PD-1 blockade upregulates Tim-3 expression in HNSCC tumor infiltrating lymphocytes

Gulidanna Shayan
University of Pittsburgh Cancer Institute

Gulidana Shayan

The following relationships exist related to this presentation:

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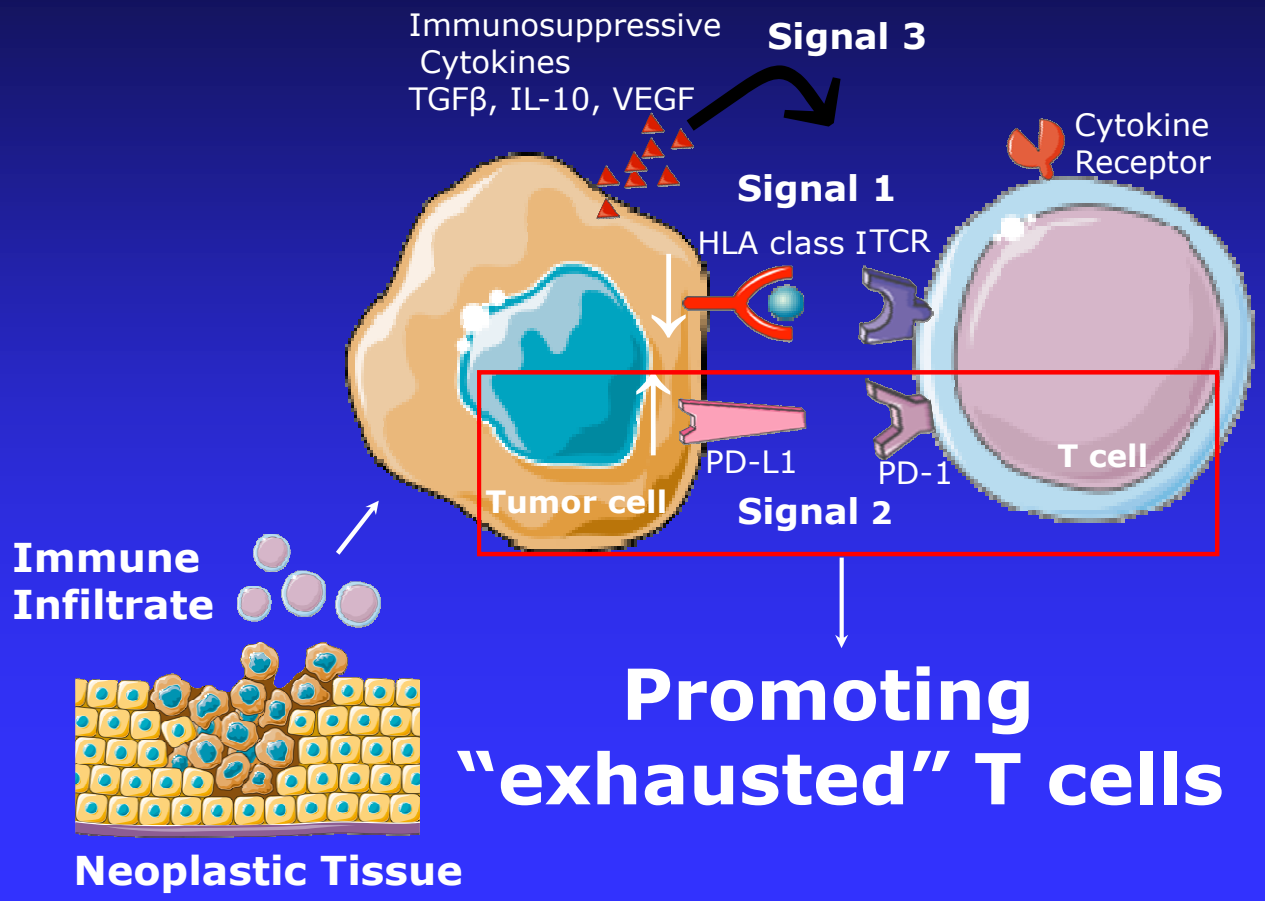
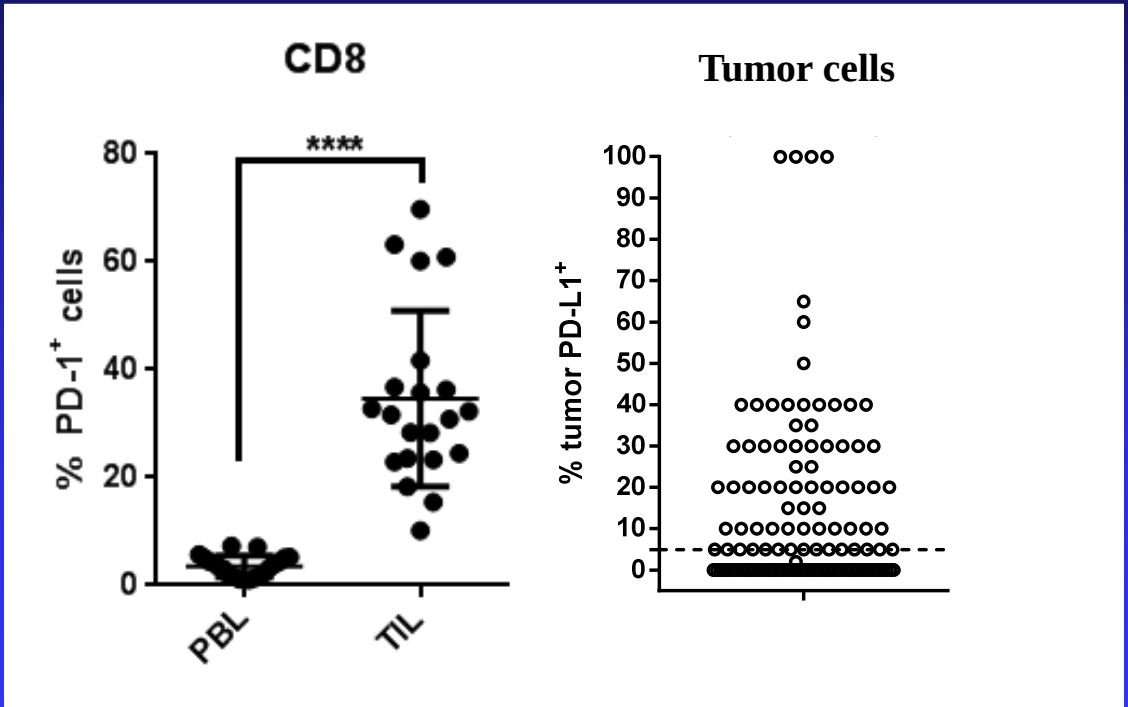
Merck, advisory board
Celgene, advisory board
Advaxis, advisory board

Tumor cell-mediated immune escape

Providing an aberrant SIGNAL 2 (co-inhibitory checkpoint receptor expression)



