Enumeral PD-1 Program Update: Differentiated Anti-PD-1 Antibody Functional Characterization in *Ex Vivo* Human Lung Biopsy Assays

November 18, 2015

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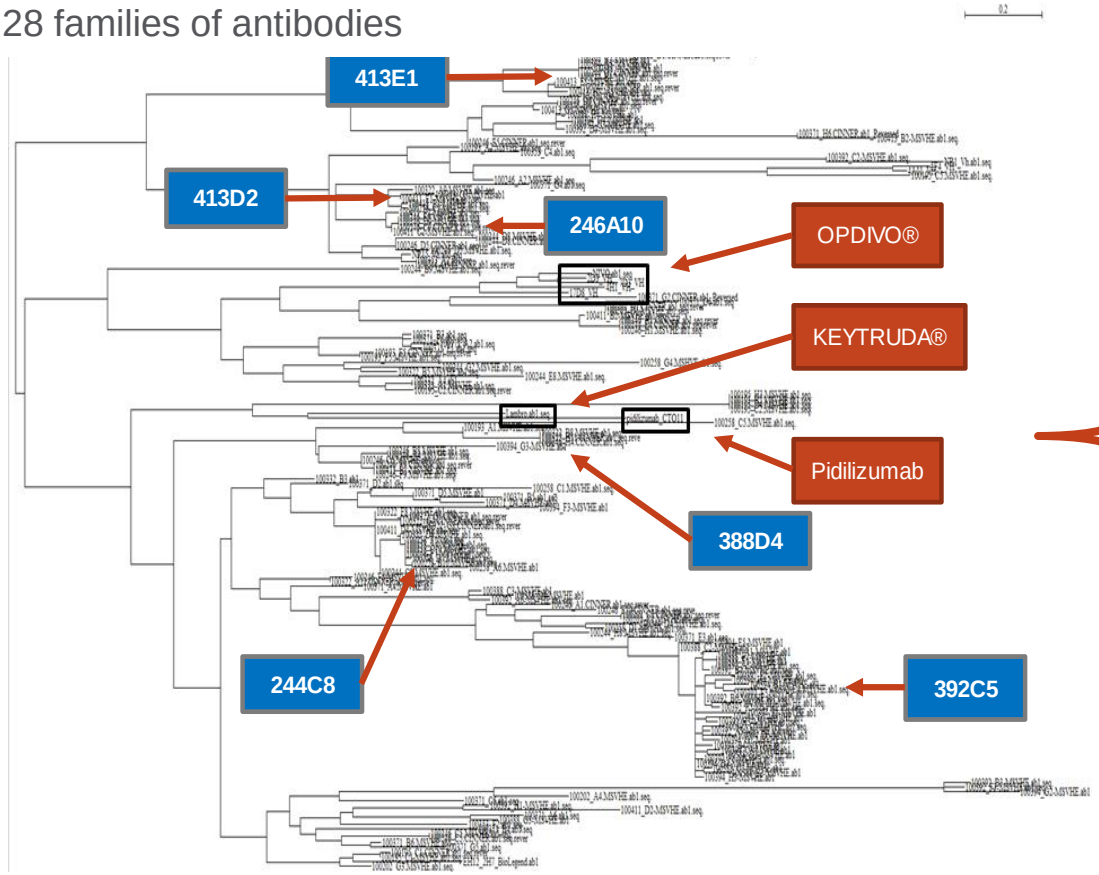
Background

- Enumeral uses a unique single cell technology platform and approach to identify functionally differentiated antibody candidates
  - Enumeral has identified two classes of anti-PD-1 antibodies with distinct modes of binding to PD-1
  - Both classes demonstrate enhancement of T cell activation via reversal of PD-1-dependent immunosuppression

Enumeral's Approach to Developing Differentiated Antibodies Starts with Diversity

Cladogram representing heavy chain AA sequences
N= 159 sequences shown
28 families of antibodies



- Enumeral antibody discovery results in exceptional diversity*
- Potential for strong IP position
- Breadth of diversity: keys to unlocking the target physiology
- Multiple potential program opportunities

*Based on ENUM evaluation of published literature

Enumeral PD-1 Program

- Enumeral has identified a novel potentially allosteric anti-PD-1 antagonist (ENUM 244C8) displaying the following properties:
  - Reversal of PD-L1-dependent immunosuppression
  - Binding to PD-1 via a novel epitope
  - Increased levels of T cell activation in cell-based assays
  - Binding to PD-1 independent of PD-L1

- ENUM 244C8 antibody and a currently-marketed anti-PD-1 antibody were tested for their ability to reverse tumor infiltrating lymphocyte (TIL) exhaustion using lymphocytes derived from human lung biopsy
  - ENUM 244C8 observed restoring T cell function to a higher level than the positive control nivolumab

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Ex Vivo Reversal of TIL Exhaustion: Methods

- NSCLC samples from staging surgeries were analyzed within 24 hours of collection
- Flow cytometry analyzed extent of T cell infiltration and co-expression of immunomodulatory receptors (PD-1 and TIM-3)
- Cells were incubated with anti-CD3/anti-CD28 antibodies for 24 hours and either negative control (isotype, Biolegend), nivolumab (Invivogen), or humanized derivatives of ENUM 388D4 and ENUM 244C8 (designated D4-1, D4-2, D4-3, C8-1, C8-2, C8-3)
- Interferon gamma production was measured (ELISA) and data is expressed as pg/mL IFN-γ.

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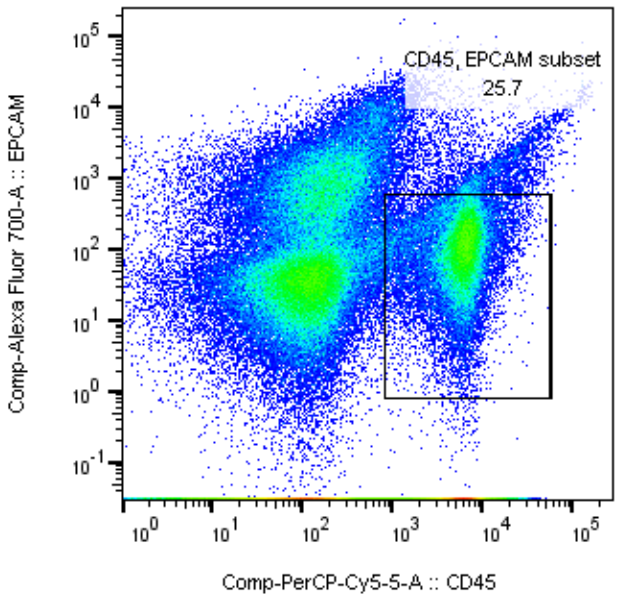
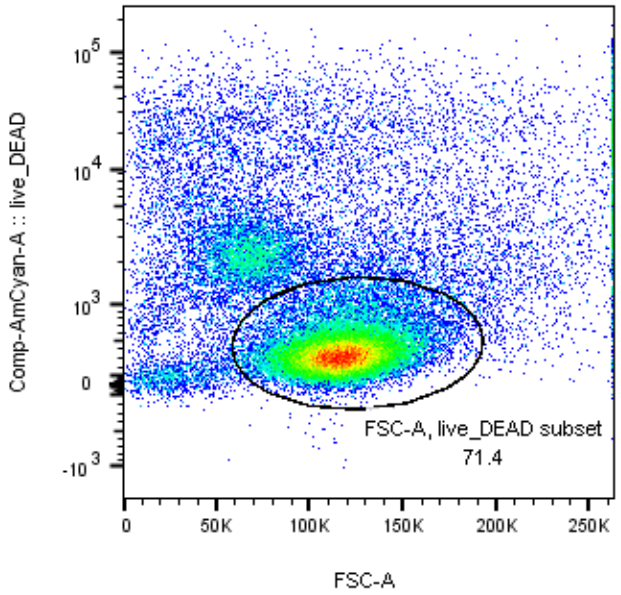
Ex Vivo Reversal of TIL Exhaustion: Example Flow Cytometry Analysis

