Enumeral PD-1 Program Update: Differentiated Anti-PD-1 Antibody Elicits Higher T Cell Activation in Ex Vivo Human Assays than a Currently Marketed Anti-PD-1 Antibody

November 3, 2015

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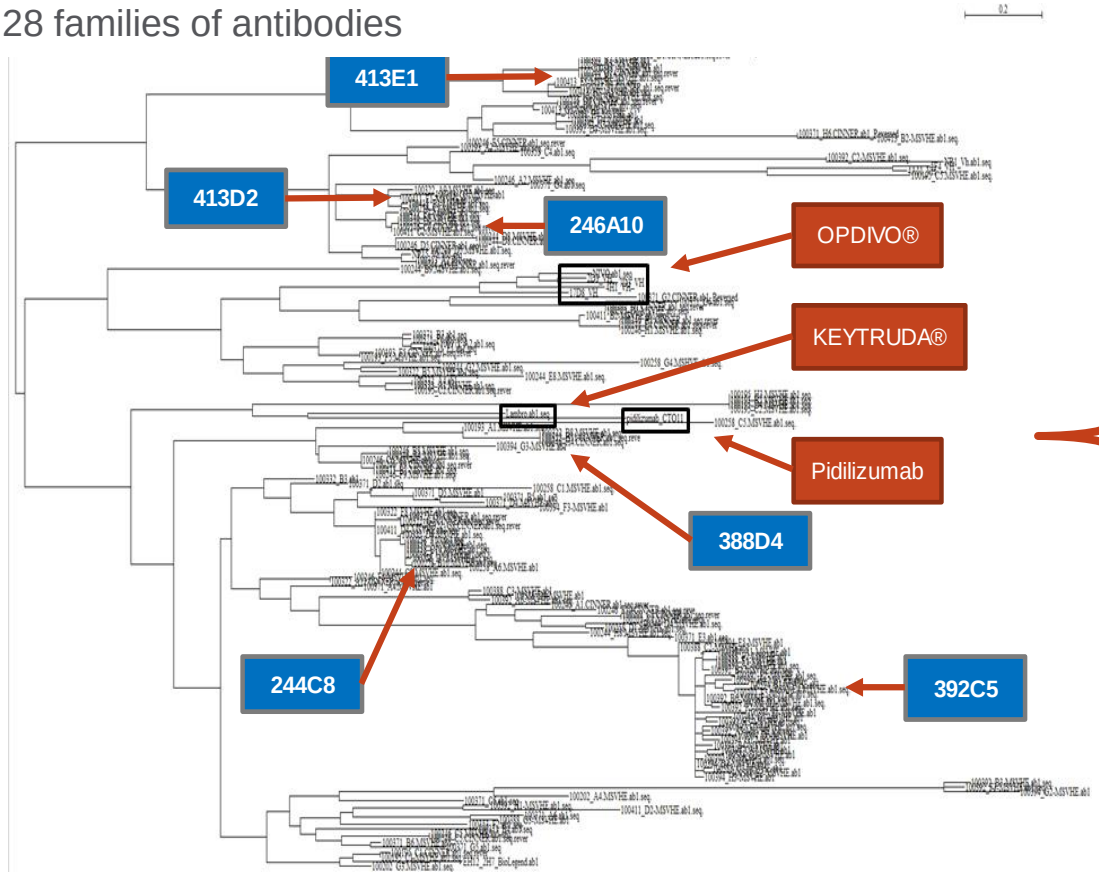
Background

- Enumeral uses a unique single cell technology platform and approach to identify functionally differentiated antibody candidates
  - Enumeral has identified two distinct 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-PD1 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-PD1 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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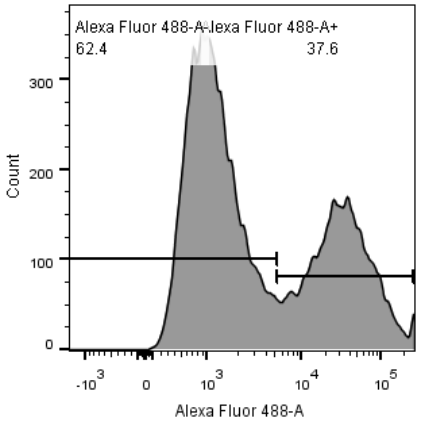
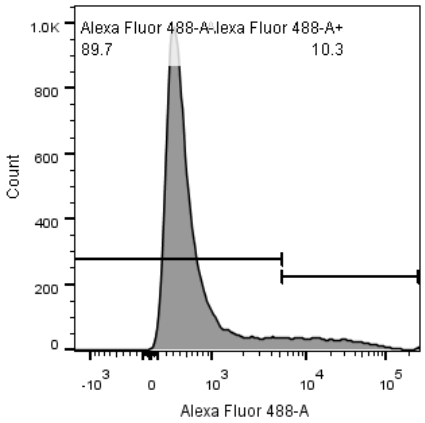
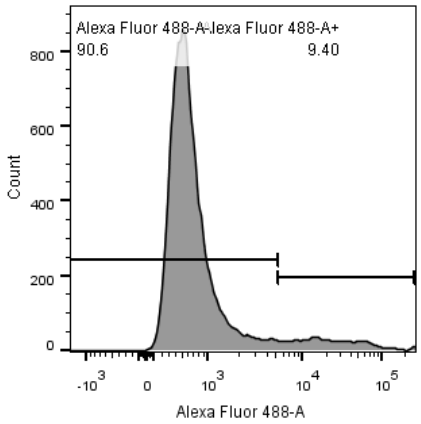
ENUM 244C8 Binding to PD-1 Does Not Displace PD-L1



