

A network diagram background with nodes and connecting lines. The nodes are represented by circles of varying sizes and colors, ranging from light blue to bright yellow. The lines are thin and light-colored, creating a complex web of connections. The background has a color gradient from orange on the left to blue on the right.

# Research and Development Program Update

December 1, 2015

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# Agenda

- Preclinical animal model studies
- Ex vivo lung biopsy studies

# Hu-NSG PDX study outline (Lung LG1306)

CD34+ engrafted Hu-NSG  
large cohorts available  
any time

NSG PDX tumor bearing mice

Harvest tumor fragments and trocar into recipients

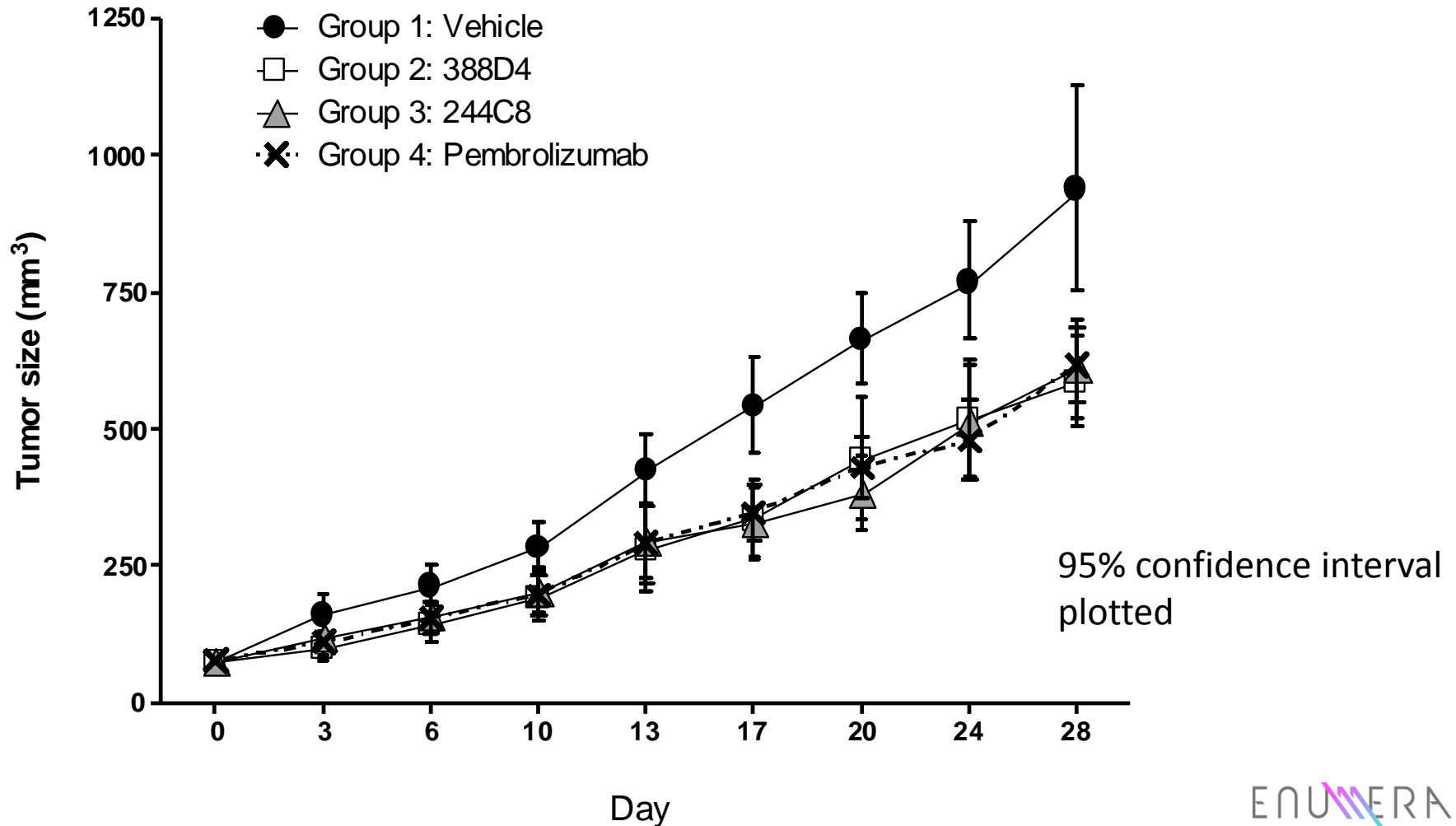
Trocar date is depending on # of tumor bearing donors mice and their tumor size

Hu-NSG (12 week post CD34+ engraftment (>25% human CD45+))

