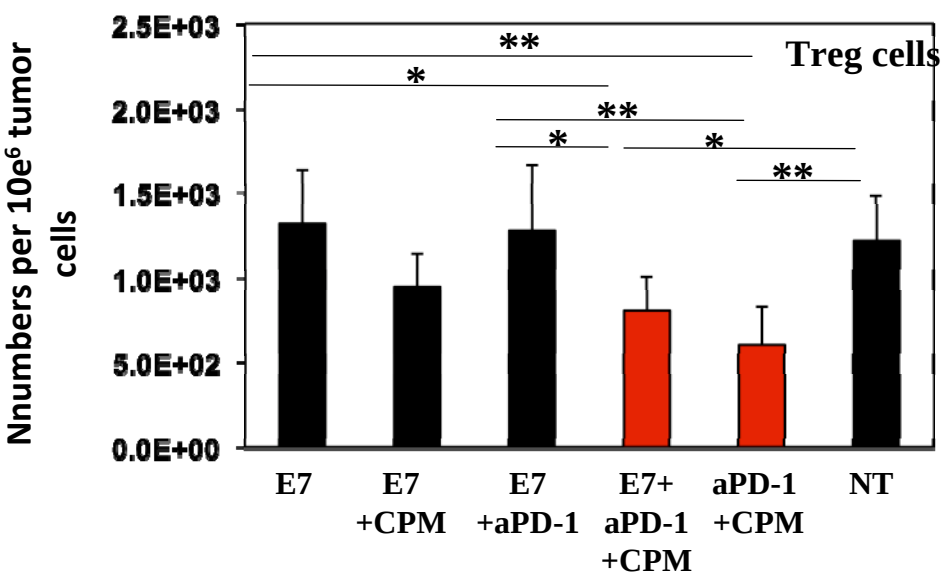




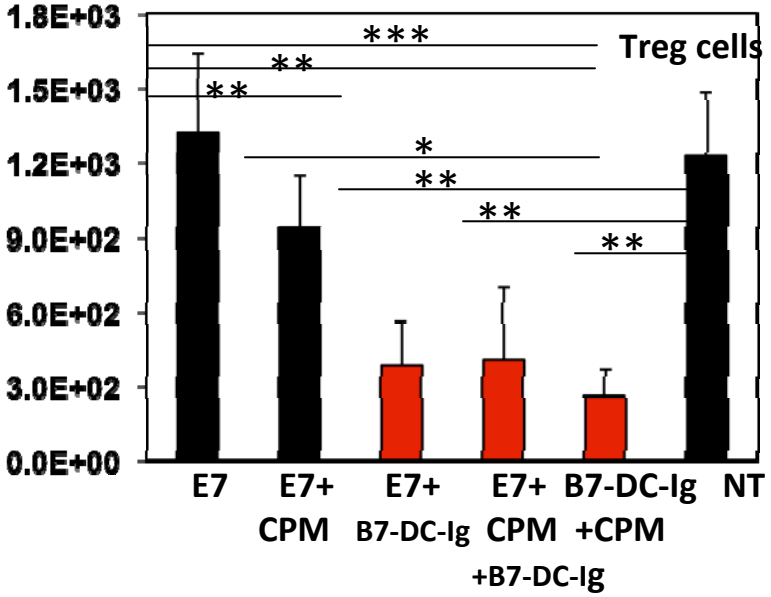
In contrast to anti-PD-1 Ab, B7-DC-Ig does not require CPM to decrease the level of Treg cells