PD-1/PD-L1 + pathway is upregulated in the HNSCC microenvironment

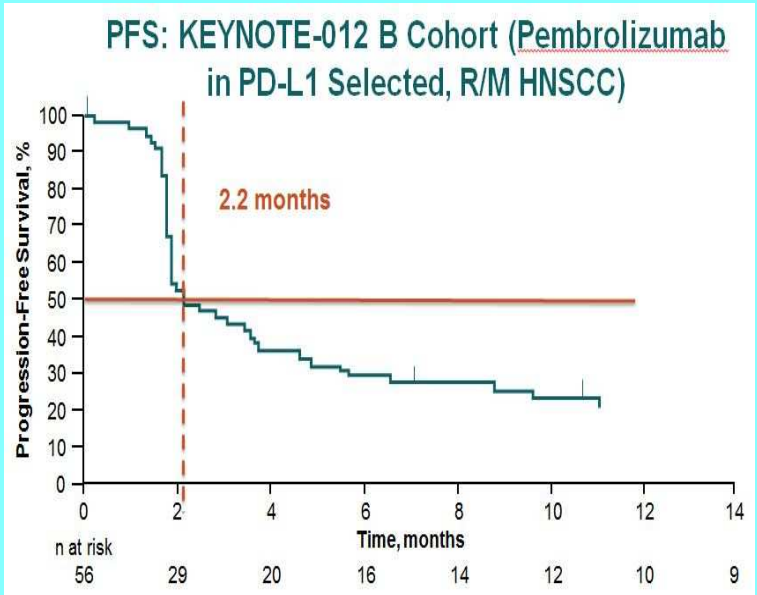


Hyun-bae Jie

Fernando Concha-Benavente

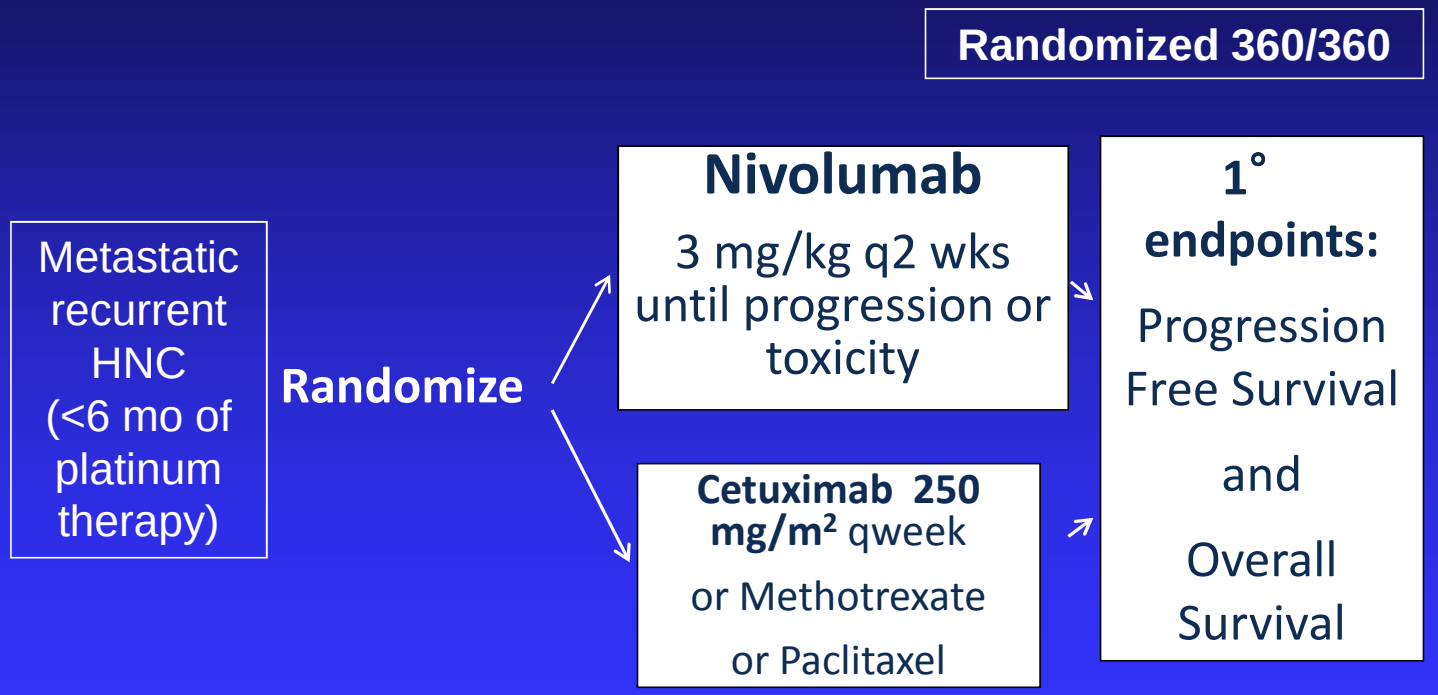
Anti-PD-1 (Pembrolizumab) KEYNOTE trial Data in HNSCC

Best overall response	Total N = 117	Total N = 56
	n (%)	n (%)
ORR	**29 (24.8)**	**11 (19.6)**
CR	1 (0.9)	1 (1.8)
PR	28 (23.9)	10 (17.9)
SD	29 (24.8)	16 (28.6)
Clinical benefit	**58 (49.6)**	**27 (48.2)**



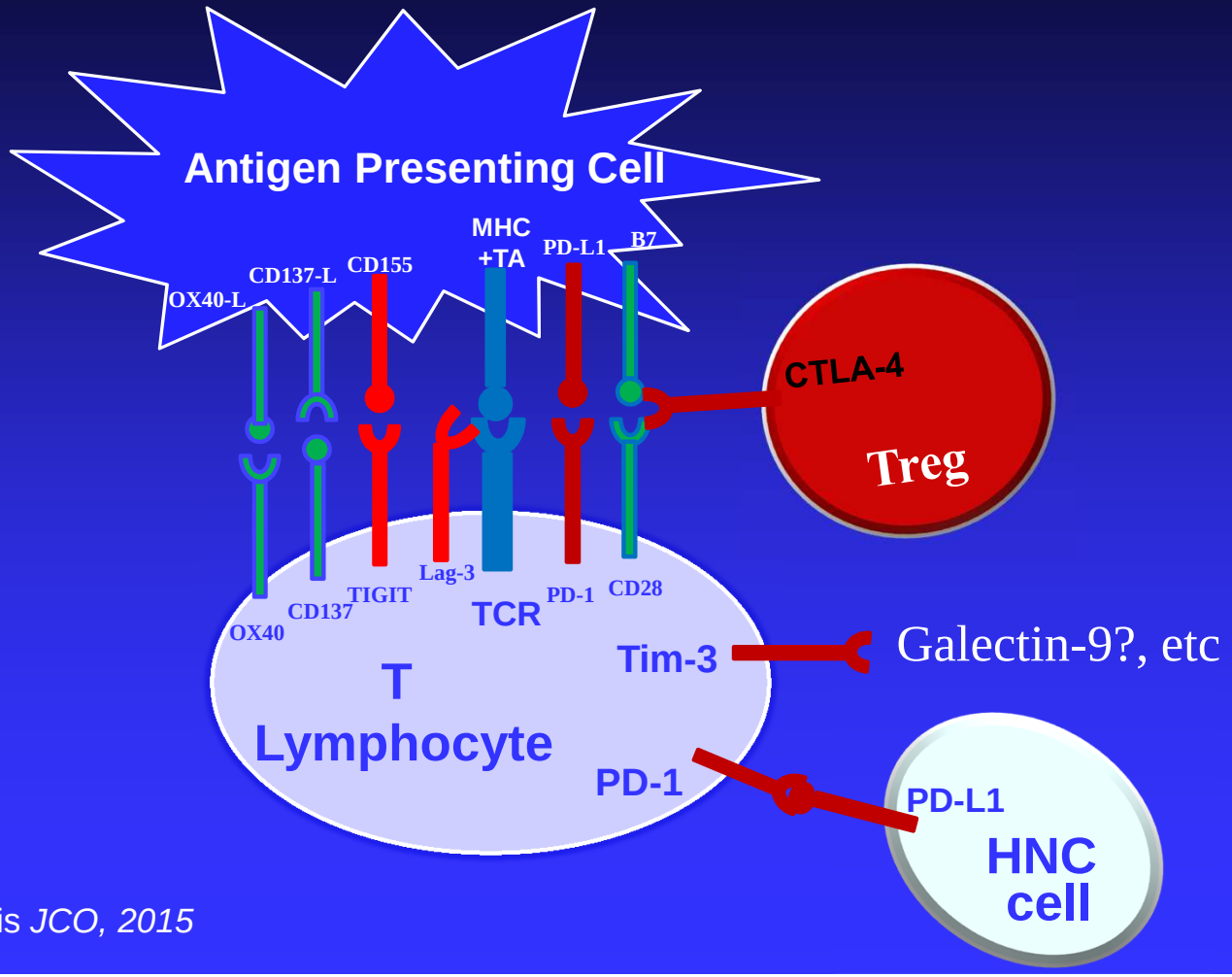
Courtesy of Seiwert T, Updated PFS for KEYNOTE B Cohort, Data Cutoff 3-23-2015, Median F/U 13.7 months

Randomized Phase III Trial of Nivolumab (anti-PD-1) in Recurrent/Metastatic HNC



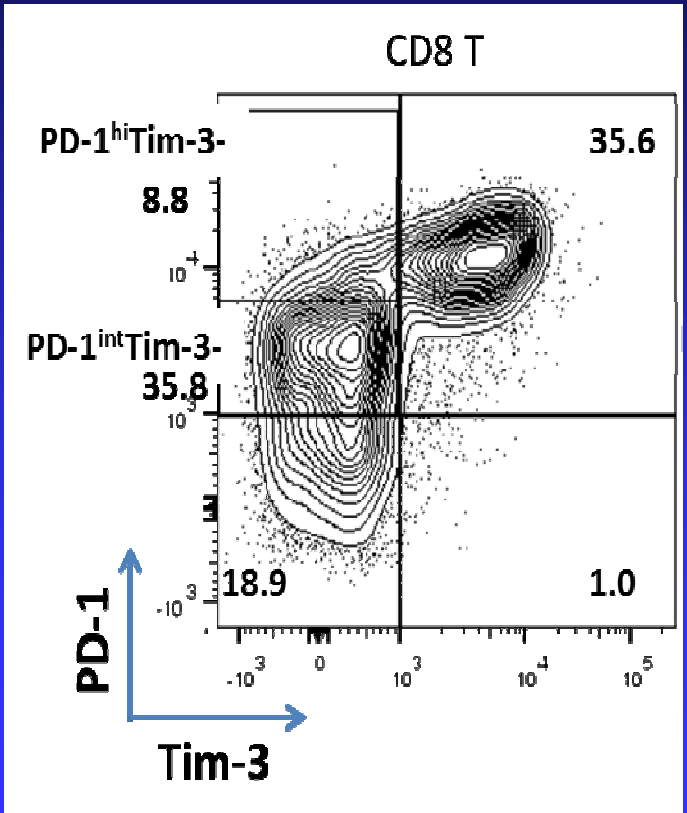
CheckMate
CHECKpoint pathway and nivoluMAb clinical Trial Evaluation