NSCLC WD36444 contains 14% T cells

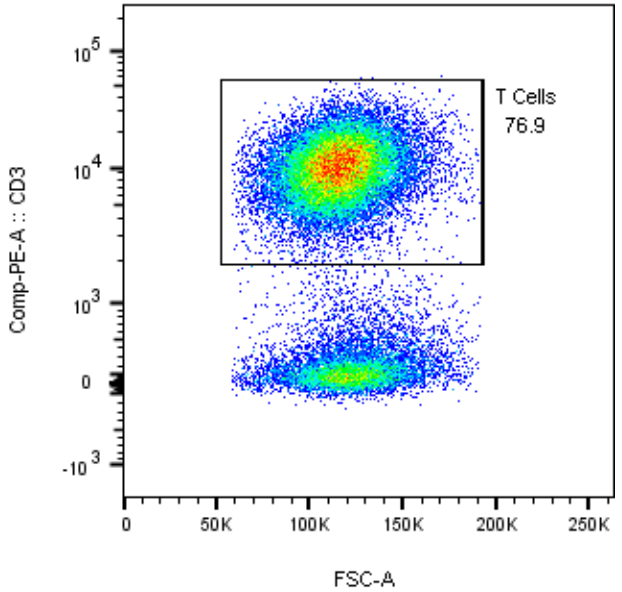
Lung sample #1 ID WD-36444



WD36444_Lungtumor 1_snapshot panel.fcs
Ungated
200000



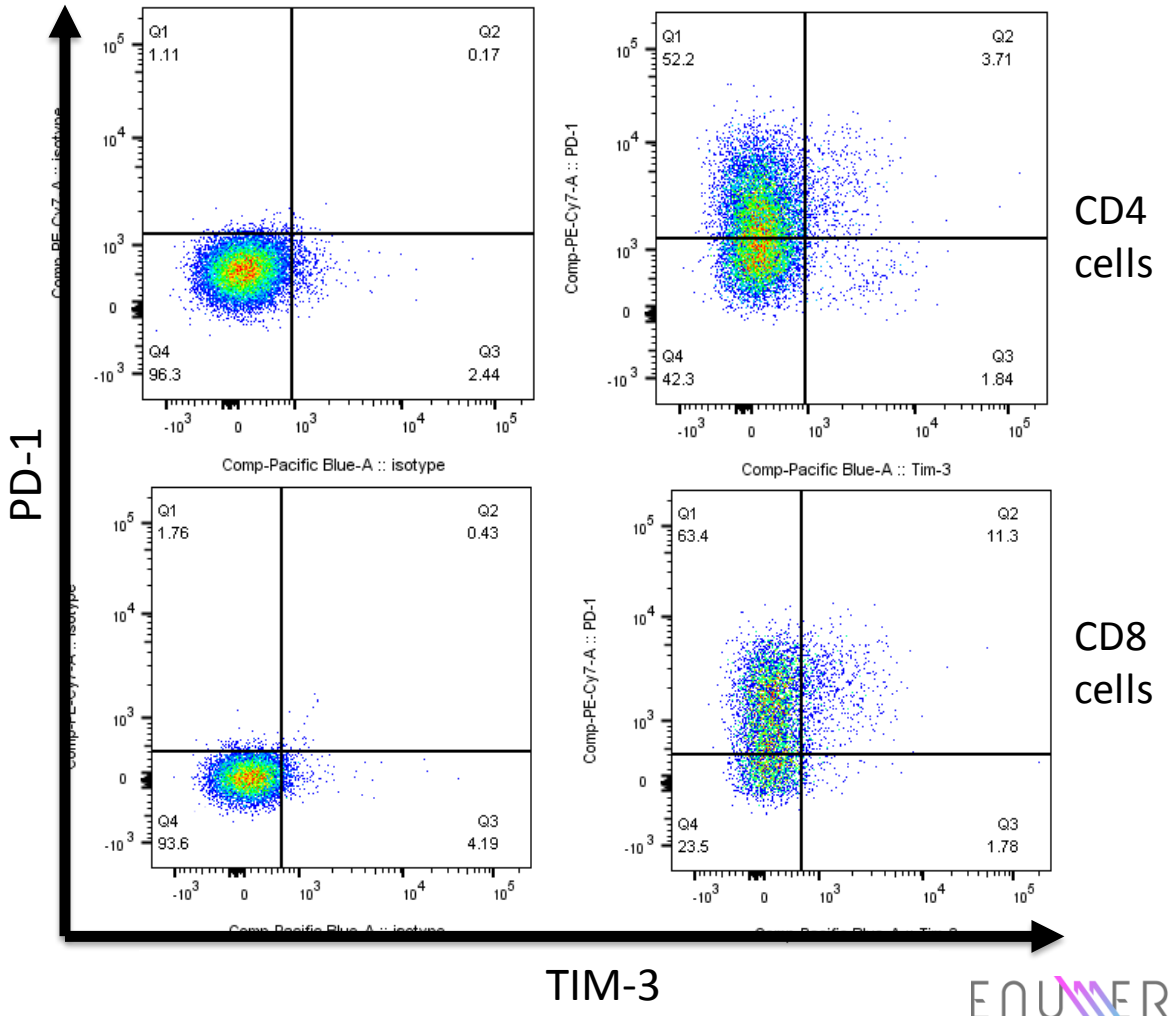
WD36444_Lungtumor 1_snapshot panel.fcs
CD45, EPCAM subset
51356