Treg

Anti-PD-1 Ab



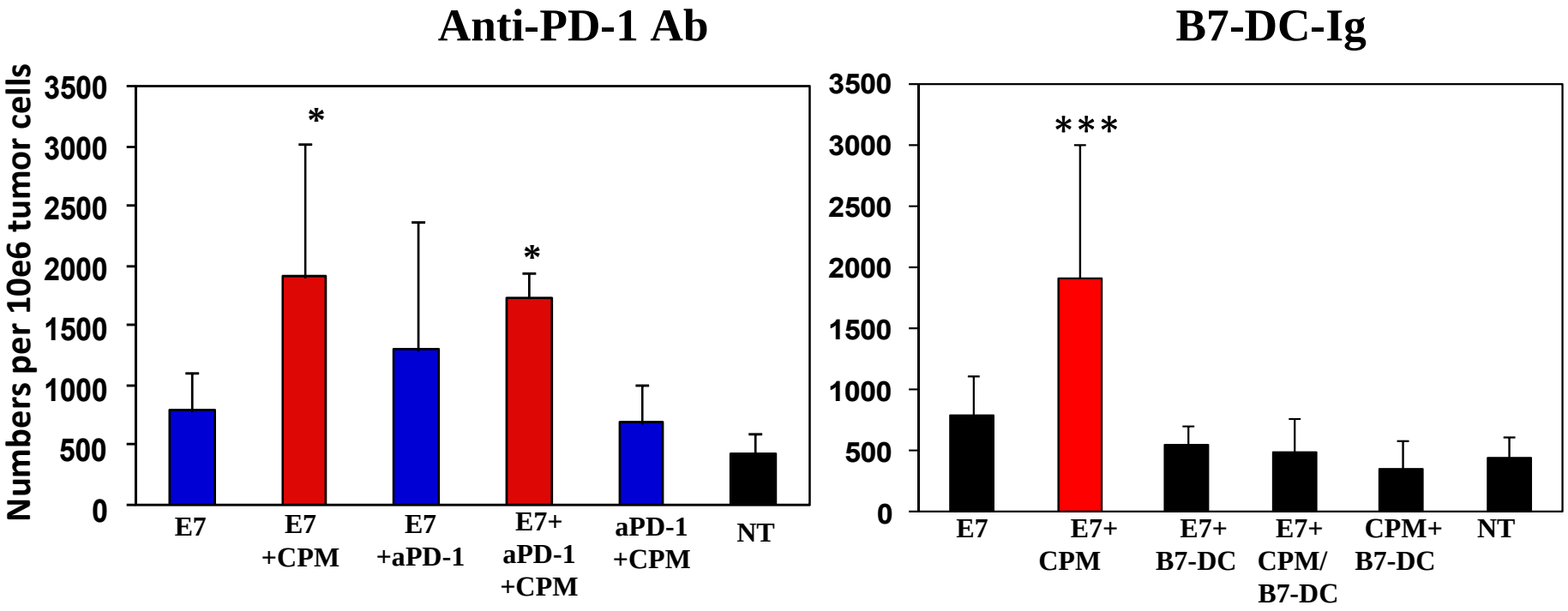
B7-DC-Ig



*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

Tconv

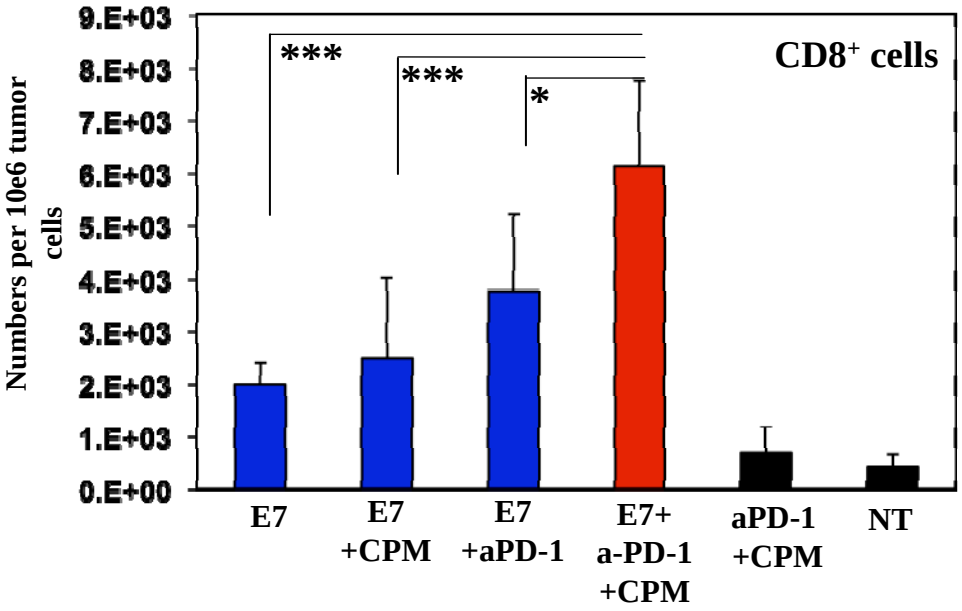


*P<0.05, ***P<0.001

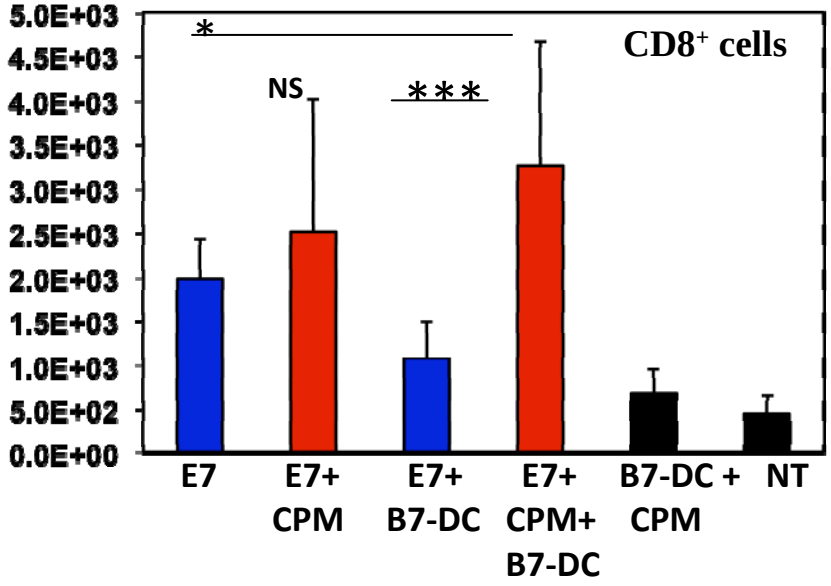
In contrast to anti-PD-1, B7-DC-Ig-based treatment does not affect CD8 T cell numbers