~ 2-3 weeks for tumor graft growth to 100 mm<sup>3</sup> size

Dosegroups of 12 animals per group are dosed and monitored for 28 days

# Tumor Growth (Mean)

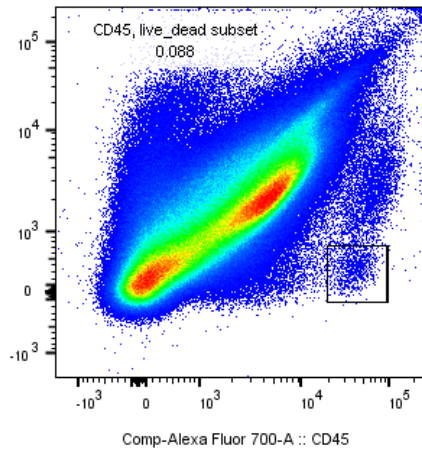


# Study Observations

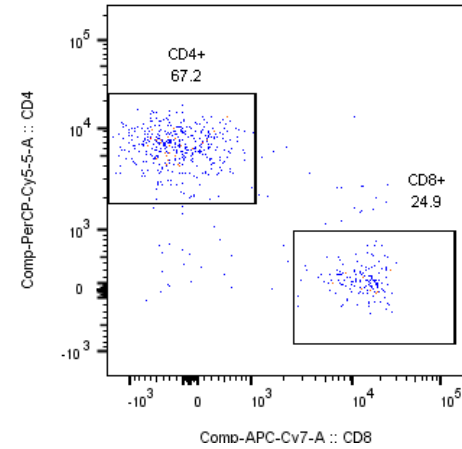
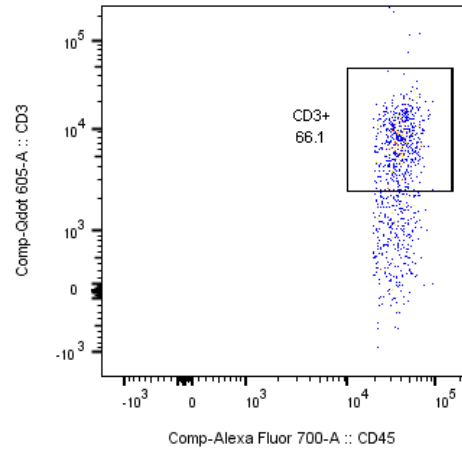
- End of study tumor biopsies collected and analyzed
- Low TIL % in tumors
  - <1% TIL in tumors
  - Suggests narrow dynamic range for immuno-modulation
- Unclear if myeloid functionality in hu-NSG model
  - Kinetics of engraftment may not recapitulate biology of myeloid cells
  - Newer NSG-SGM3 model thought to rectify this

# T Cell Infiltration from hu-NSG/PDX Tumors

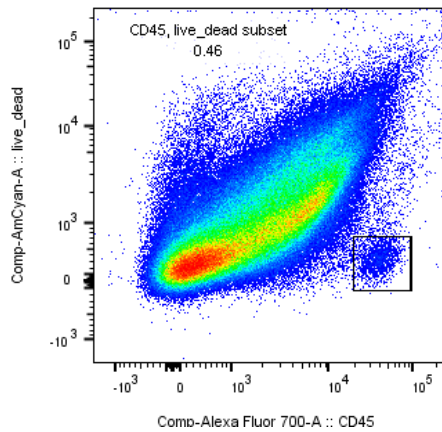
## Vehicle Control



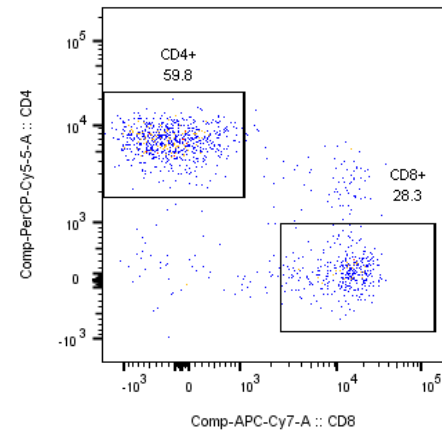
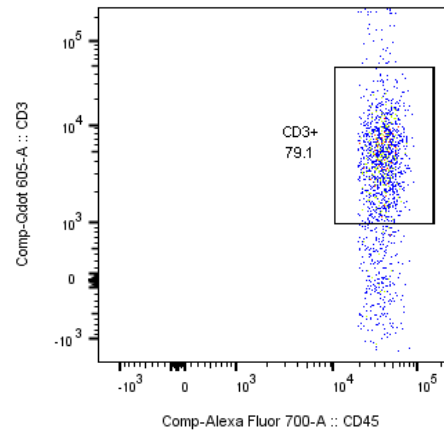
CD3+ Freq. of Total : 0.058



## Vehicle Control



CD3+ Freq. of Total : 0.37



# Summary

- Enumeral anti-PD1 antibodies 388D4 and 244C8 demonstrate activity in an accepted *in vivo* model of immunomodulation
  - huNSG lung PDx model used
  - Tumor growth inhibition similar to that observed with pembrolizumab
- Initial characterization of tumor biopsy suggests low levels of TIL infiltration
  - Model has a narrow dynamic range for measuring activity of novel immunomodulators and may not reflect effects on non-T cell compartments

# PD-1 Blockade in NSCLC Tumor Samples

Complex IMR biology

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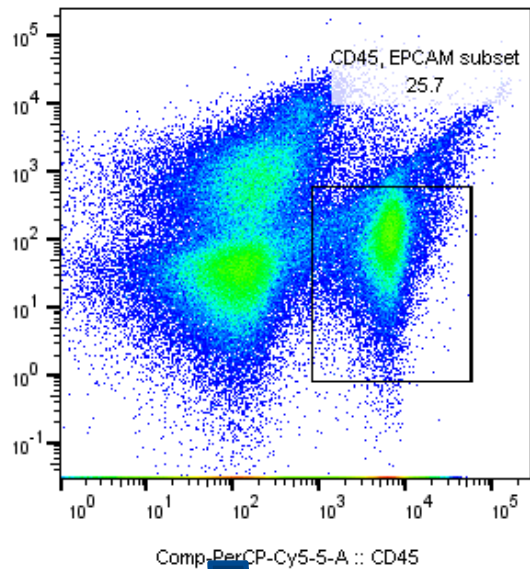


# Overview: Data Annotation

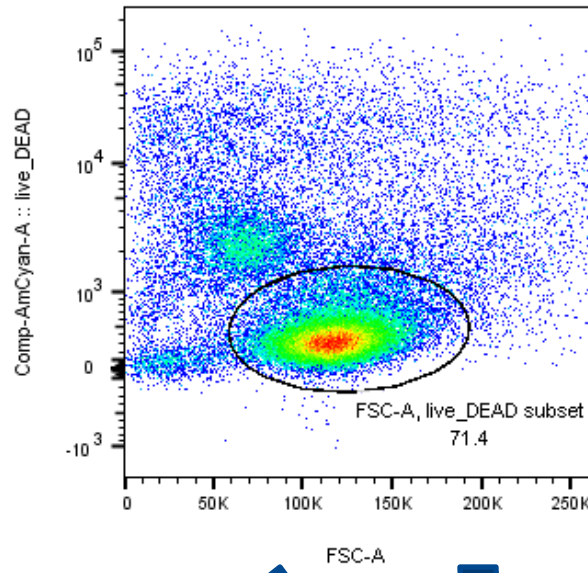
- The following slides show data obtained from two different lung biopsies obtained from patients undergoing staging surgeries
- Characterization of baseline IMR expression on TILs:
  - TIM-3<sup>lo</sup>/TIGIT<sup>lo</sup> - WD36444
  - TIM-3<sup>hi</sup>/TIGIT<sup>hi</sup> - WD36988