WD36444_Lungtumor 1_snapshot panel.fcs
FSC-A, live_DEAD subset
36686

Ex Vivo Reversal of TIL Exhaustion: Co-expression of Immunomodulatory Receptors (IMRs)

- WD36444 CD4+ and CD8+ TILs express exhaustion markers (IMRs)
- PD-1 expressed on 55% of CD4 cells and 75% of CD8 cells
- 'IMR X' expressed 5.5% of CD4 cells and 13% of CD8 cells



Ex Vivo Reversal of TIL Exhaustion: Variability Across Patients

NSCLC tumor biopsies demonstrate varying degrees of lymphocyte infiltration and PD-1 expression on T cells

Tumor Identifier	% EpCAM-CD45+	%CD3+	%CD4$^+$ PD-1$^+$	%CD8$^+$ PD-1$^+$	%CD4$^+$ TIM3$^+$	%CD8$^+$ TIM3$^+$
WD-36444*	25.7	14	55	75	5.5	13
WD-36571*	10.3	6.3	47	64	1.8	<1
WD-36686*	21.6	17	55	84	6	16
WD-36790*	16.8	10.4	38	68	1	5.2
WD-36904*	12.8	7	63	72	9.5	24.5
M115801A2*	3.4	2.9	79	84	22	16
WD-36923	1.6	0.9	53	51	27	n/a
WD-36988*	8.9	7	58	93	22	62
M4150952	5.4	3	78	79	26	15
M1151877A	15.9	12.8	57	71	11	24

- Data from flow cytometry analysis
  - Lymphocyte infiltration ranged from 1.6% - 25.7%
  - T cell infiltration ranged from 0.9% - 17%

*Data on functional reversal of exhaustion reported on following slides

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Ex Vivo Reversal of TIL Exhaustion: Experimental Questions

- Tumor biopsy from n=10 patients found to harbor TILs that express exhaustion markers including PD-1.

1. What is the activity of the T cells following activation?
   - If cells do not produce IFN-γ in response to TCR triggering (anti-CD3 + anti-CD28), cells are “exhausted”.

2. Is cellular activity modified by the addition of an anti-PD-1 antibody?

3. Do nivolumab and ENUM antibodies behave differently in this experiment?

4. Do different classes of anti-PD-1 antibody exhibit additive effects on reversal of TIL exhaustion?

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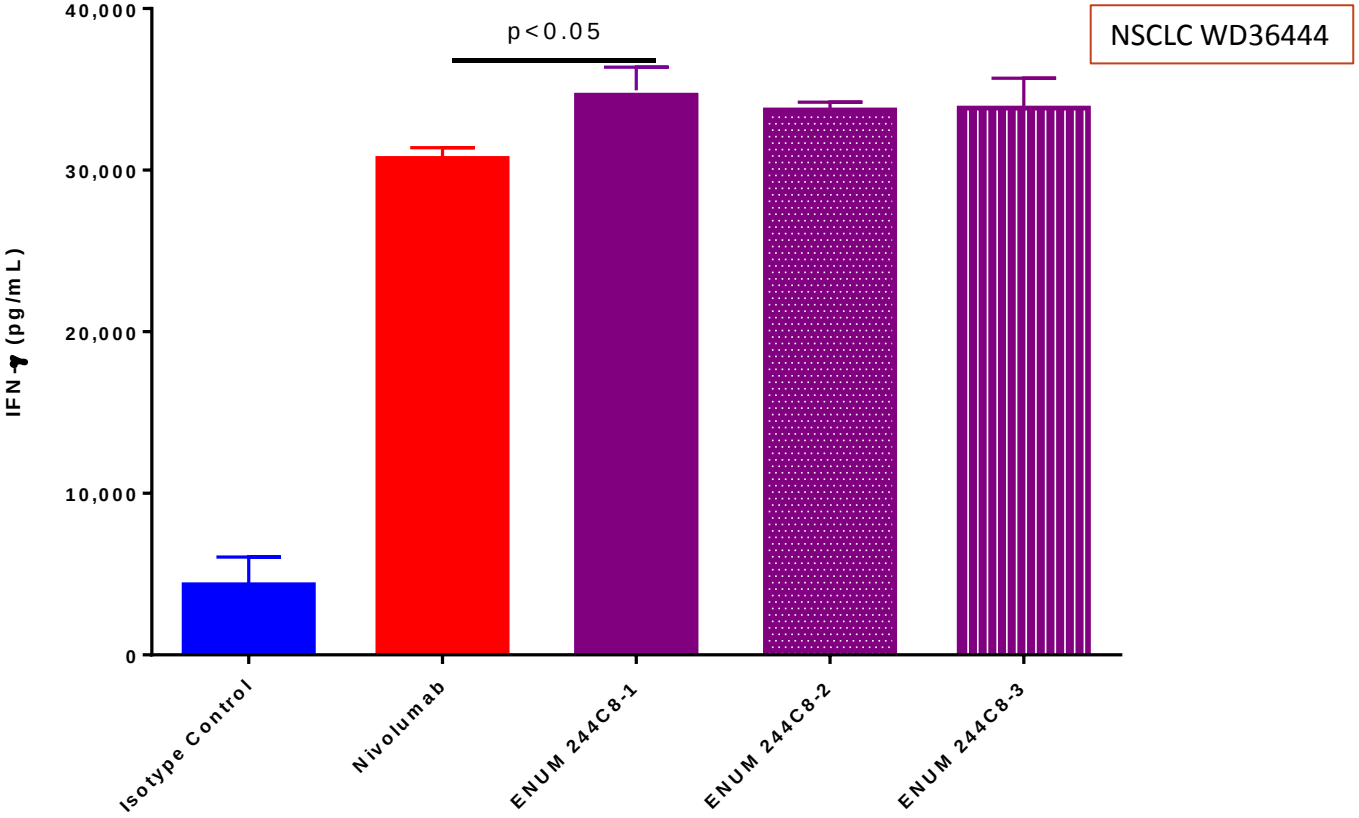
Ex Vivo Reversal of TIL Exhaustion: PD-1 blockade can restore function to T cells derived from human lung biopsy

ENUM 244C8 elicits increased interferon-γ secretion compared to nivolumab



Functional modification of TILs with PD-1 blockade using NSCLC tumor WD36444 containing 25.7% lymphocytes. The indicated antibodies used at 20 ug/mL. ENUMC8-1, ENUMC8-2, and ENUMC8-3 are humanized variants of ENUM 244C8. p<0.05 for ENUMC8-1, ENUMC8-2, and ENUMC8-3 compared to nivolumab.