CD8

Anti-PD-1 Ab

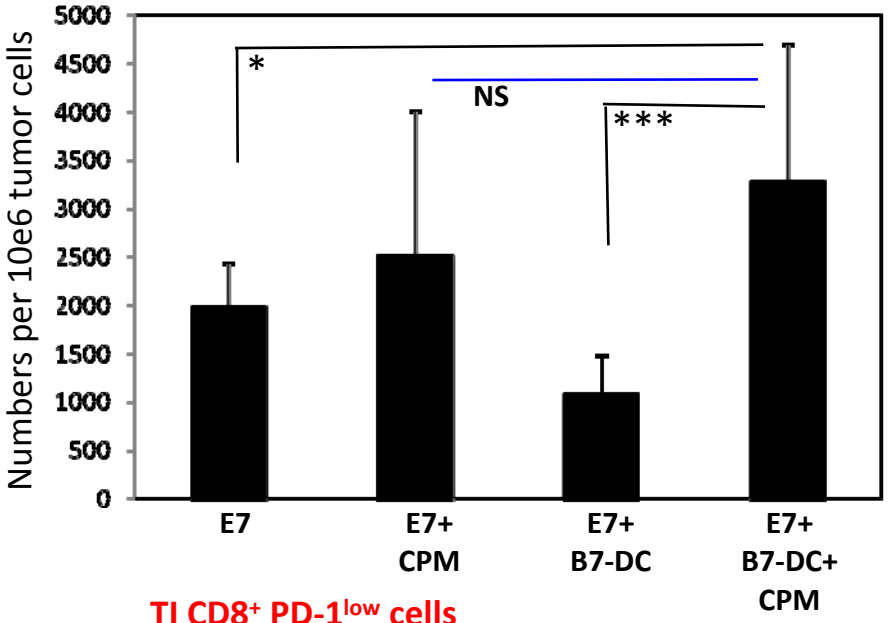


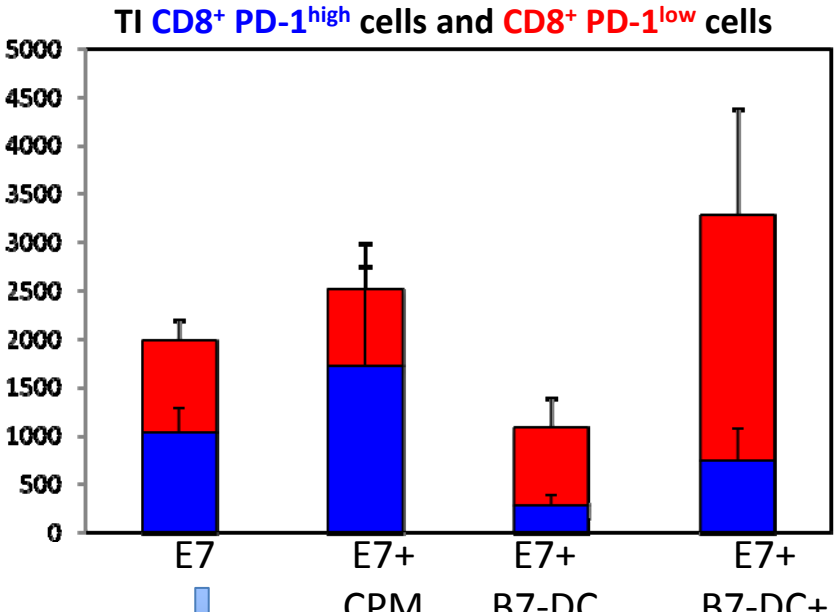
B7-DC-Ig

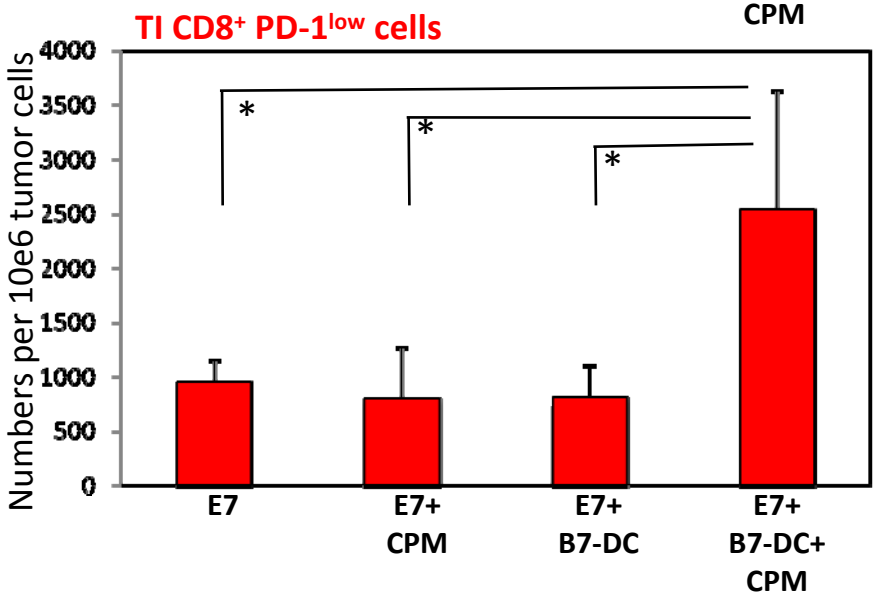