# NSCLC WD36444 Contains 14% T Cells

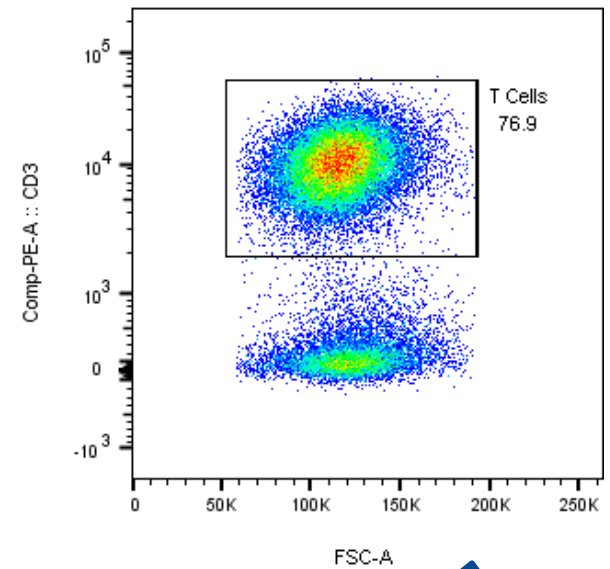
CD45+ cells



Live/dead cells

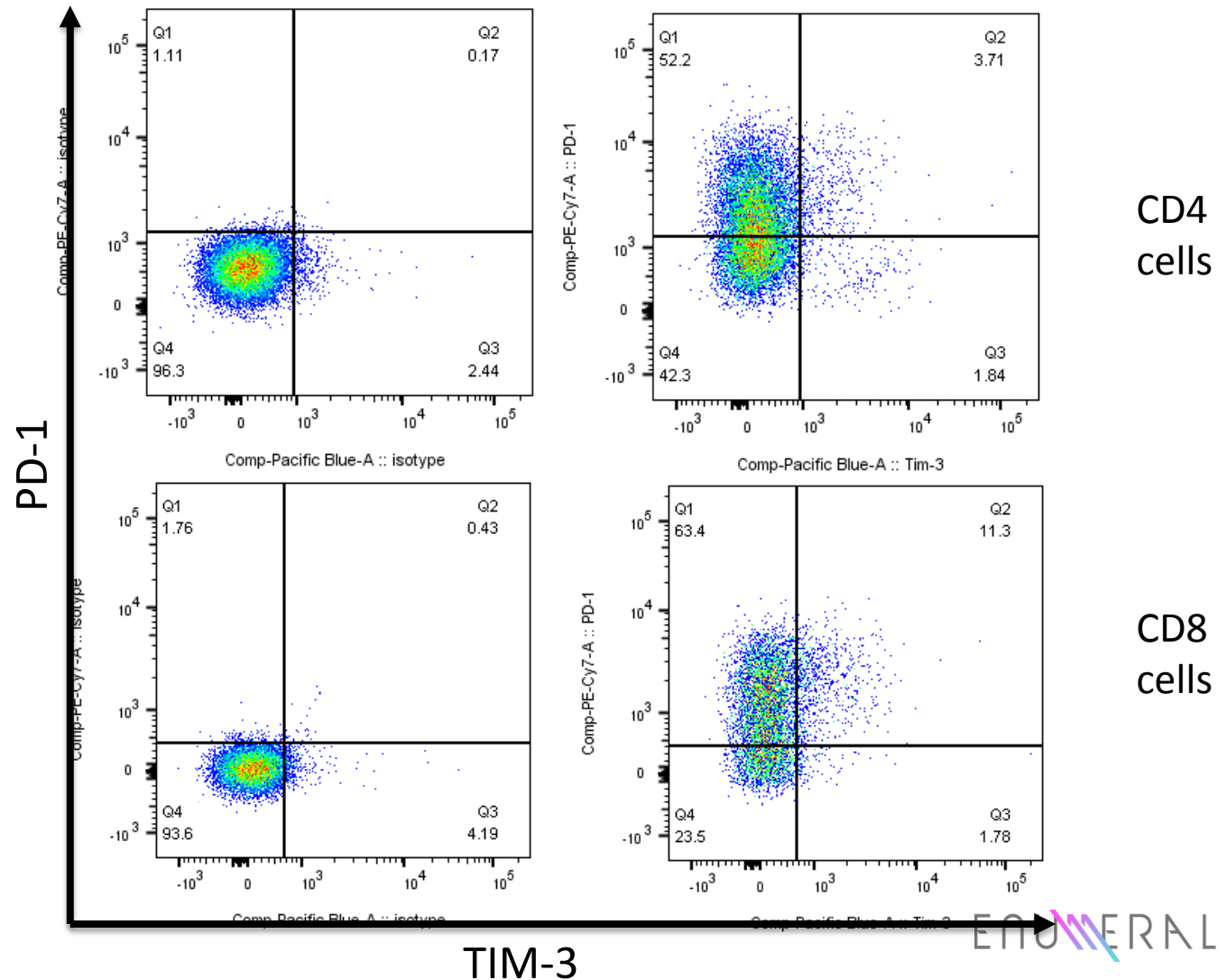


T cells

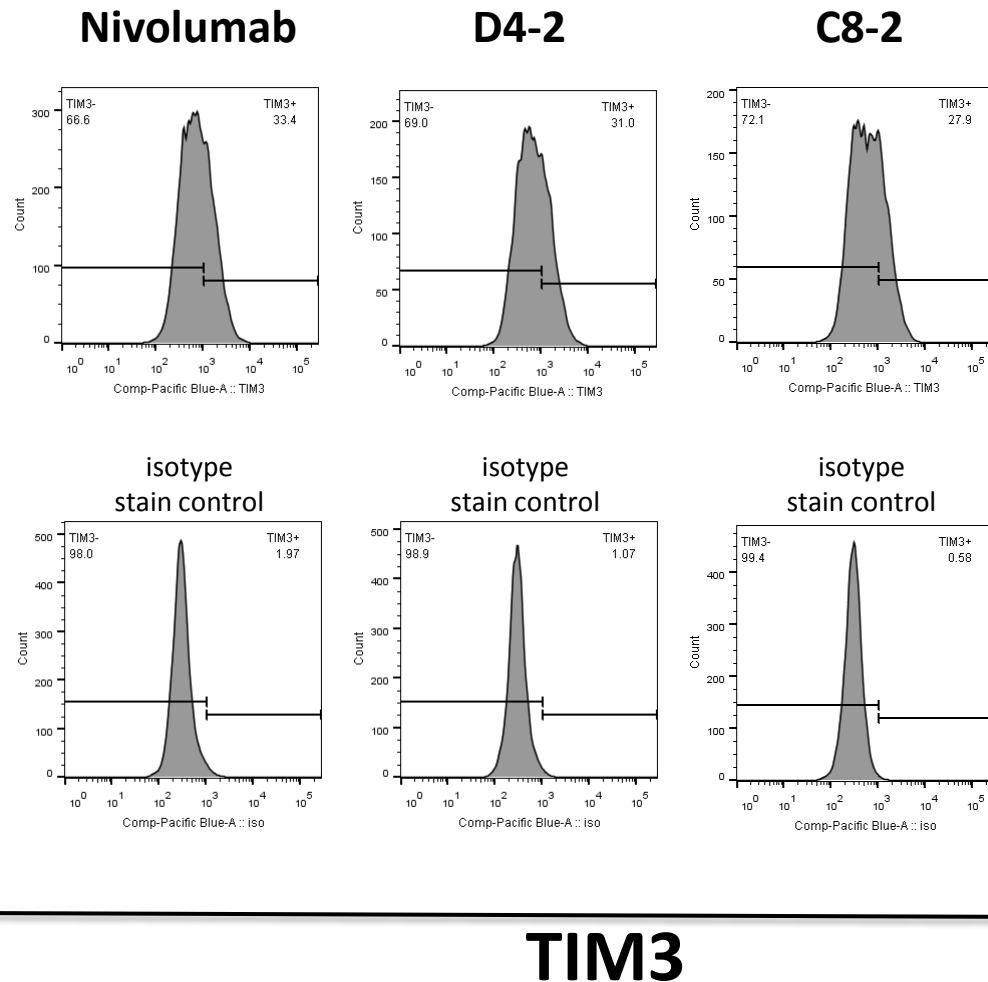


# WD36444 CD4 and CD8+ TILs Express PD1<sup>hi</sup>, but TIM-3<sup>lo</sup>

- PD-1 is expressed on 55% of CD4 cells and 75% of CD8 cells