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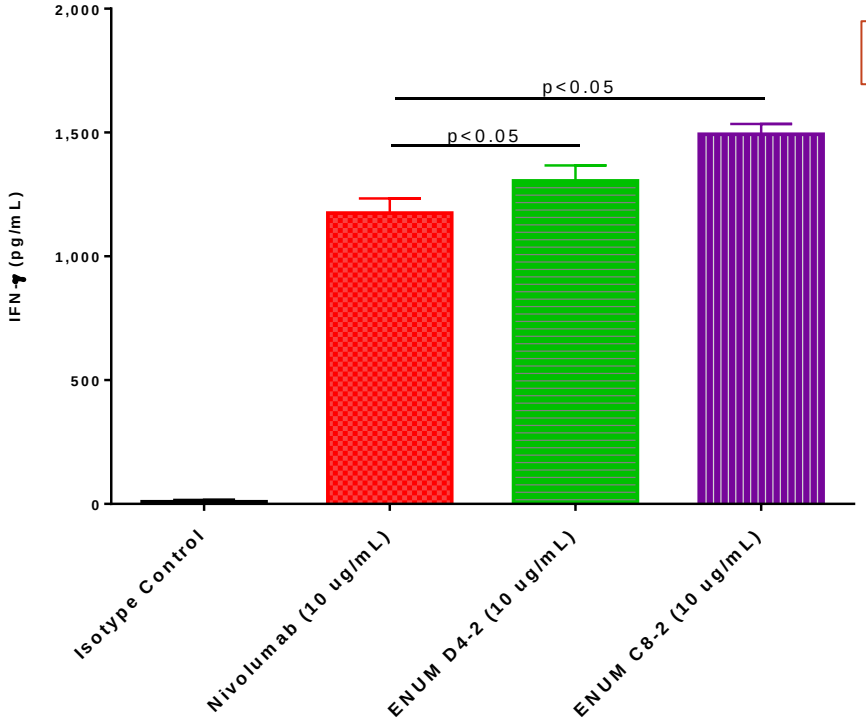
Ex Vivo Reversal of TIL Exhaustion: PD-1 blockade can restore function to T cells derived from human lung biopsy

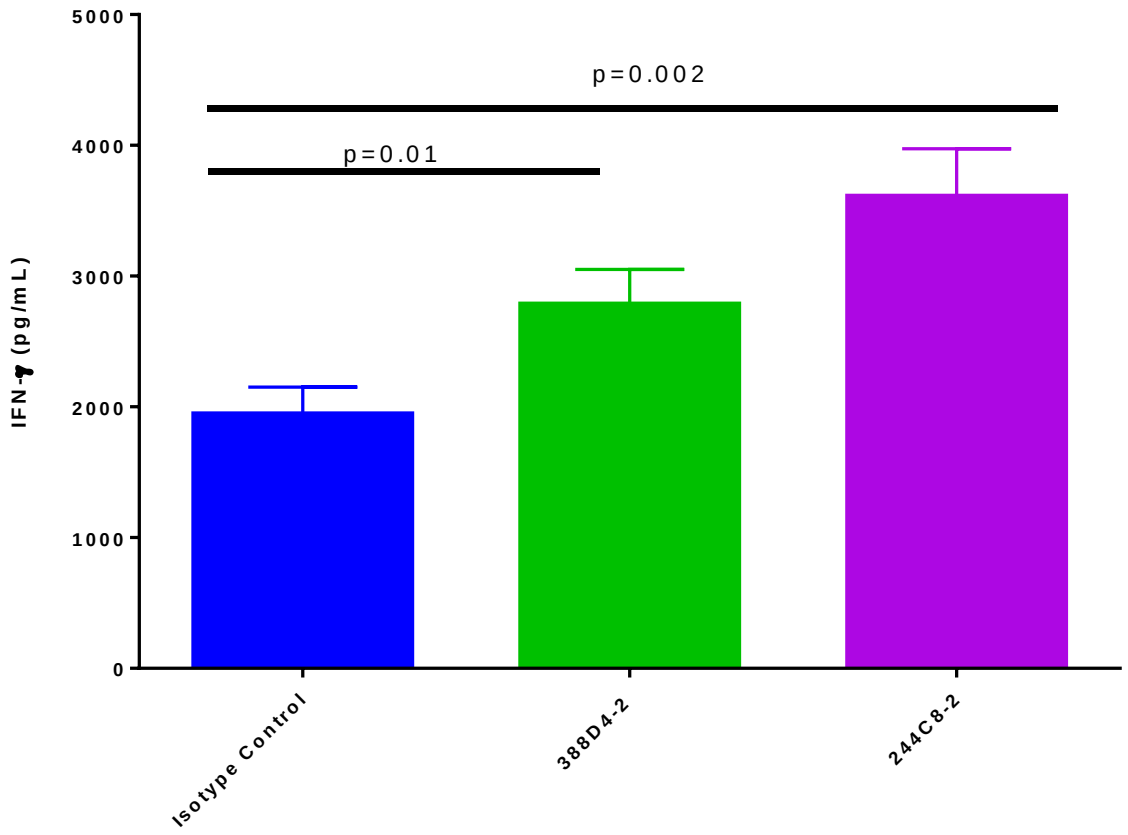
ENUM 244C8 elicits increased interferon-γ secretion compared to nivolumab



Functional modification of Tumor infiltrating lymphocytes with PD-1 blockade. 3e5 WD36571 tumor cells containing 10 % lymphocytes were activated in the presence of anti-CD3+anti-CD28 and the indicated antibodies.

Ex Vivo Reversal of TIL Exhaustion: PD-1 blockade can restore function to T cells derived from human lung biopsy

ENUM 244C8 elicits increased interferon-γ secretion compared to ENUM 388D4



NSCLC WD36988

Functional modification of Tumor infiltrating lymphocytes with PD-1 blockade. 3e5 WD36988 tumor cells containing 8.9 % lymphocytes were tested with the indicated antibodies at the listed concentrations.