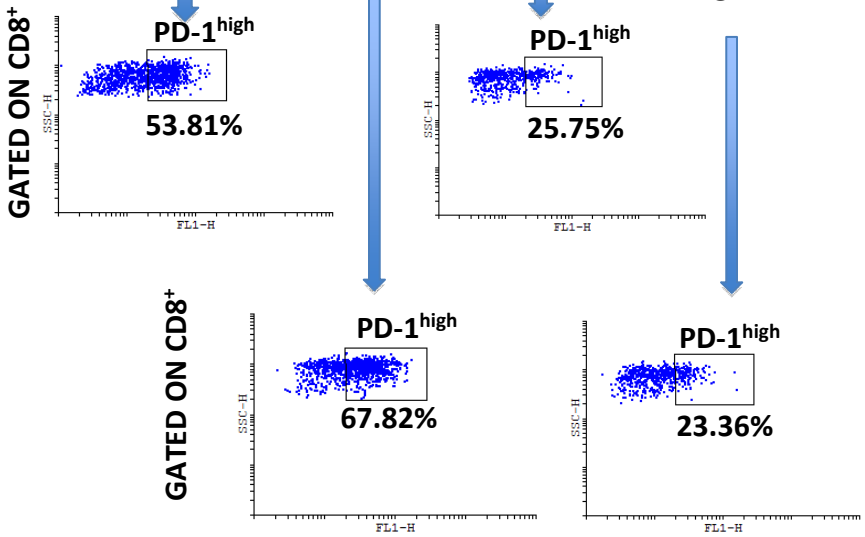
*P<0.05, ***P<0.001

B7-DC-Ig decreases the level of tumor infiltrated PD-1^{high} CD8^{+}T cells

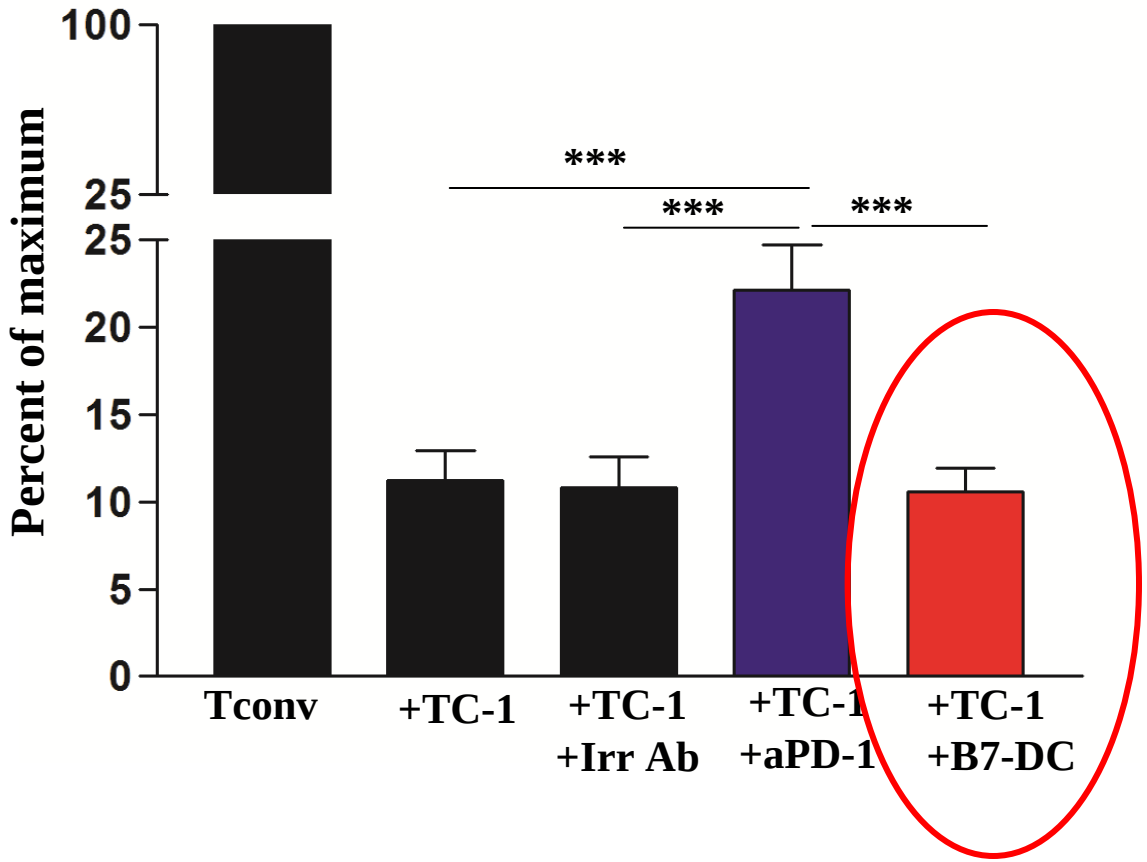