- **TIM-3 is expressed on 5.5% of CD4 cells and 13% of CD8 cells**
- Cells dually expressing both markers are observed



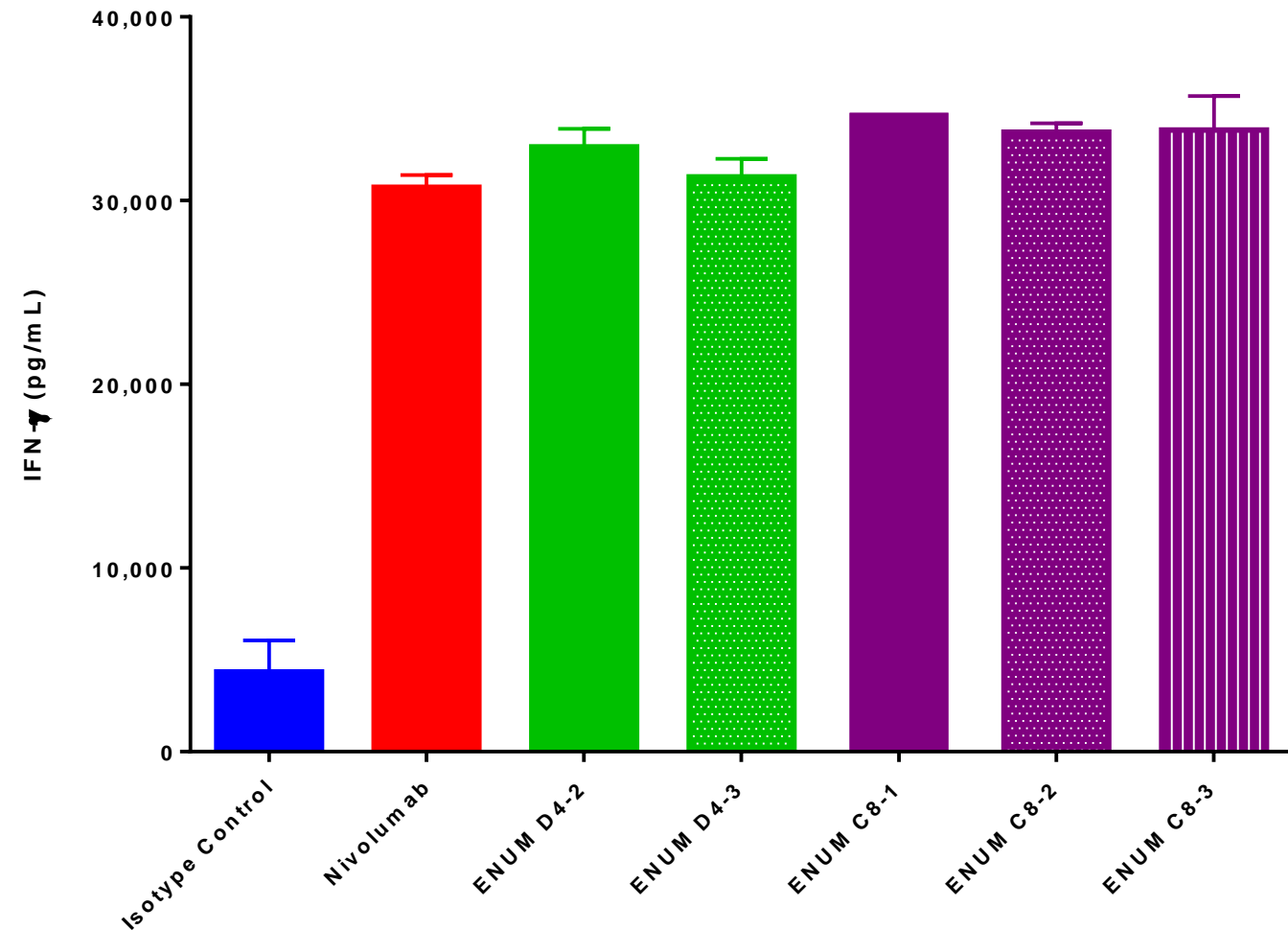
# Ex Vivo PD-1 Blockade Can Drive Expression of TIM-3 on TILs Derived From Lung Cancer Biopsy