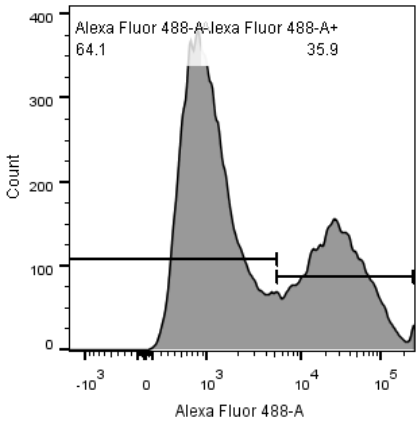
Negative control
mIgG1K



Positive control
EH12.2H7



388D4



244C8

ENUM 388D4, but not ENUM 244C8, blocks the binding of soluble PD-L1 to cells expressing PD1. HEK293 cells expressing PD1 were incubated with 10ug/mL of isotype, EH12.2H7, 388D4 or 244C8 antibodies. Cells were washed and then stained with soluble PD-L1-Ig protein labeled with Alexa488. Cells were washed and analyzed for PD-L1 binding by FACS analysis.

Antibody 244C8 behaves like the isotype negative control, and inhibition of PD-L1 binding to cells expressing PD1 is not observed.

Antibody ENUM 388D4 is a 'conventional' binder to PD1 with anti-PD-1 antagonist immune activation properties.

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
- Cells were incubated with anti-CD3/anti-CD28 antibodies for 24 hours and either negative control (isotype), nivolumab, or ENUM 244C8
- Interferon gamma production was measured (ELISA) and data is expressed as fold increase normalized to isotype control

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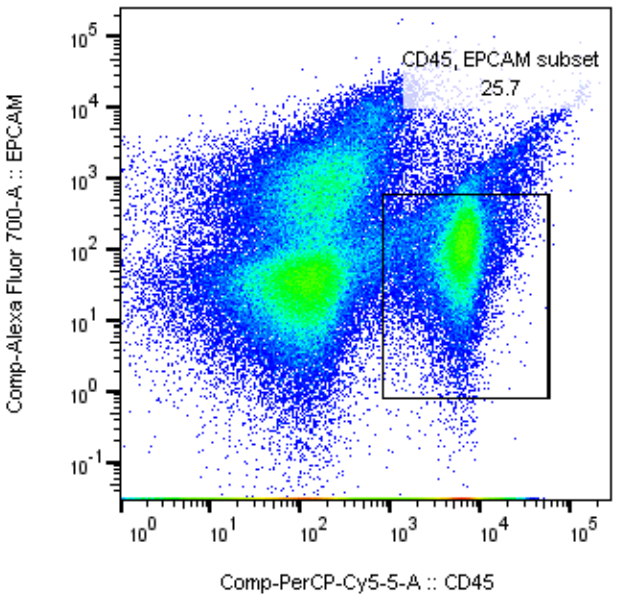
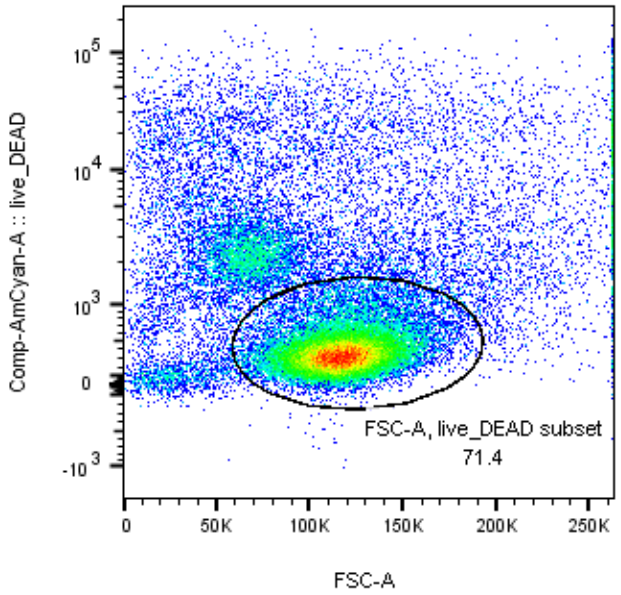
Ex Vivo Reversal of TIL Exhaustion: Example Flow Cytometry Analysis