The Immune Synapse



Ferris *JCO, 2015*

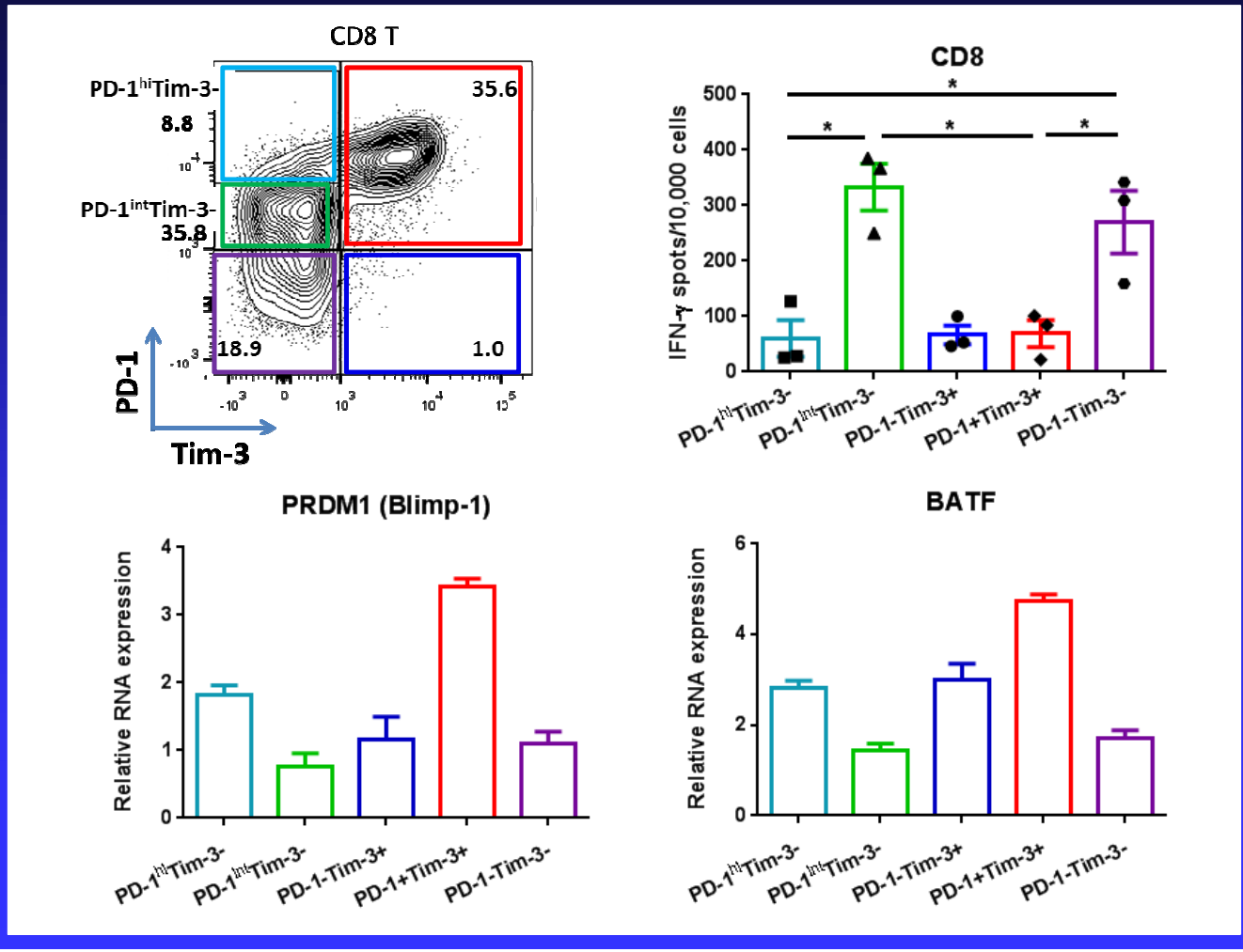
Multiple inhibitory receptors are co-expressed by HNSCC patient TIL



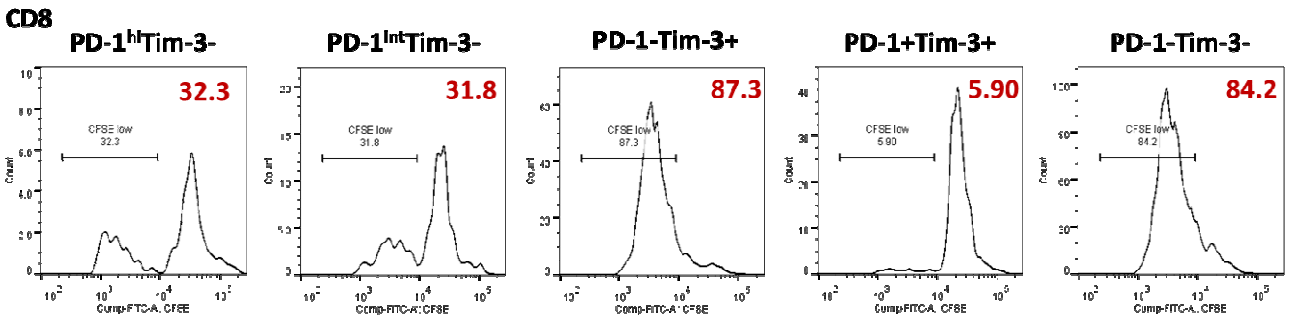
- Nearly half of the PD-1^{+} CD8 T cells are Tim-3^{+}
- Tim-3 expression correlates with a higher PD-1 expression level

Jing Li

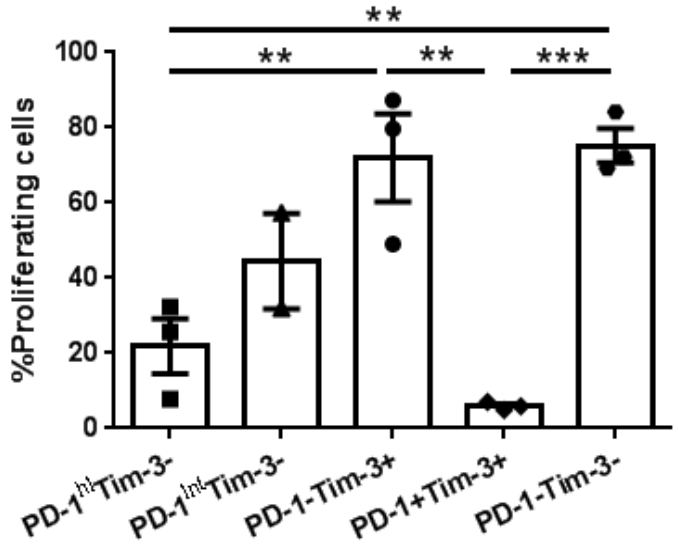
Differential expression of PD-1 and Tim-3 marks activation vs exhaustion status of T cells in the TME



PD-1^{hi}Tim-3^{-} and PD-1^{+} Tim-3^{+} TIL subsets do not proliferate upon TCR stimulation



CD8+
T cells



Anti-PD-1 + Anti-Tim-3 yields higher antitumor response

- ***Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity***
  Kaori Sakuishi, Ana C. Anderson et al. J. Exp. Med. 2010