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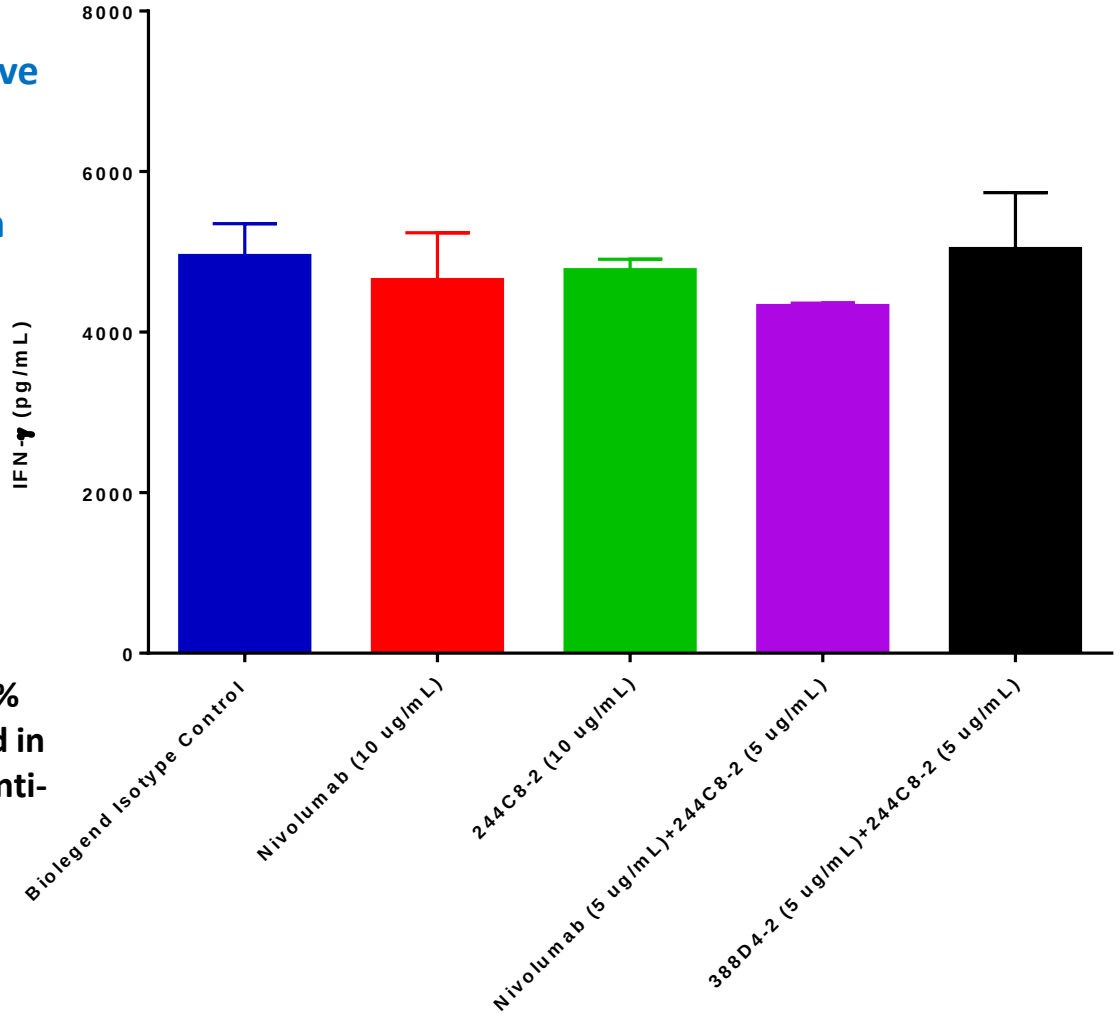
Ex Vivo Reversal of TIL Exhaustion: responsiveness of TILs to PD-1 blockade varies among patients

Despite presence of active TIL infiltrate anti-PD-1 antibodies did not elicit enhanced T cell function



NSCLC WD36904

Functional modification of NSCLC Tumor infiltrating lymphocytes with PD-1 blockade. 3e5 WD36904 tumor cells containing 12.8% lymphocytes were activated in the presence of anti-CD3+anti-CD28 and the indicated antibodies at the listed concentrations.

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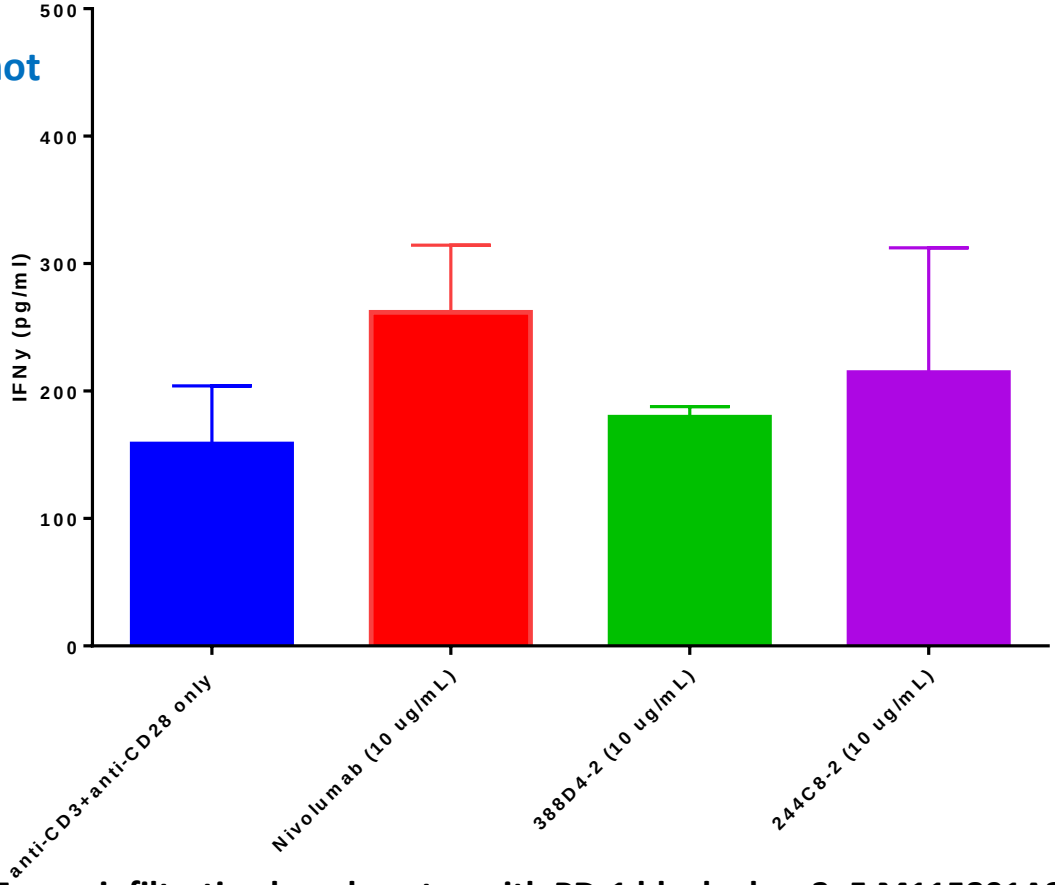
Ex Vivo Reversal of TIL Exhaustion: responsiveness of TILs to PD-1 blockade varies among patients

Anti-PD-1 antibodies did not elicit enhanced T cell function in a low TIL-infiltrate tumor biopsy

NSCLC M115801A2



Functional modification of Tumor infiltrating lymphocytes with PD-1 blockade. 3e5 M115801A2 tumor cells containing 3.4 % lymphocytes were activated in the presence of anti-CD3+anti-CD28 and the indicated antibodies. No statistically significant differences were observed in this experiment.