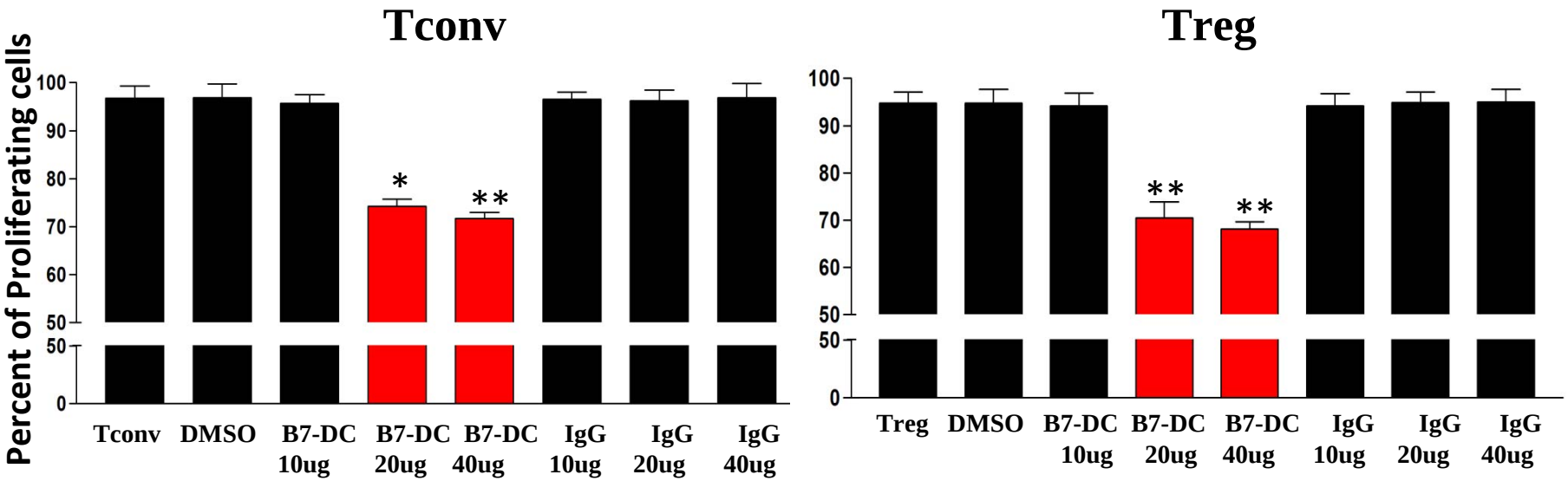


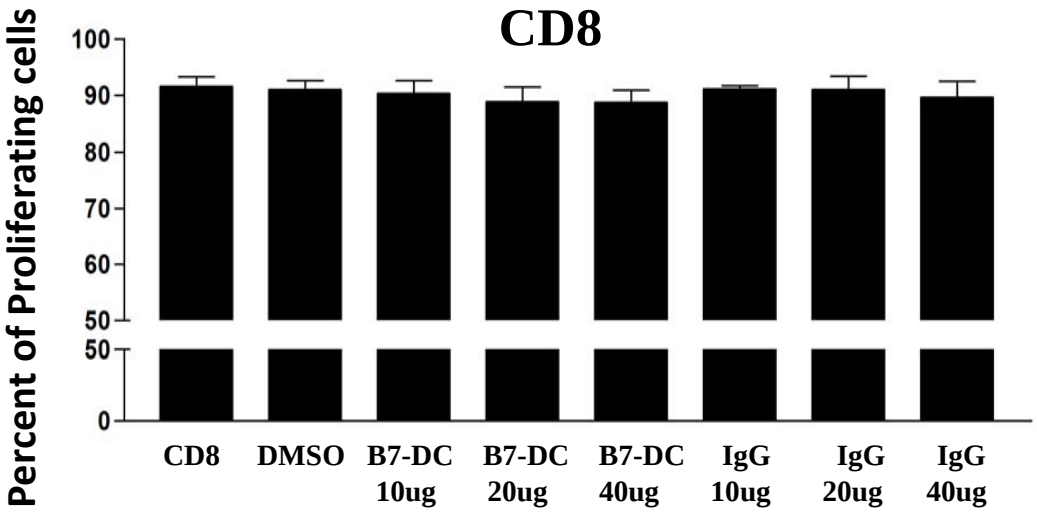
In contrast to anti-PD-1 Ab, B7-DC-Ig doesn't overcome tumor-induced suppression