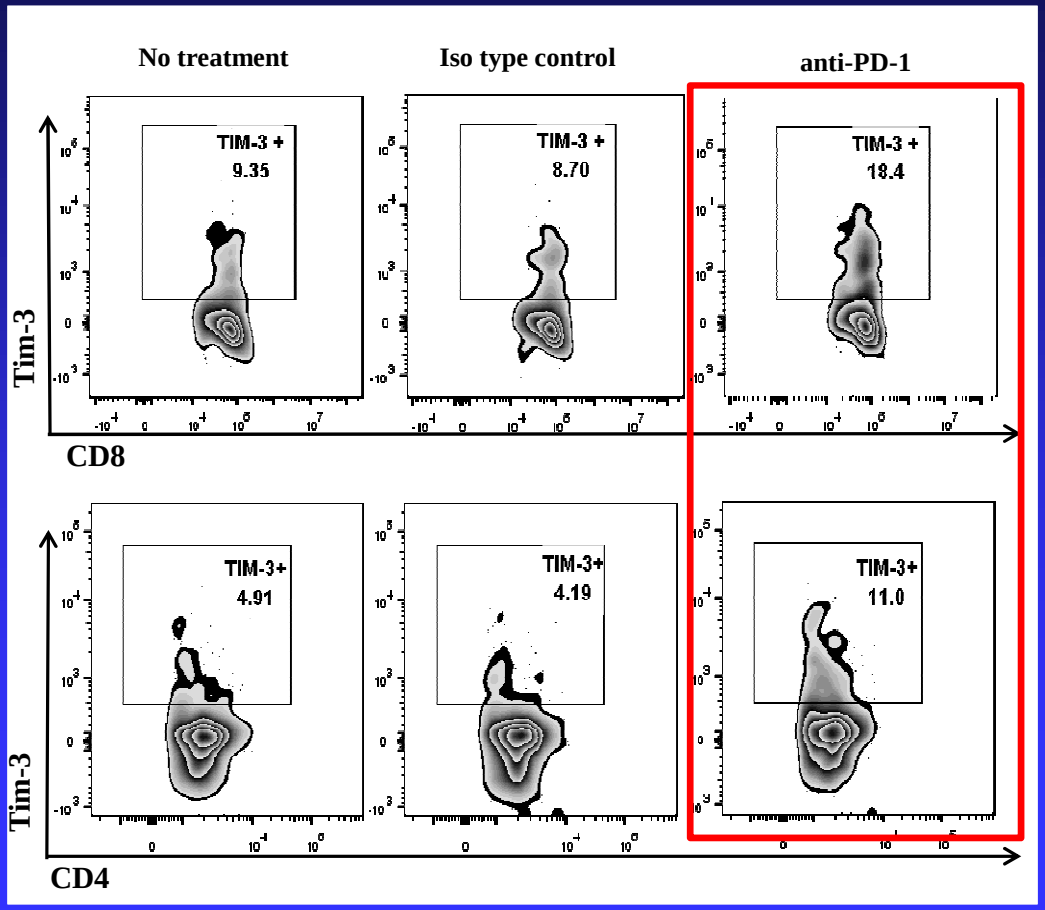
- ***Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen–specific CD8+ T cell dysfunction in melanoma patients***
  Julien Fourcade, Hassane M. Zarour et al. J. Exp. Med. 2010

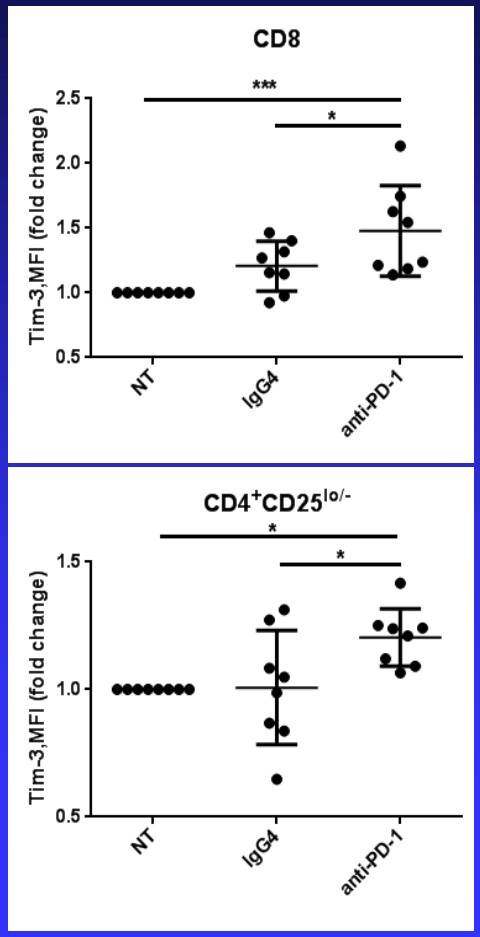
- **Question 1: Is Tim-3 upregulated in response to anti-PD-1 therapy in HNSCC?**

- ***Selective Effects of PD-1 on Akt and Ras Pathways Regulate Molecular Components of the Cell Cycle and Inhibit T Cell Proliferation***
  Nikolaos Patsoukis, Vassiliki A. Boussiotis et al. Sci. Sig. 2012

- **Question 2: Is there down stream cross-talk between PD-1 and Tim-3 signaling pathway?**

Tim-3 is upregulated after PD-1 blockade in vitro in human HNSCC TIL

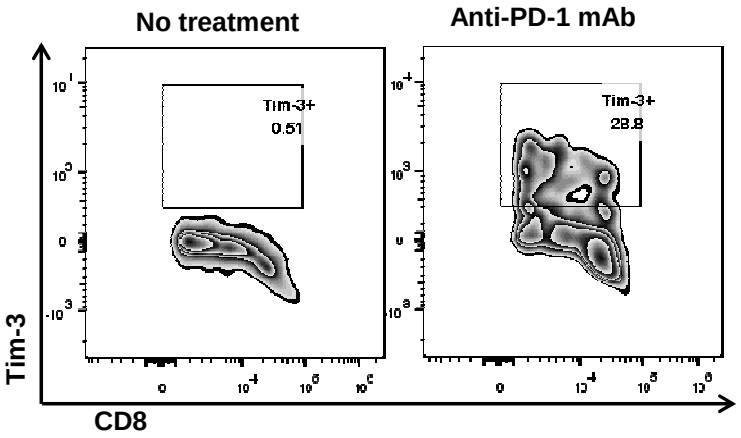


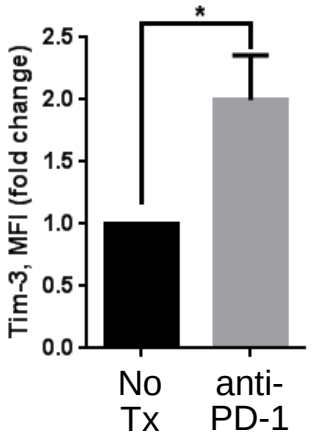


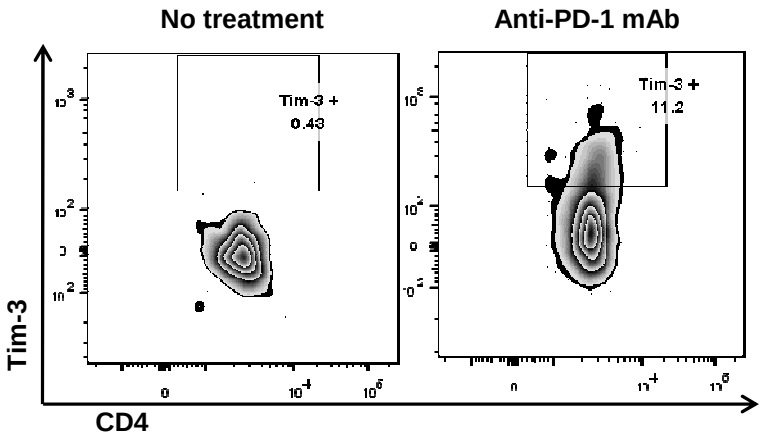
Tim-3 is upregulated on TIL from anti-PD-1 treated mice