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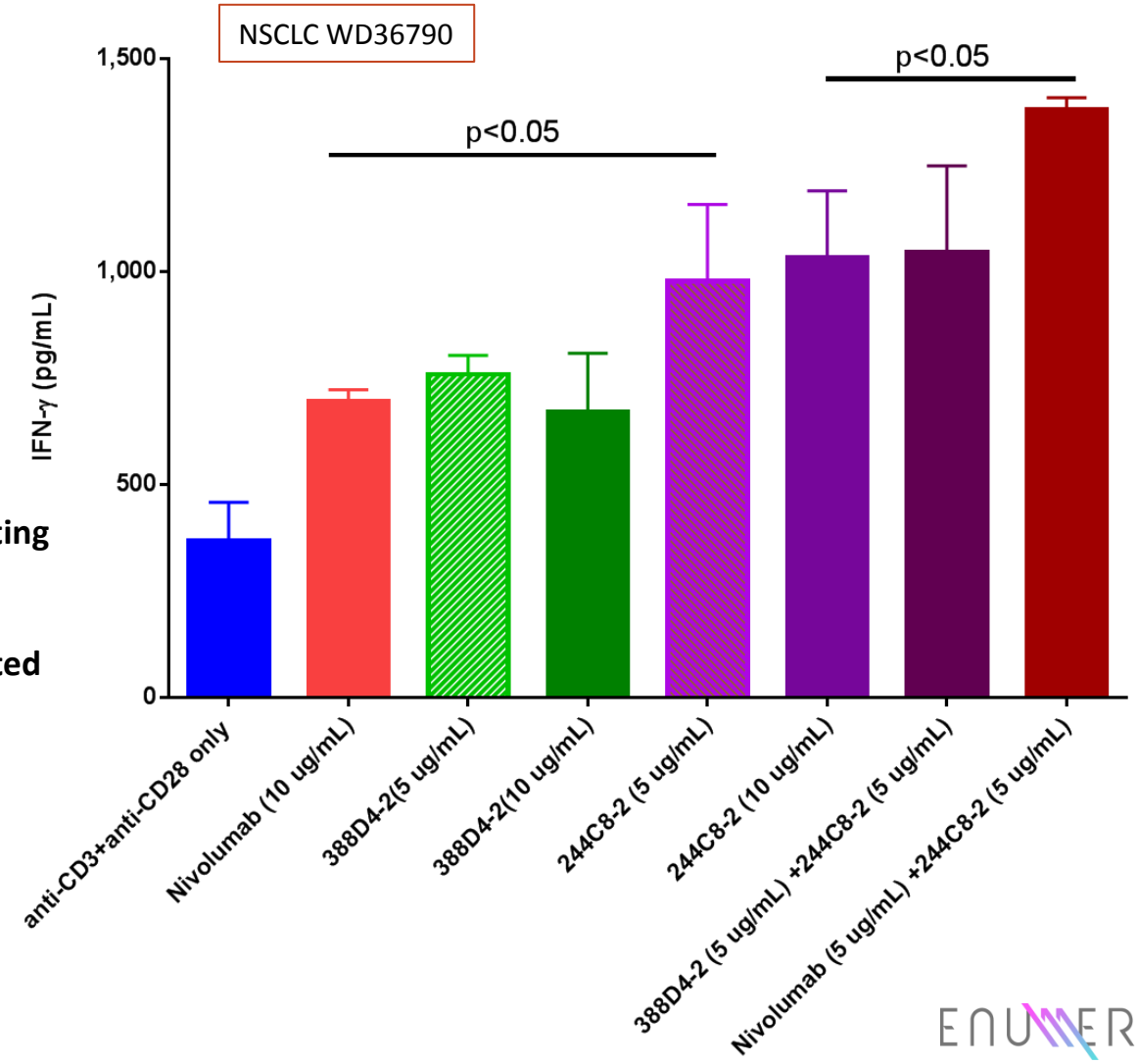
Ex Vivo Reversal of TIL Exhaustion: additive effects of two anti-PD-1 antibodies

ENUM 244C8 elicits increased interferon-γ secretion and can augment nivolumab activity



Functional modification of Tumor infiltrating lymphocytes with PD-1 blockade. 3e5 WD36790 tumor cells containing 16.8 % lymphocytes were tested with the indicated antibodies at the listed concentrations.

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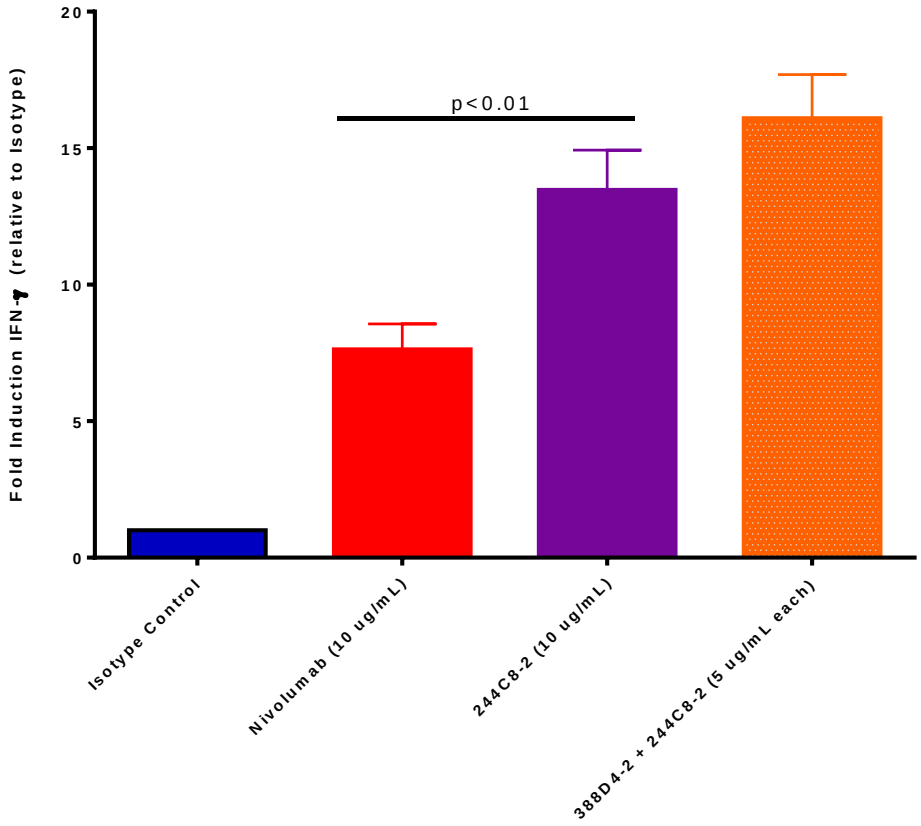
Ex Vivo Reversal of TIL Exhaustion: additive effects of two anti-PD-1 antibodies

ENUM 244C8 elicits increased interferon-γ secretion and can augment nivolumab activity



Functional modification of NSCLC Tumor infiltrating lymphocytes with PD-1 blockade. 3e5 WD36686 tumor cells containing 21.6% lymphocytes were activated in the presence of anti-CD3+anti-CD28 and the indicated antibodies. p<0.01 for ENUM C8-2 vs nivolumab (all at 10 ug/mL); not statistically significant for ENUM D4-2 + ENUM C8-2 (5ug/mL each) vs ENUM C8-2.