of stimulated Tconv cell proliferation



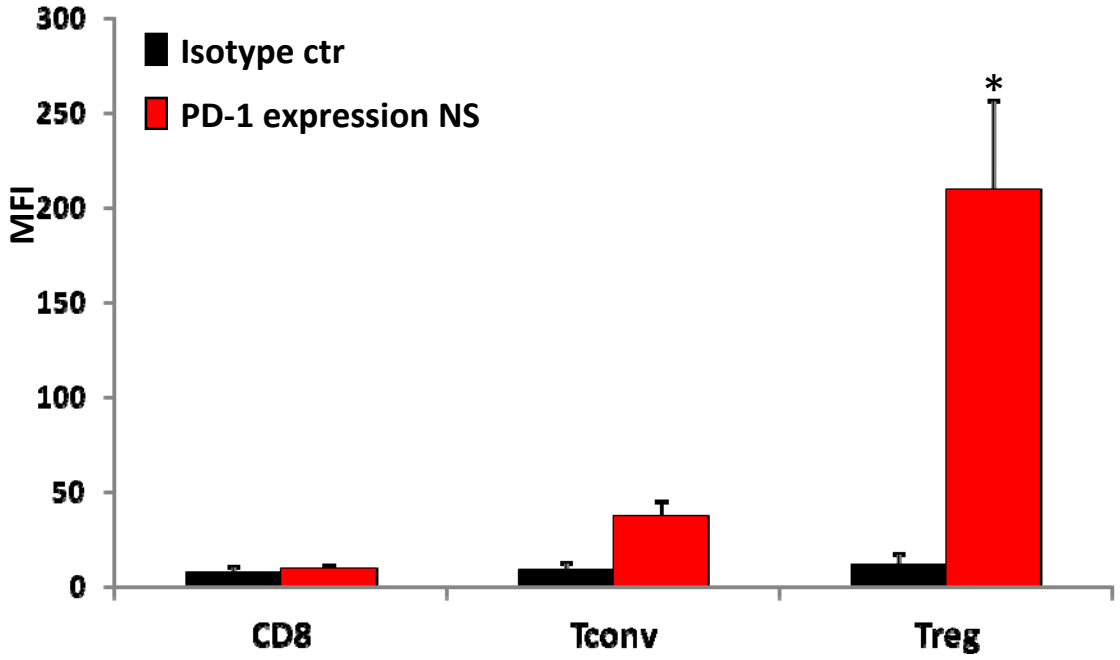
No blocking of PD-1/PD-L1 interaction. Due to low affinity of AMP-224 binding to PD-1