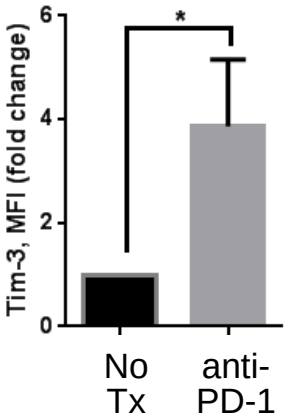
$CD8^+$ TIL

$CD4^+/CD25^-$ TIL



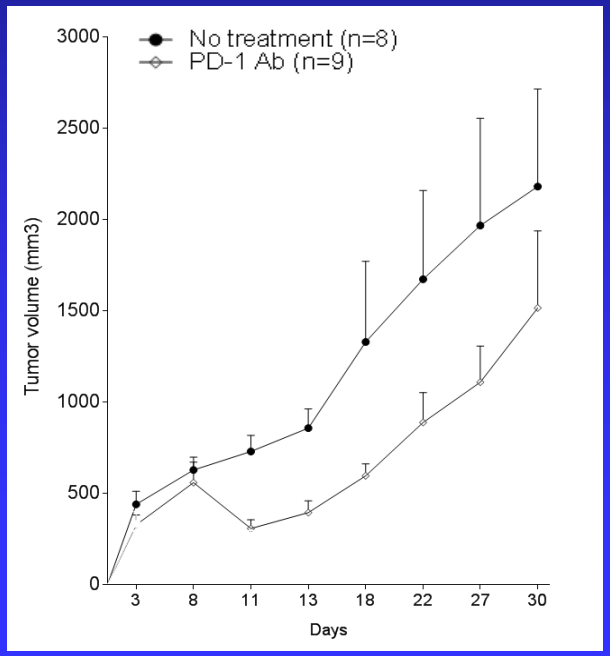






Tim-3^{+} T cells are upregulated in splenocytes of anti-PD-1 treated group

Mouse orthotopic HNC model



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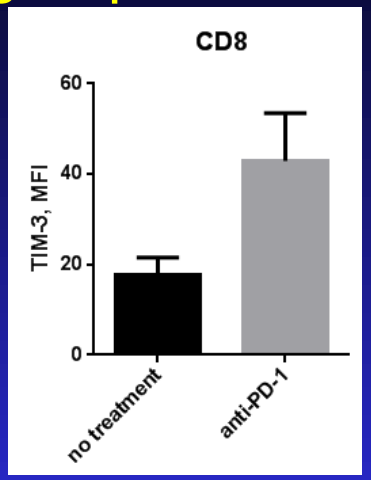
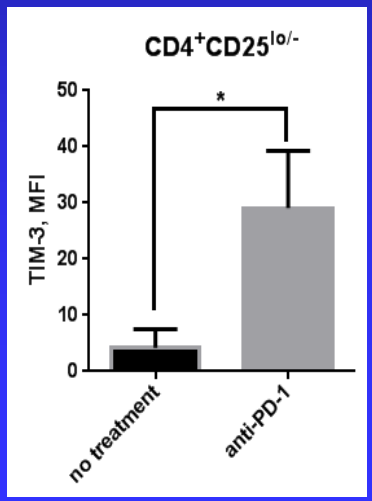
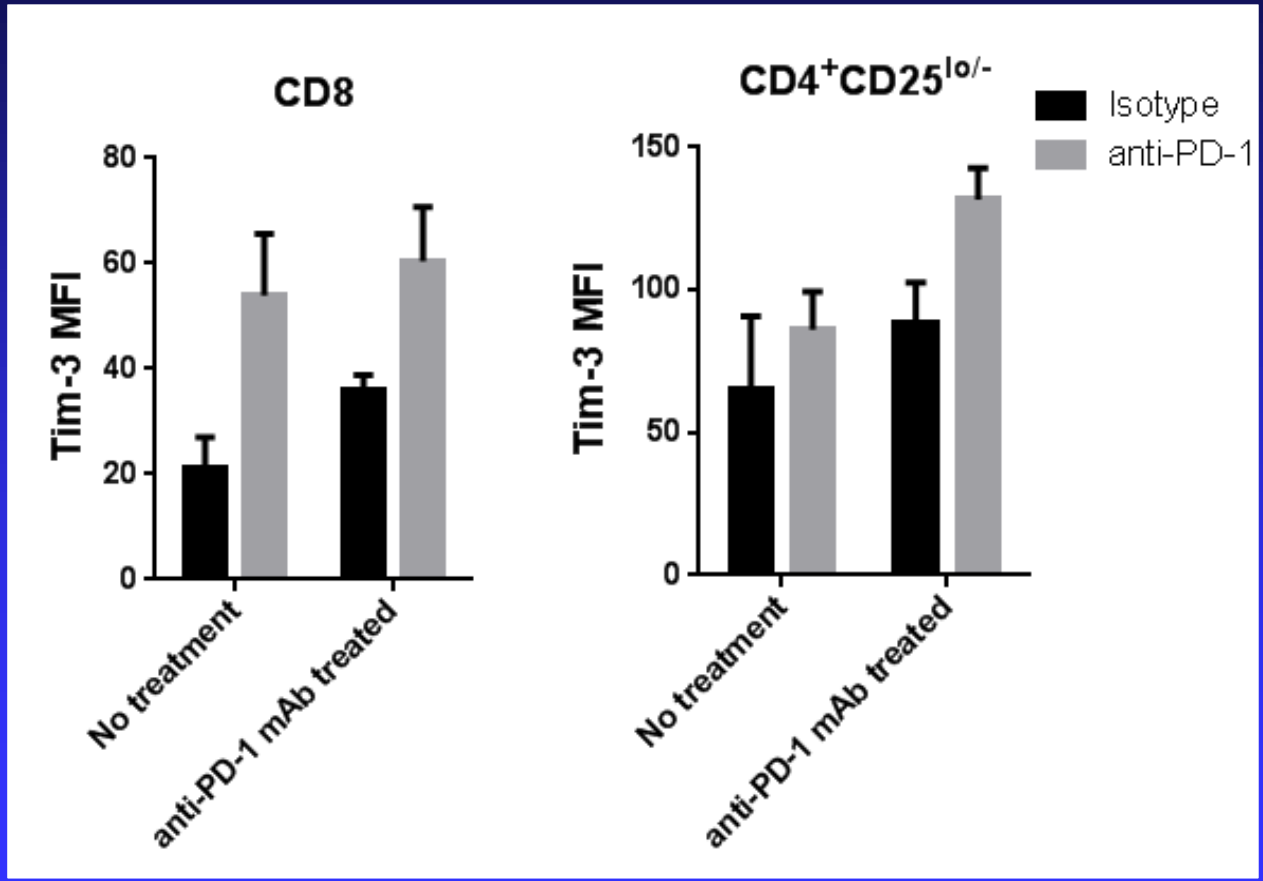


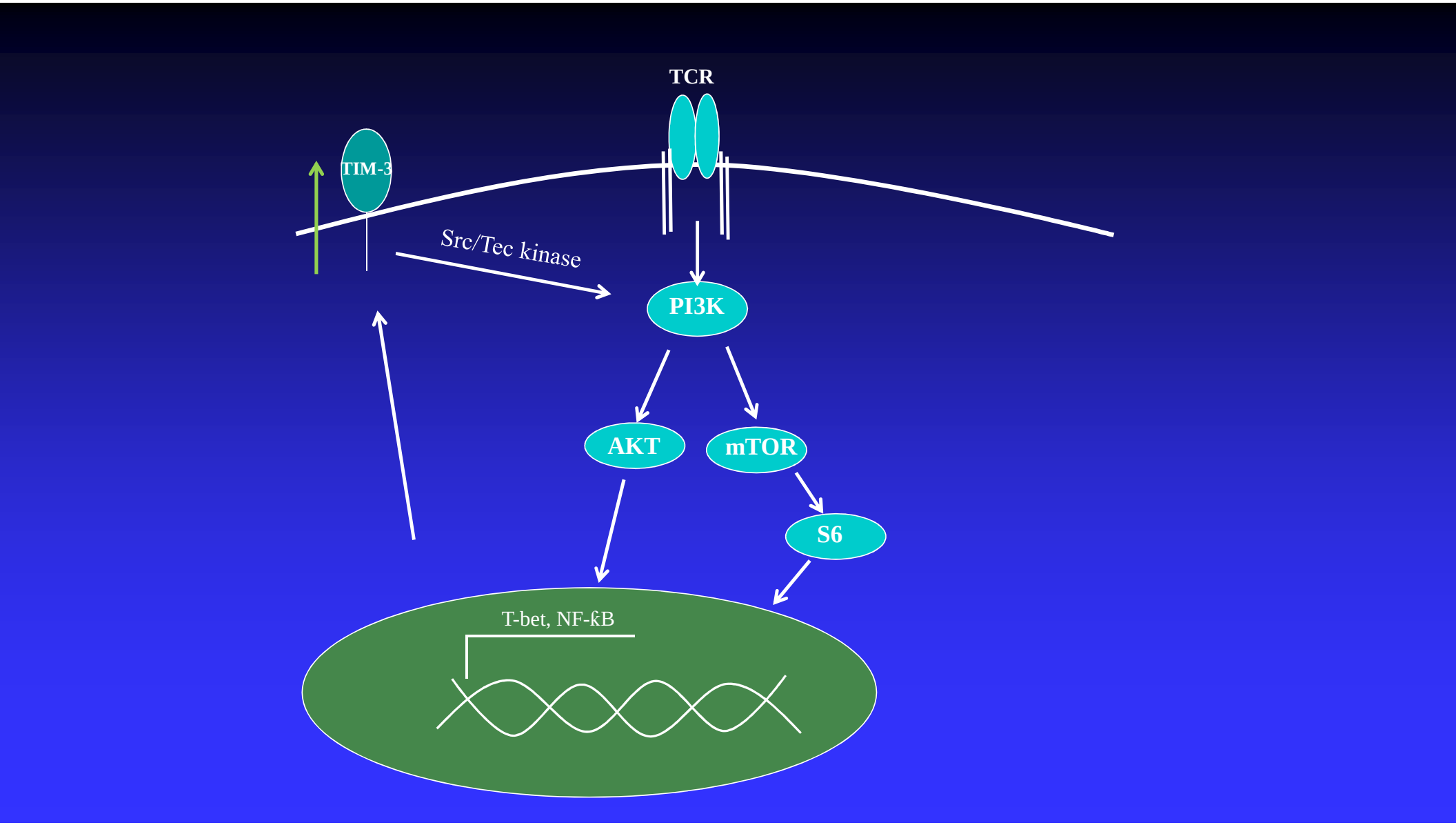


(splenocytes)