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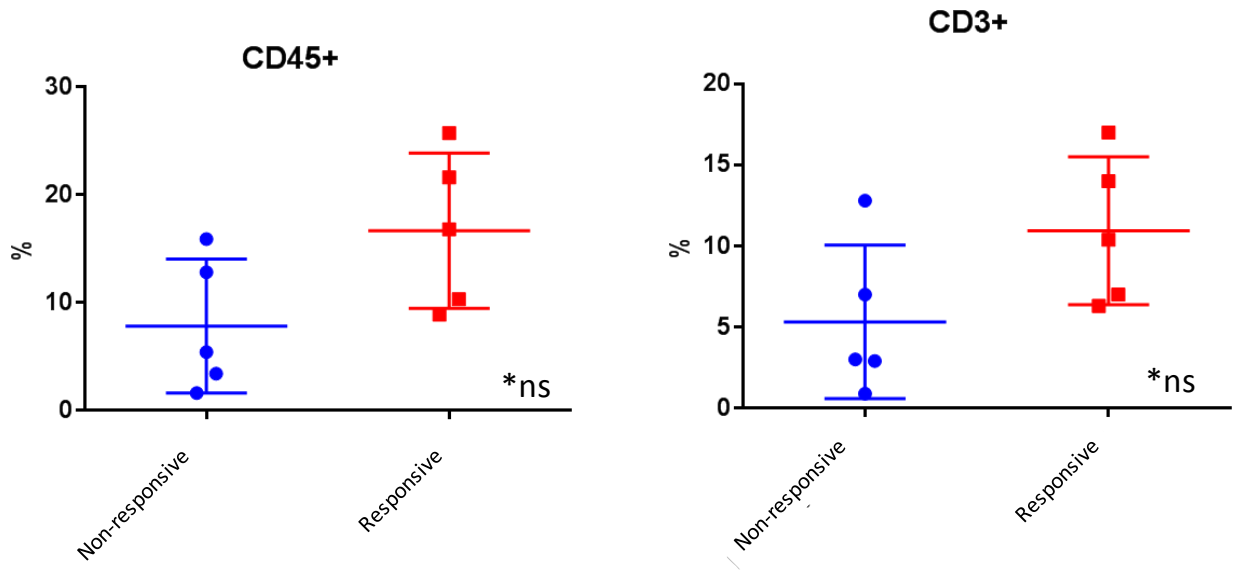
Ex Vivo Reversal of TIL Exhaustion: Summary of Experimental Observations

- Tumor biopsy from n=10 patients were found to harbor TILs that express exhaustion markers including PD-1.
- IFN-γ production was measured as an indicator of reversal of T cell exhaustion following incubation with anti-CD3/anti-CD28 and various anti-PD-1 antibodies.
  - Example data shown on slides 10-16

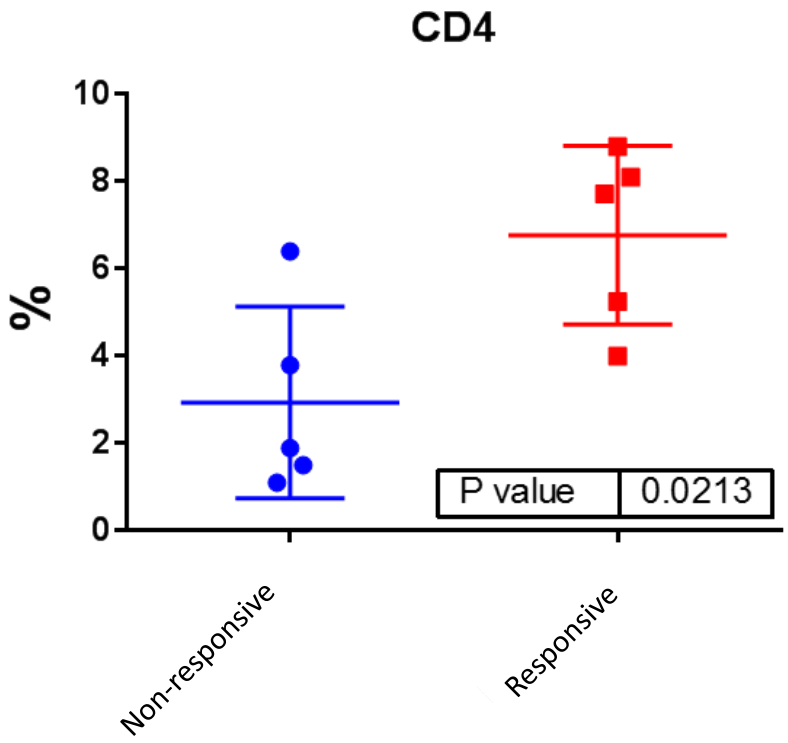
1. T cell activity following CD3/CD28 stimulation varied from 200 to 30,000 pg/mL across the 10 samples;
2. 50% of biopsies assayed were found to harbor TILs responsive to anti-PD-1 antibody using IFN-γ production as a readout
3. ENUM 244C8 elicited a higher level of IFN-γ production from responsive TILs than Nivolumab or ENUM 388D4;
4. ENUM 244C8 used in combination with other antibodies had an additive effect on production of IFN-γ from TILs in 3 of 5 responsive samples

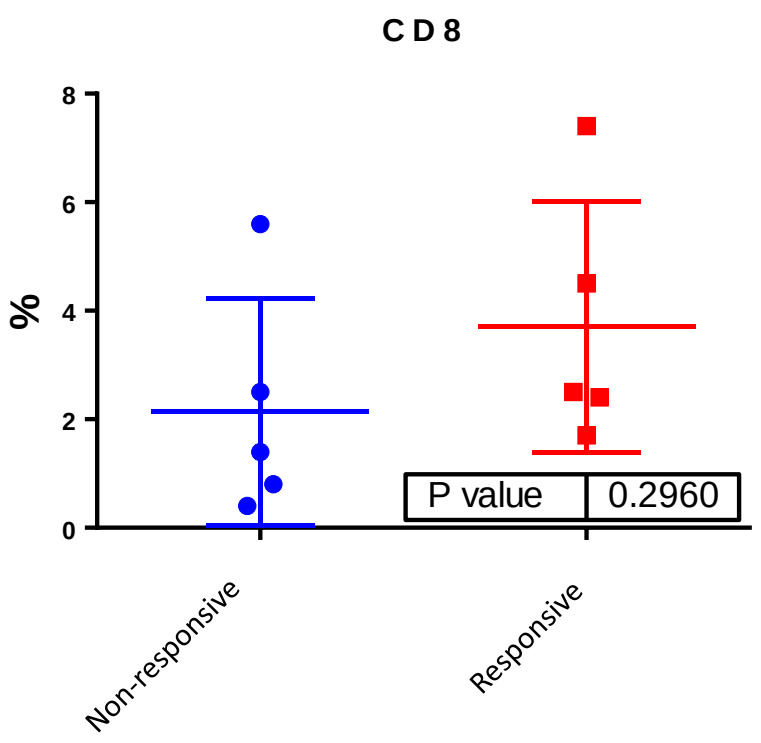
Ex Vivo Reversal of TIL Exhaustion: Responsiveness Correlated with TIL Infiltrates