WD-36444

Condition	CD3 <sup>+</sup> Tim-3%
isotype	<1
Nivolumab	31.4
D4-2	29.9
D4-3	34.7
C8-1	39.5
C8-2	27.3
C8-3	42.5

# Ex Vivo PD-1 blockade Can Reverse TIL Exhaustion

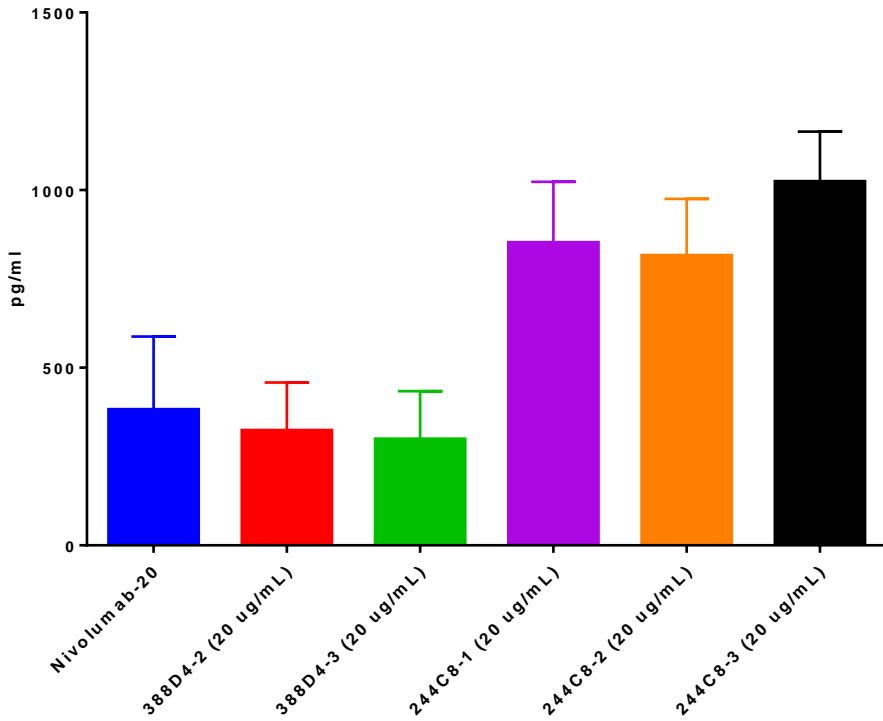


**Experimental Protocol:**  
300,000 cells purified from Tumor WD3644 were stimulated with anti-CD3+anti-CD28 in the presence of 20 ug/mL isotype control or the indicated anti-PD1 antibody.

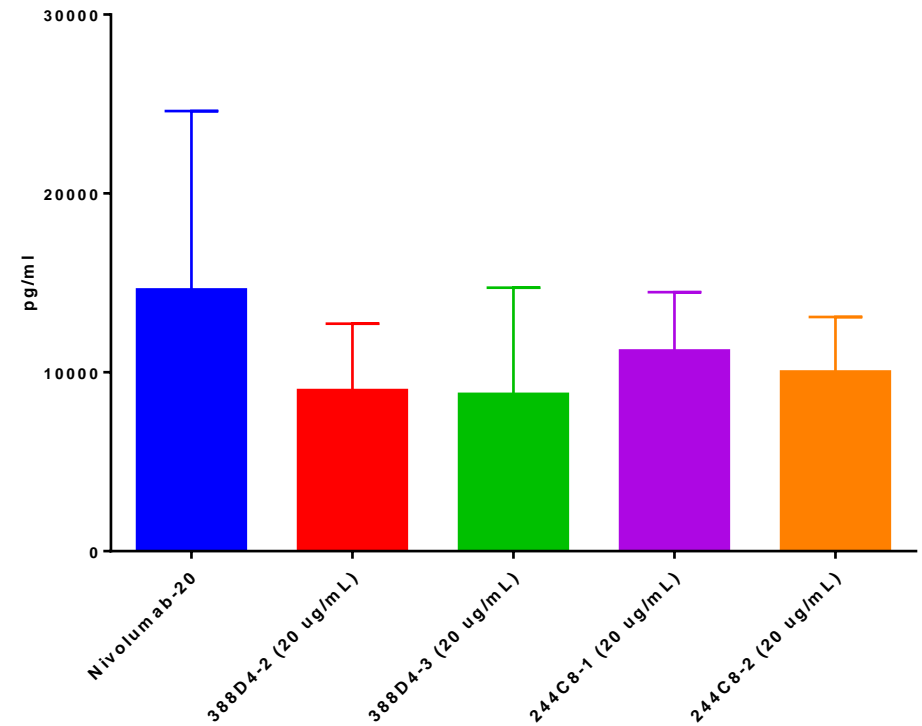
Supernatants were analyzed by **ELISA** after 24 hours of Activation.