Differential effect of B7-DC-Ig on proliferation of different T cell subsets

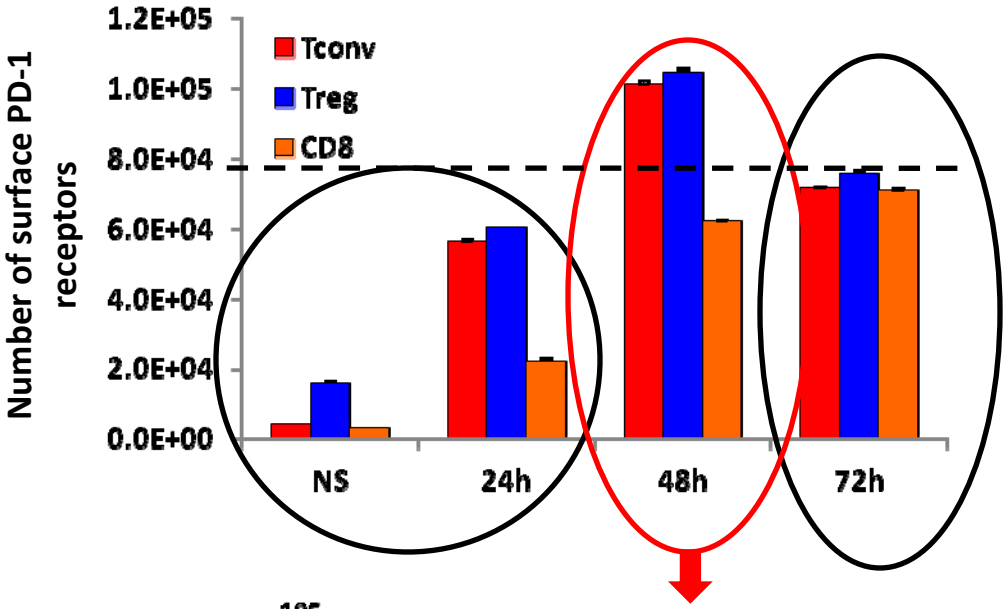


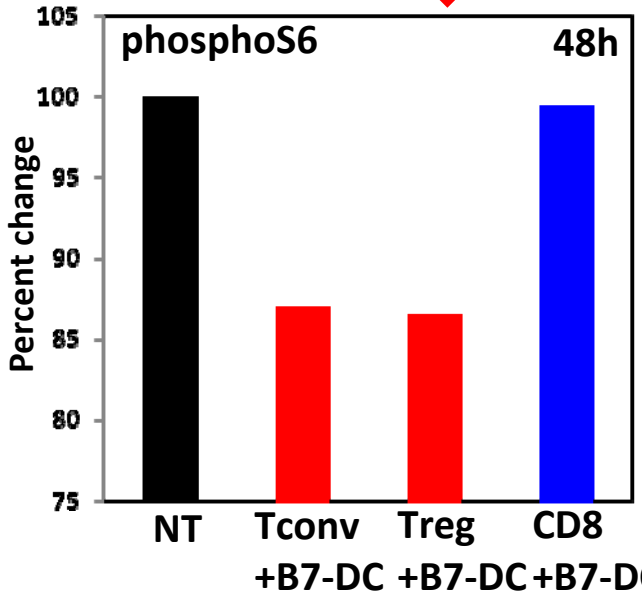


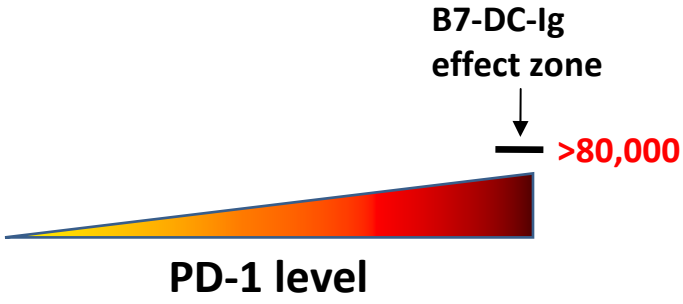
Surface PD-1 expression on different T cell subsets at non-stimulated stage