- Each experiment was evaluated and the TILs were determined to be responsive or non-responsive to anti-PD-1 antibodies
  - Responsiveness was scored based on whether statistically significant elevation in IFN-γ production was observed
- Response is characterized by CD45 and CD3 expression in NSCLC tumor biopsy-derived TILs

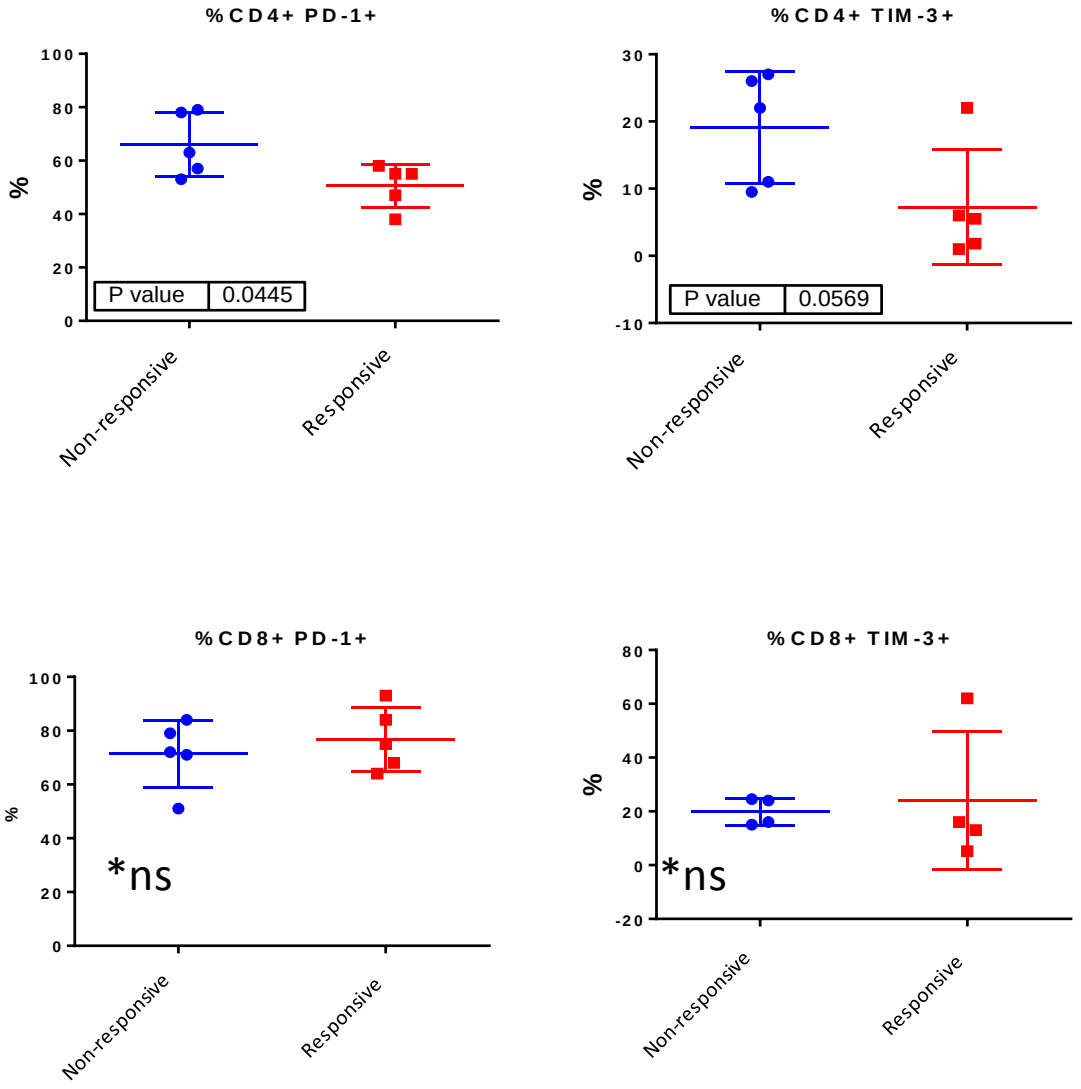


Ex Vivo Reversal of TIL Exhaustion: Responsiveness Correlated with Increased CD4 expression





Ex Vivo Reversal of TIL Exhaustion: hypo-responsiveness associated with increased $CD4^+PD\text{-}1^+$ and $CD4^+TIM\text{-}3^+$ levels



Summary of NSCLC findings to date

- NSCLC tumors contain varying levels of TILs that are heterogeneous in expression levels of lineage markers (CD4, CD8) and immunomodulatory receptors (PD-1, TIM-3)

- Experimental Summary: 50% of samples analyzed found to harbor TILs responsive to PD-1 blockade *ex vivo*.
  - Samples were found to respond to nivolumab, ENUM 388D4 and ENUM 244C8, a pharmacologically distinct potentially allosteric PD-1 antagonist antibody.
  - ENUM 244C8 elicited a higher level of IFN-γ production from TILs than Nivolumab or ENUM 388D4, and these effects were found to be additive certain samples.

- IFN-γ production in response to anti-PD-1 antibodies correlated with higher levels of TIL infiltration and lower levels of PD-1, and TIM-3 on CD4 cells.

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Conclusions

- ENUM 244C8 is a pharmacologically distinct humanized anti-PD-1 antibody that binds PD-1 non-competitively with PD-L1
- Incubation with anti-CD3/anti-CD28 antibodies results in limited reversal of exhaustion of NSCLC tumor-derived T cells; PD-1 blockade is required to enhance IFN-γ secretion.
- ENUM 244C8 can augment *ex vivo* IFN-γ secretion by TILs to a greater level than nivolumab and may display additive effects.
- Enumeral's platform enables generation of broad antibody diversity that can translate into functionally distinct and potentially improved therapeutic candidates.

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