# Selective IL-12 Induction with 244C8 in TIM-3<sup>lo</sup> NSCLC

WD-36444  
IL-12 p70

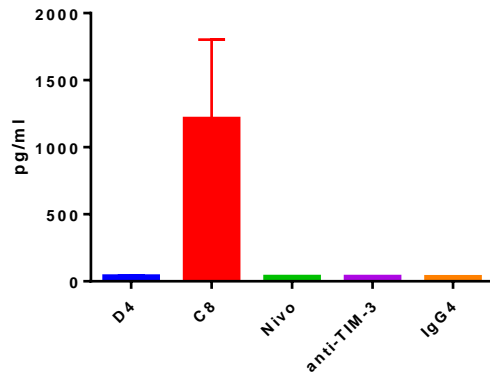


WD-36444  
TNF- $\alpha$

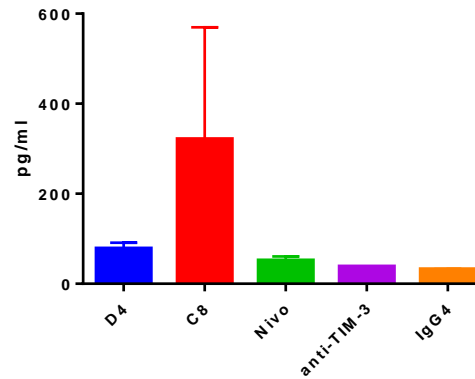


# IL-12 Induction is Consistent and Specific to 244C8-based PD-1 Blockade

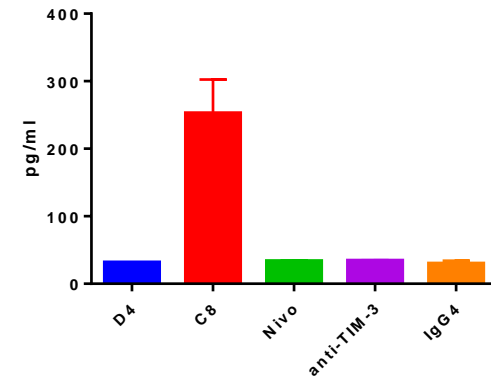
IL-12 MLR donor 26 day 2.5



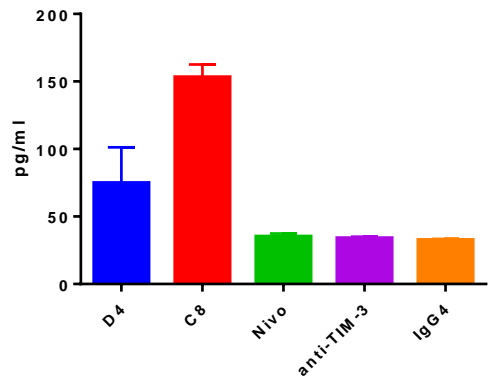