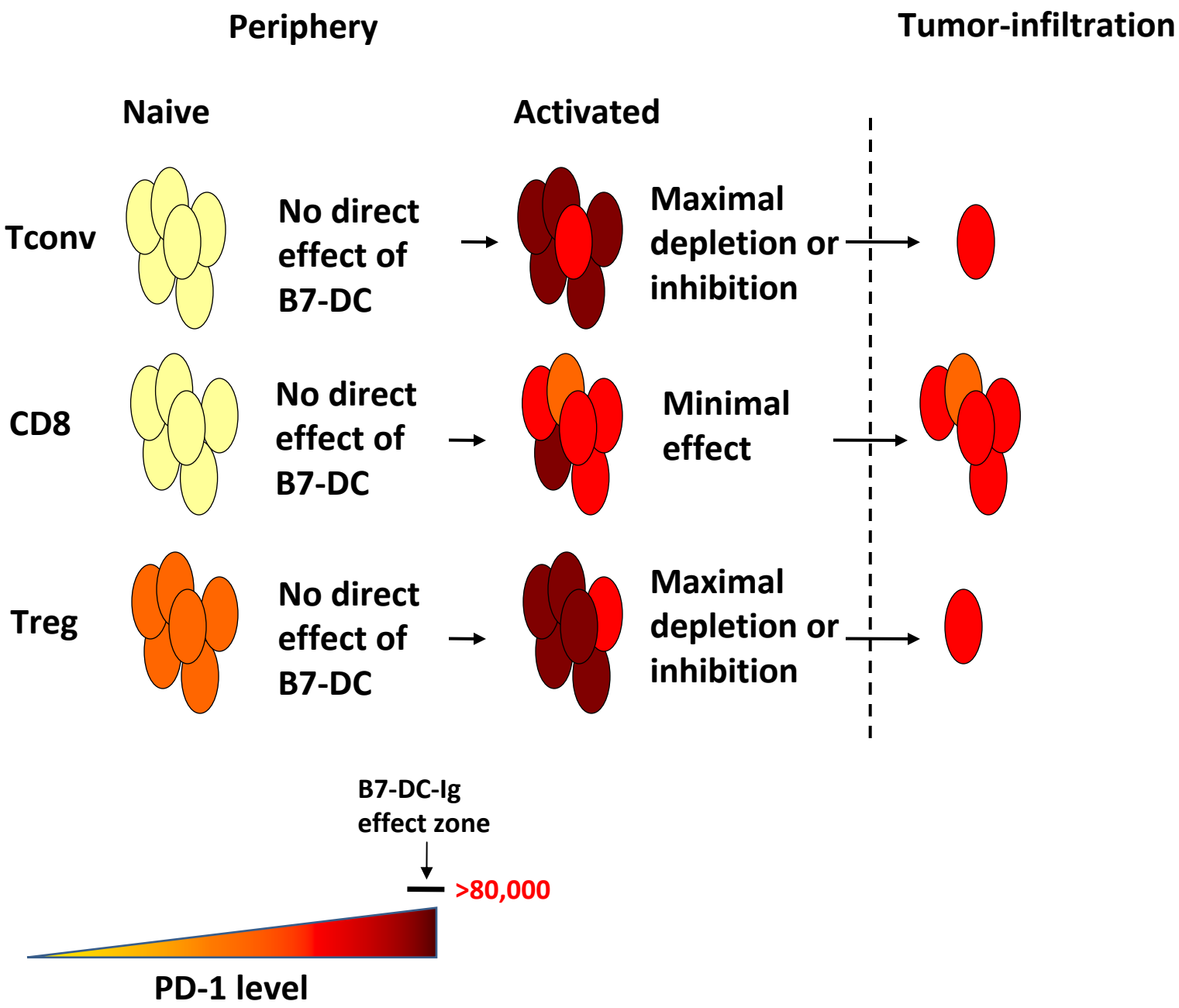


Number of surface PD-1 molecules on different T cell subsets at different time-points after stimulation








Periphery
Tumor-infiltration
Naive
Activated
Tconv
No direct effect of B7-DC
Maximal depletion or inhibition
CD8
No direct effect of B7-DC
Minimal effect
Treg
No direct effect of B7-DC
Maximal depletion or inhibition
B7-DC-Ig effect zone
>80,000
PD-1 level

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