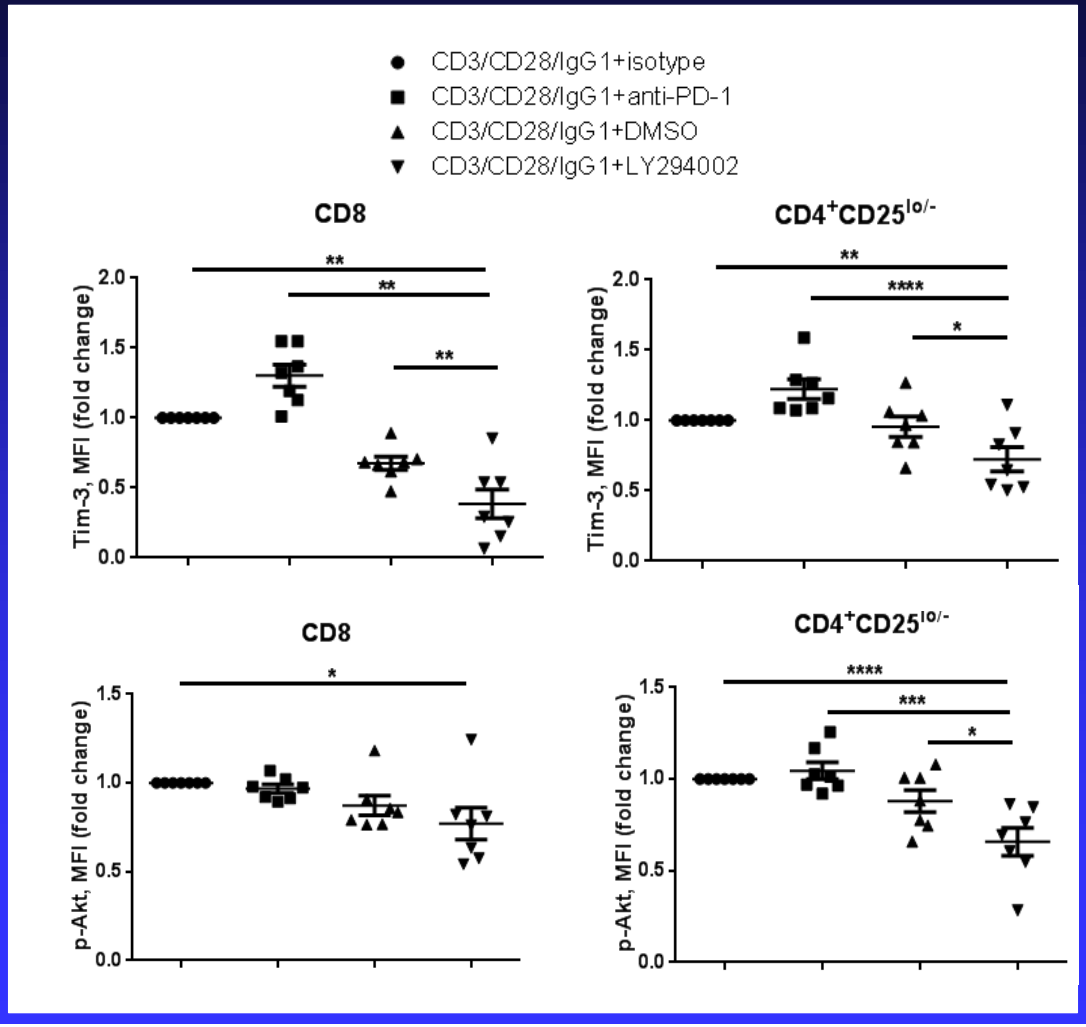
Tim-3 is further upregulated in mouse splenocytes after ex vivo PD-1 blockade



(mouse splenocytes)

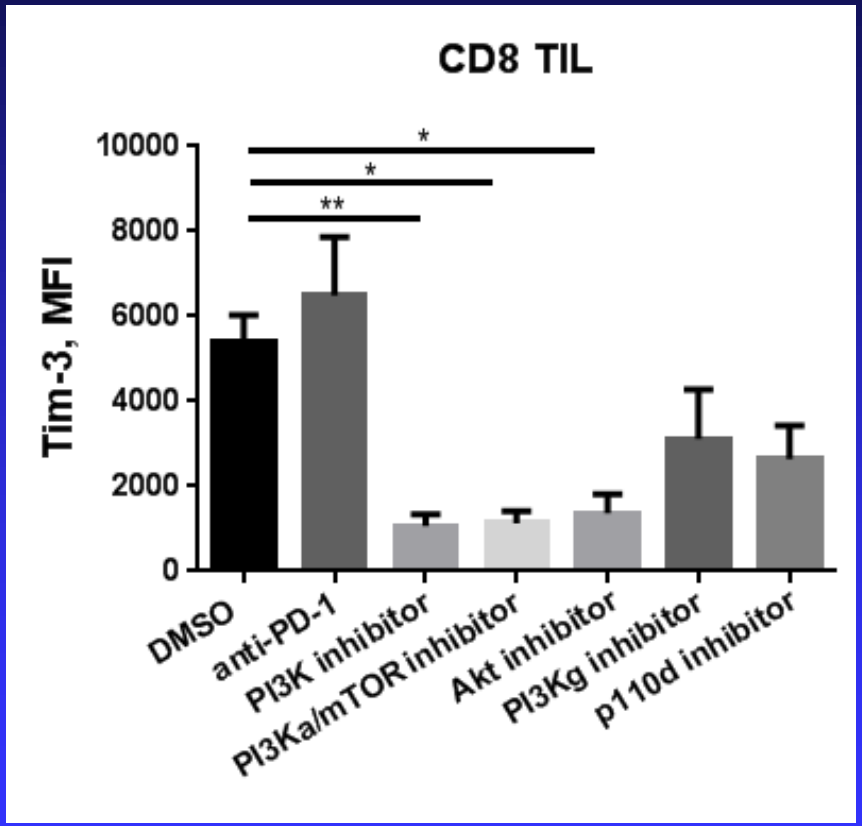

TCR
TIM-3
Src/Tec kinase
PI3K
AKT
mTOR
S6
T-bet, NF-ƙB

Tim-3 upregulation after TCR stimulation is PI3K dependent



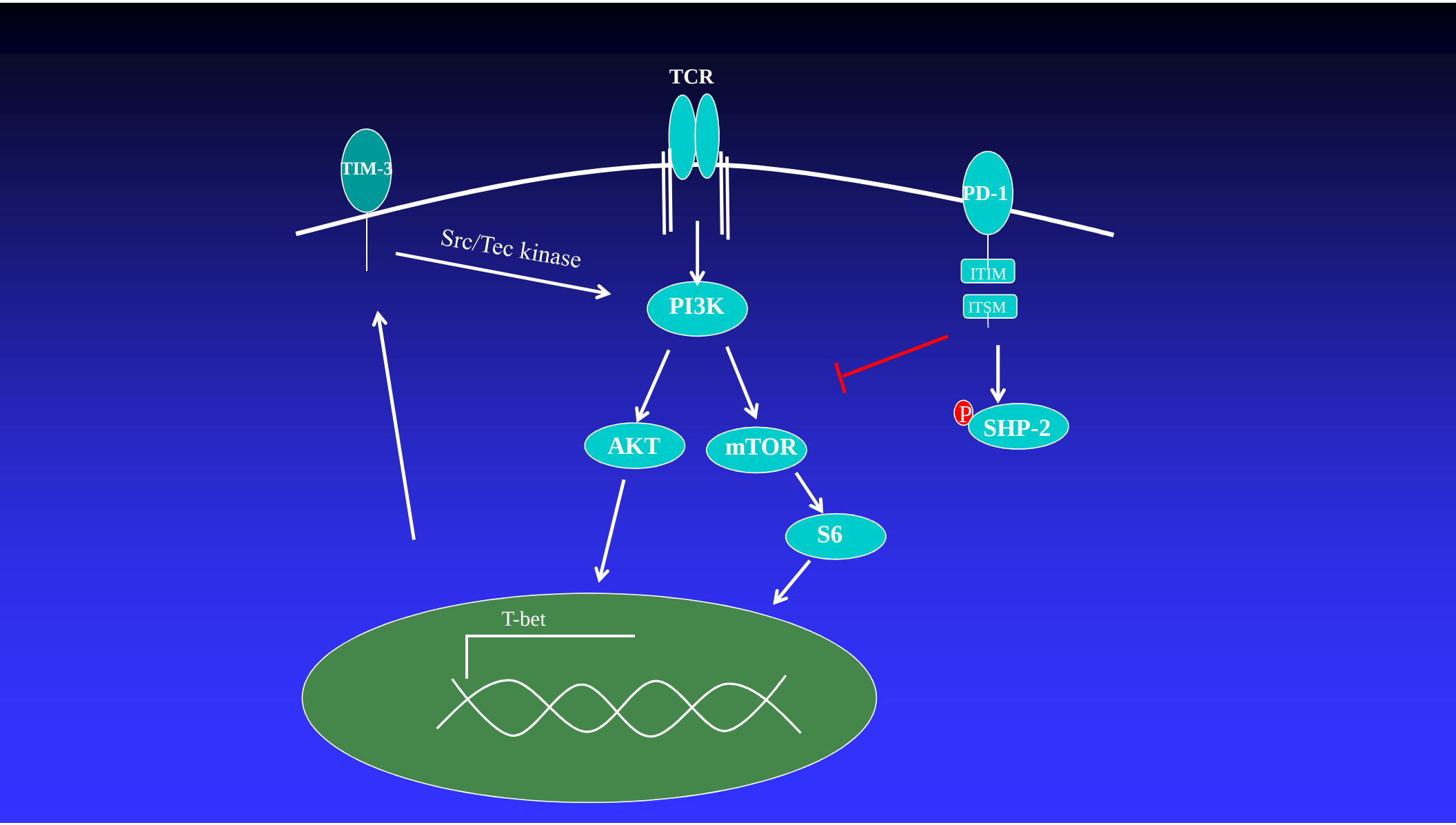
(TIL)