IL-12 MLR donor 26 day 5.5



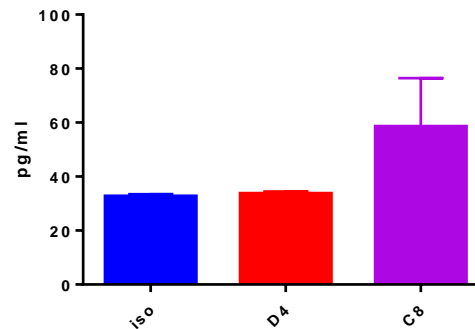
IL-12 MLR donor 23 day 2.5



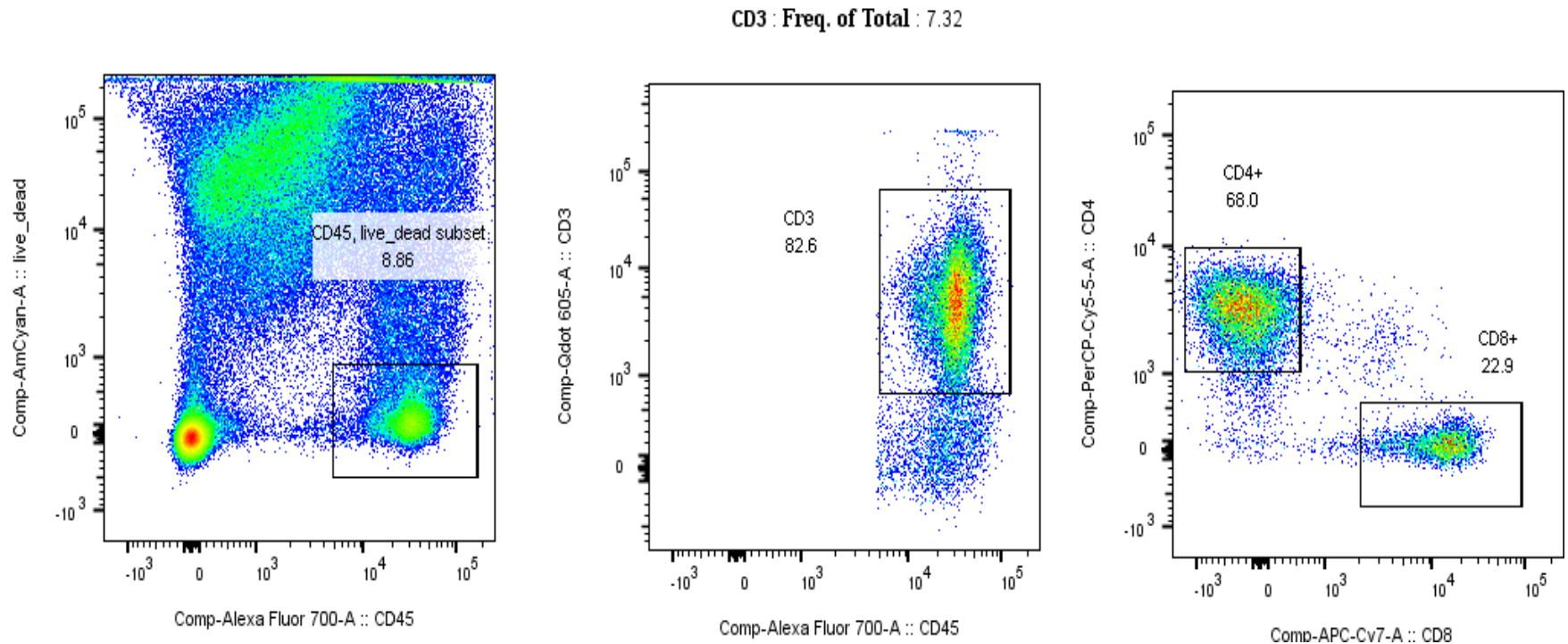
IL-12 MLR donor 23 day 5.5



WD-36988  
IL-12

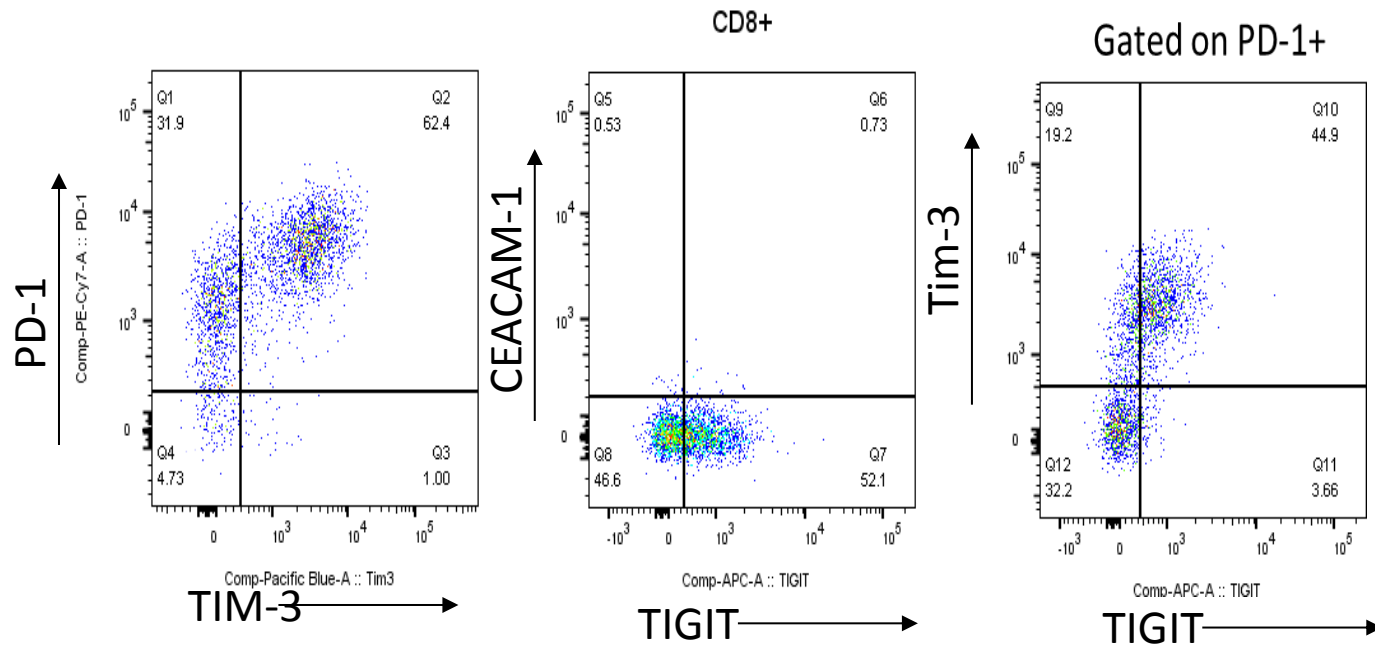


# NSCLC WD36988 Contains 7% T Cells





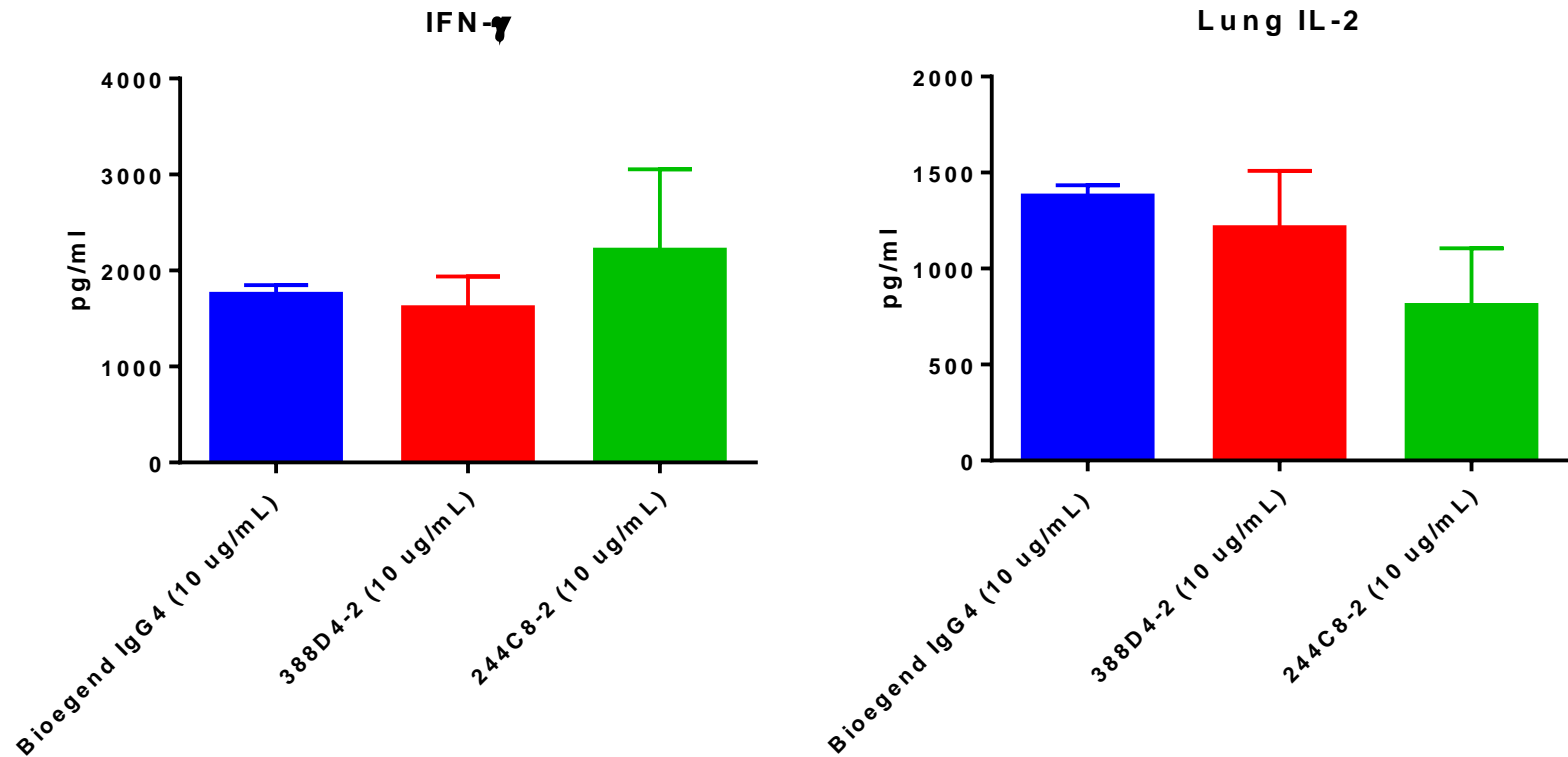
# CD8<sup>+</sup> TILs Express PD-1, Tim-3, TIGIT