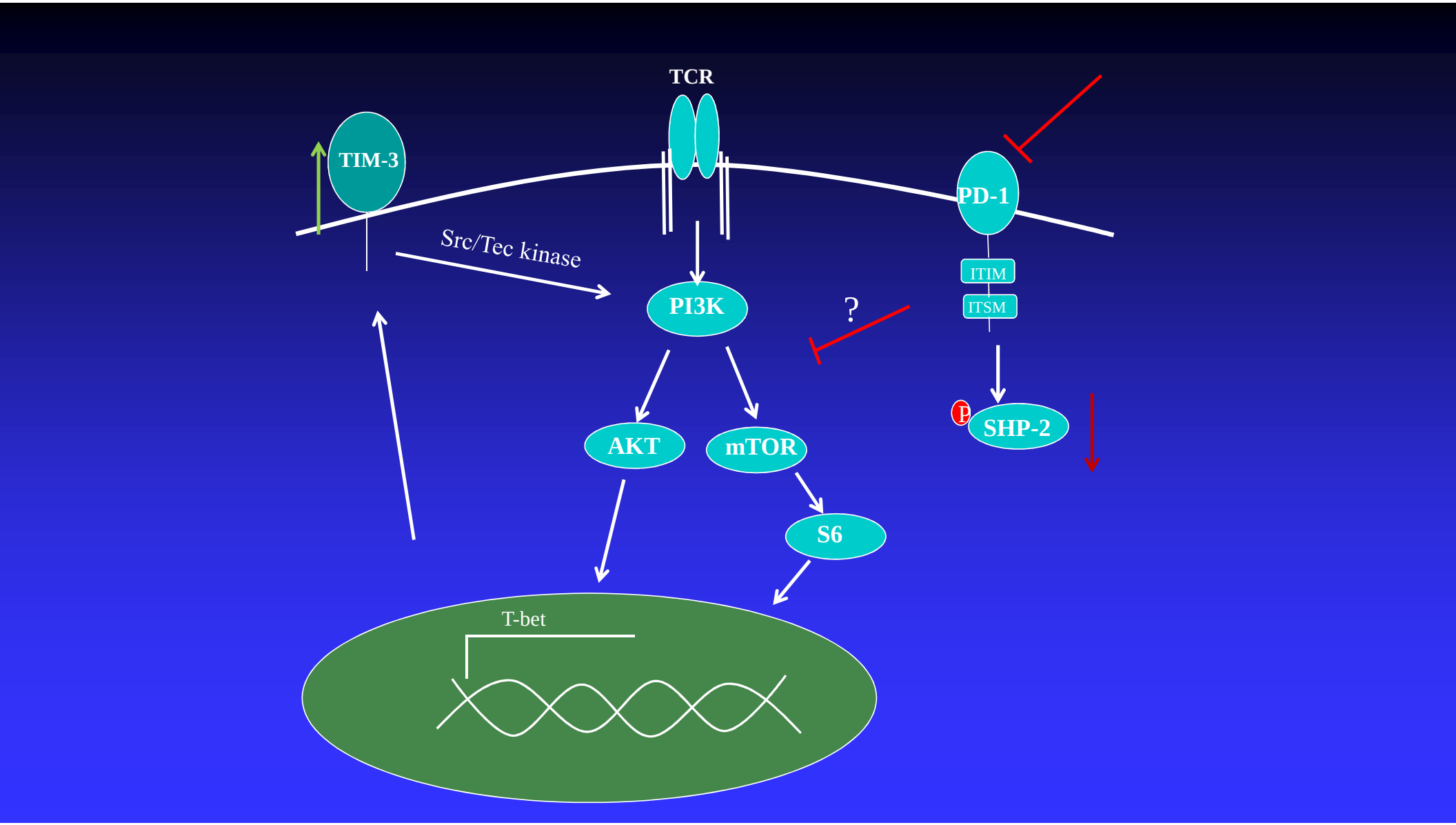
Tim-3 upregulation after TCR stimulation is PI3K-Akt-mTOR dependent



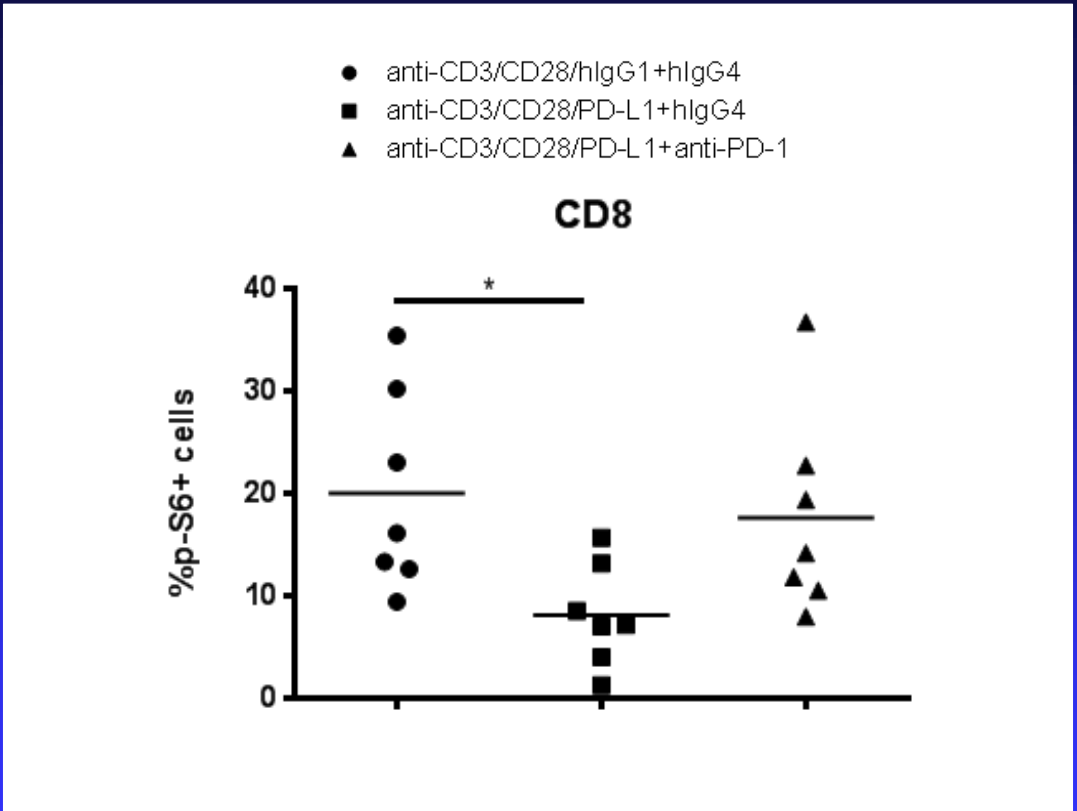
Inhibitors
*LY294002(**PI3K**)*
*CAY10626(**PI3Kα/mTOR**)*
*AS04-1164(**PI3Kγ**)*
*CAL-101(**p110δinhibitor**)*

(TIL)