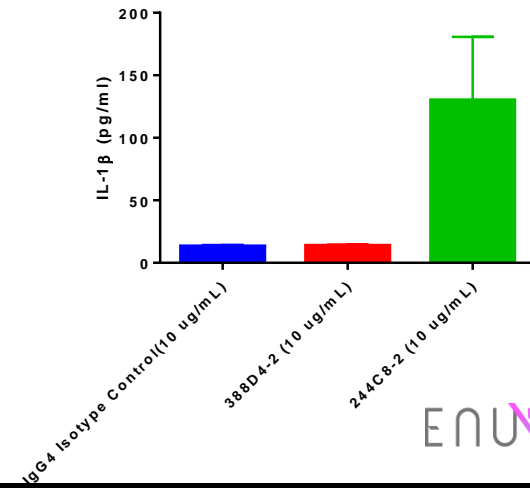
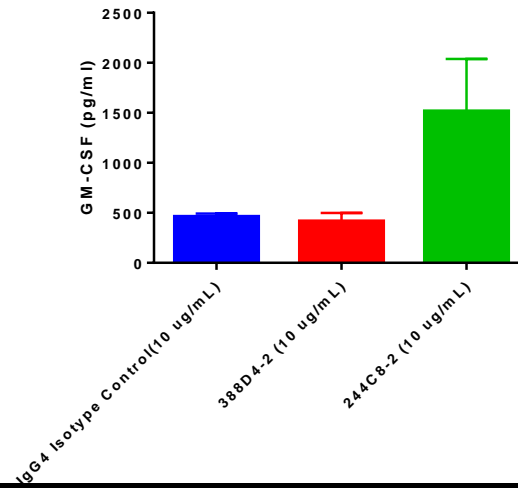
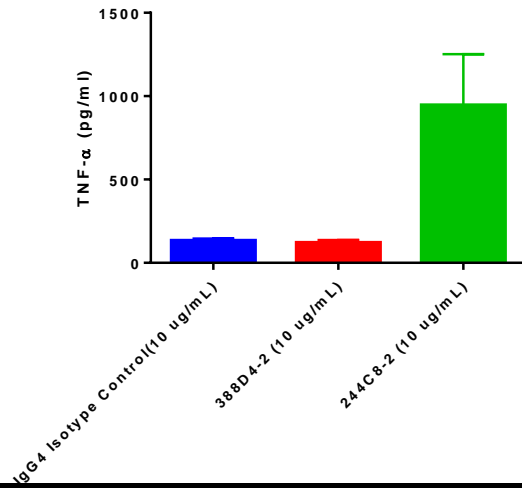
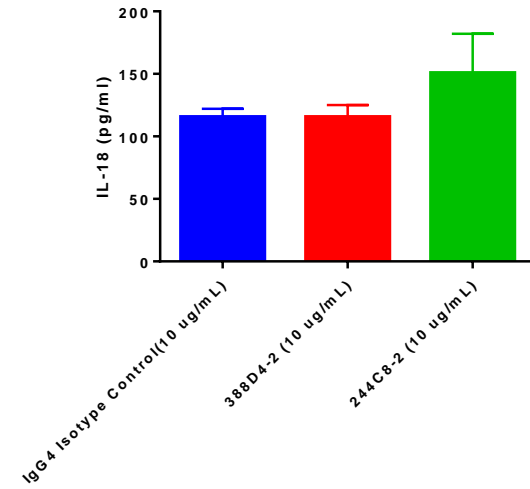
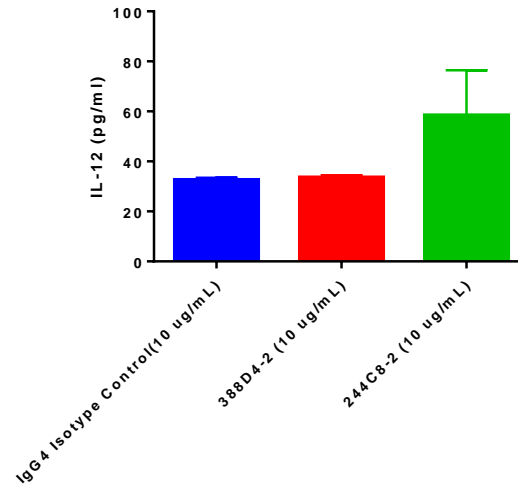
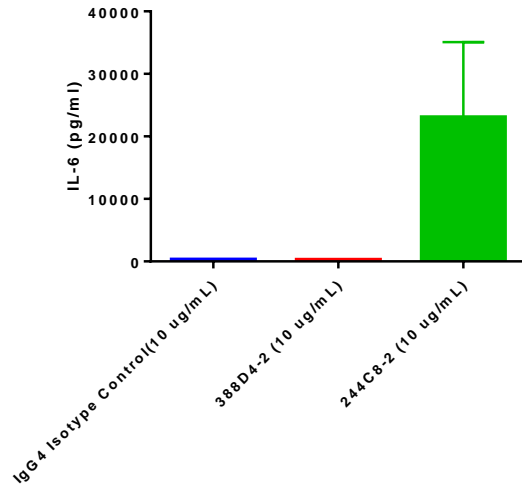
93% of CD8<sup>+</sup> express PD-1  
 62% of CD8<sup>+</sup> express TIM-3  
 No CD8<sup>+</sup> express CEACAM-1  
 52 % of CD8<sup>+</sup> express TIGIT

70 % of PD-1<sup>+</sup>  
 TIM3<sup>+</sup> cells are  
 also TIGIT<sup>+</sup>

# Ex Vivo PD-1 Blockade can still increase IFN $\gamma$ production in TIM-3<sup>hi</sup>/TIGIT<sup>hi</sup> T cells



# 244C8 Specific PD-1 Blockade Generates a Different Molecular Signature than 388D4 in an NSCLC tumor with TIM-3<sup>hi</sup>/TIGIT<sup>hi</sup> T cells



# Summary

- NSCLC tumors generally have PD-1<sup>hi</sup> T cell infiltrates, however the level of additional checkpoint markers varies considerably
- PD-1 biology appears to be linked to TIM-3 receptor expression and both markers appear to be important in T cell exhaustion in NSCLC tumors
- PD-1 blockade can be biologically distinct between anti-PD-1 antibodies
  - Enumeral 244C8 is an anti-PD-1 antibody that may promote release of non T cell (myeloid) derived anti-tumor cytokines



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