TCR
TIM-3
PD-1
Src/Tec kinase
ITIM
ITSM
PI3K
AKT
mTOR
S6
P
SHP-2
T-bet

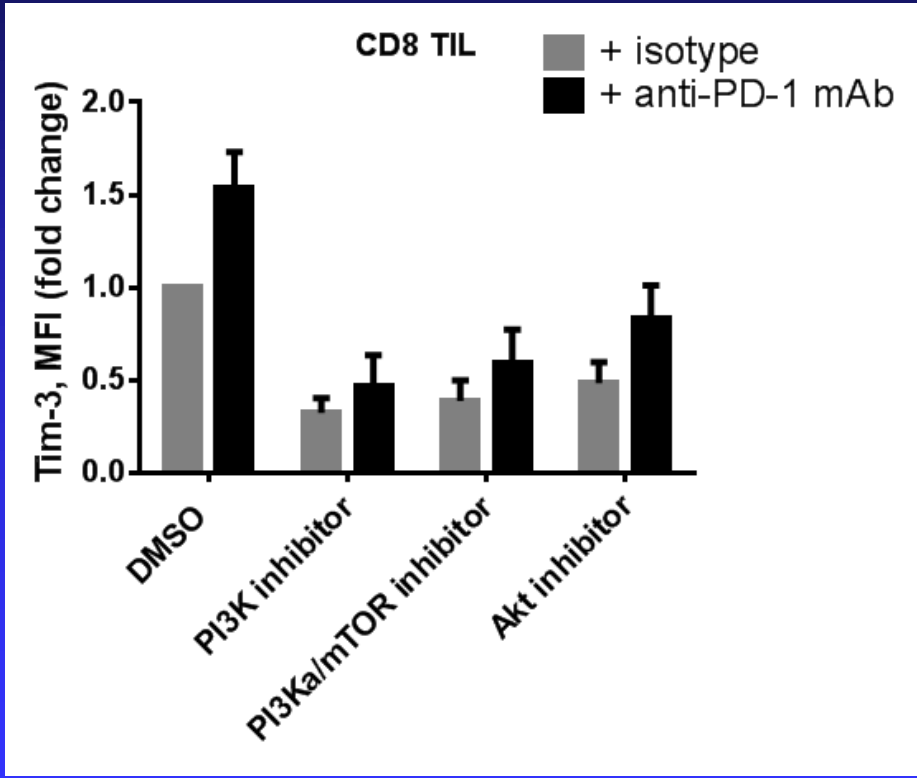

TCR
TIM-3
PD-1
Src/Tec kinase
ITIM
ITSM
PI3K
?
AKT
mTOR
P
SHP-2
S6
T-bet

PD-1 blockade upregulates p-S6 expression



PD-1/SHP-2 Inhibits Tc1/Th1 Phenotypic Responses and the Activation of T Cells in the Tumor Microenvironment
Jing Li, Robert Ferris. Cancer Res. 2015 Feb 1;75(3):508-18

Tim-3 upregulation after anti-PD-1 may not only be PI3K-Akt dependent



Inhibitors:
*LY294002(**PI3K**)*
*CAY10626(**PI3Kα/mTOR**)*

Lessons and Take Home Messages

- Multiple inhibitory signals exist in the tumor microenvironment, disrupting crucial pathways required for optimal T cell activation and tumor lysis
- Restoration of full T cell activation is necessary by reversing co-inhibitory signals in the tumor microenvironment
- Correlating Tim-3 upregulation in anti-PD-1 treated patients with clinical response may help elucidate its potential role in escape from anti-PD-1 therapy

Is Tim-3 upregulation an additive exhaustion marker in HNSCC TIL?

- Role of Tim-3 as a positive signal, or an additive negative signal, is unresolved

- Elucidate downstream cross-talk between PD-1 and Tim-3

- In general, how does blockade of one ICR affect expression of other receptors and through what mechanism

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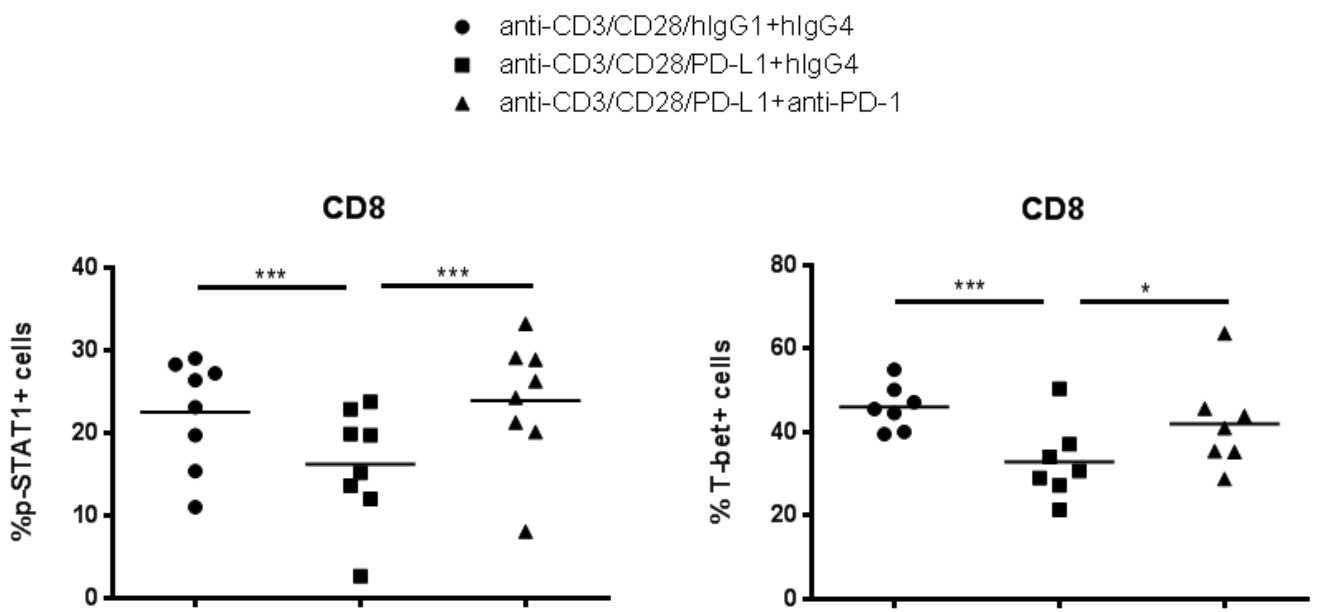
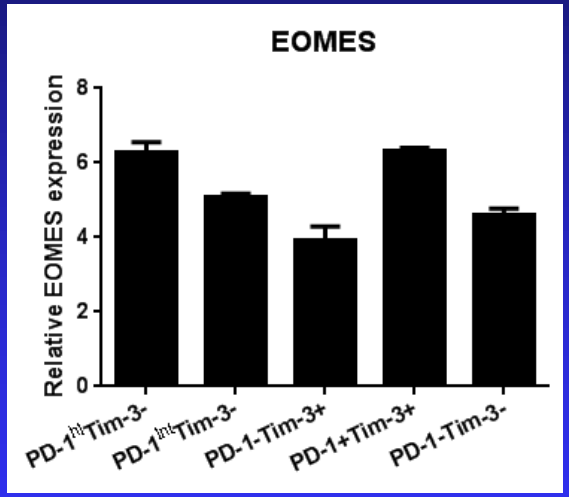
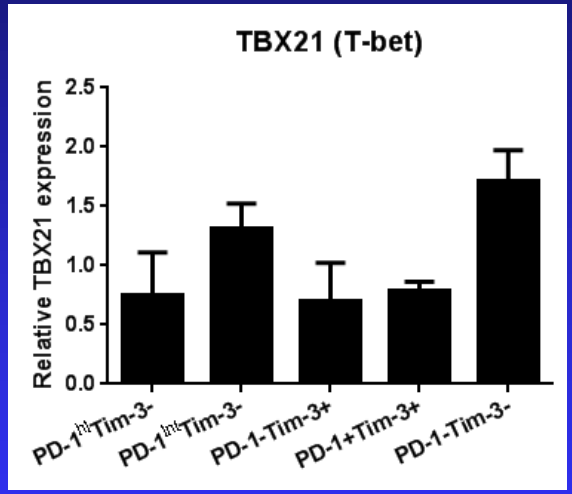

anti-CD3/CD28/hIgG1+hIgG4
anti-CD3/CD28/PD-L1+hIgG4
anti-CD3/CD28/PD-L1+anti-PD-1
CD8
%p-STAT1+ cells
40
30
20
10
0
***
***
CD8
% T-bet+ cells
80
60
40
20
0
***
*


EOMES
Relative EOMES expression
8
6
4
2
0
PD-1hiTim-3-
PD-1intTim-3-
PD-1-Tim-3+
PD-1+Tim-3+
PD-1-Tim-3-


TBX21 (T-bet)
Relative TBX21 expression
2.5
2.0
1.5
1.0
0.5
0.0
PD-1hiTim-3-
PD-1intTim-3-
PD-1-Tim-3+
PD-1+Tim-3+
PD-1-Tim-3-