NSCLC WD36444 contains 14% T cells

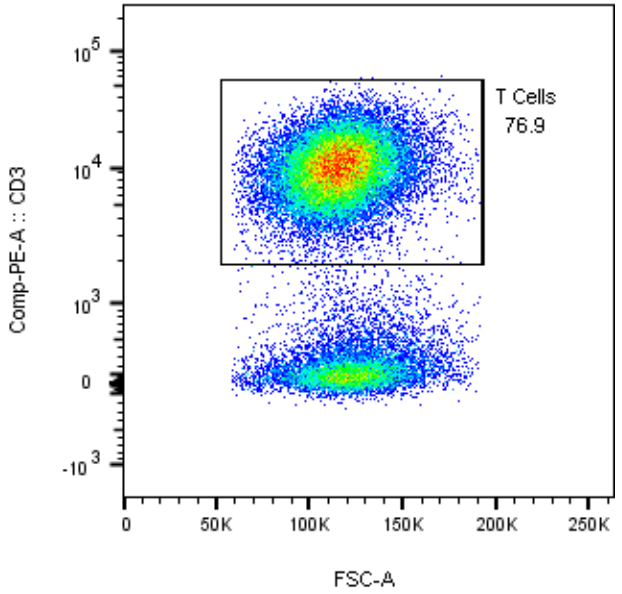
Lung sample #1 ID WD-36444



WD36444_Lungtumor 1_snapshot panel.fcs
Ungated
200000



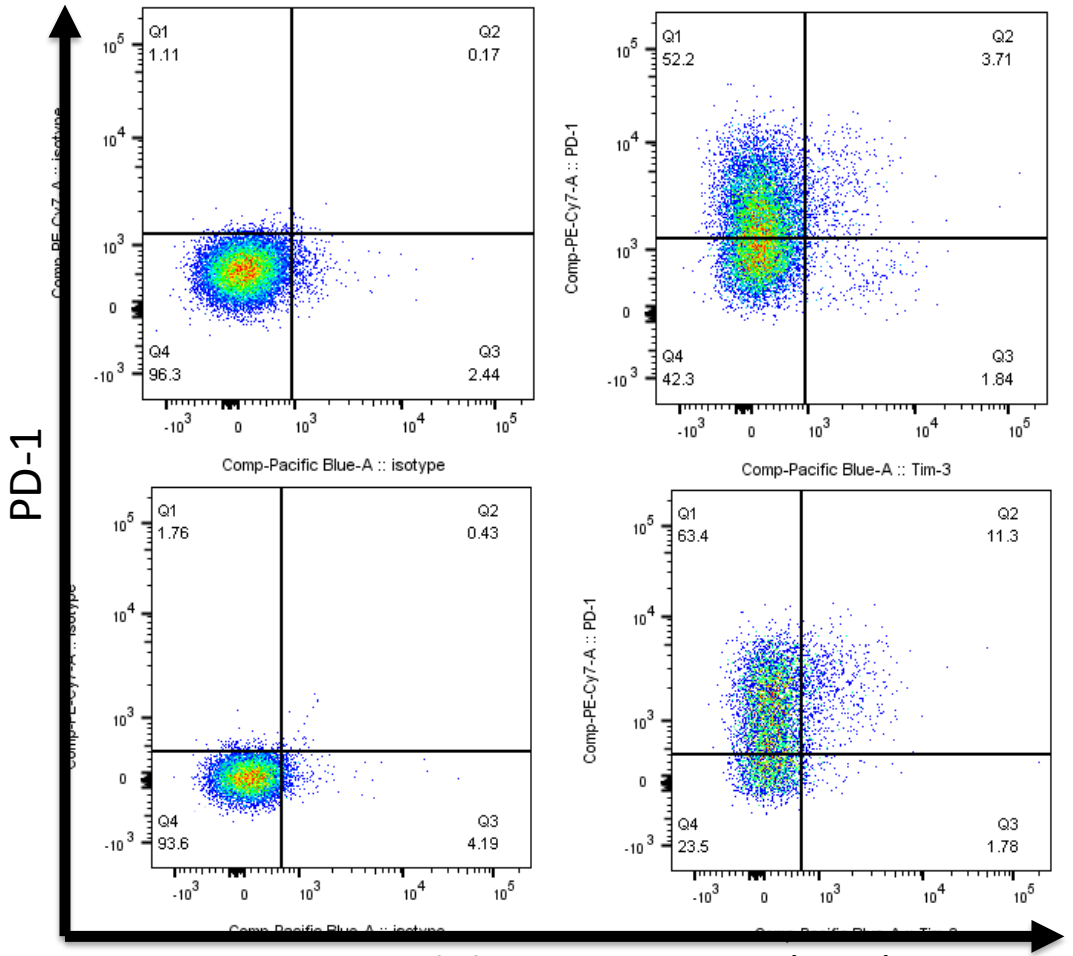
WD36444_Lungtumor 1_snapshot panel.fcs
CD45, EPCAM subset
51356



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

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

- 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^{+} PD1^{+}	%CD8^{+} PD1^{+}
WD-36444*	25.7	14	55	75
WD-36571	10.3	6.3	47	64
WD-36686*	21.6	17	55	84
WD-36790*	16.8	10.4	38	68
WD-36904	12.8	7	63	72
M115801A2	3.4	2.9	79	84
WD-36923	1.6	0.9	53	51
WD-36988	8.9	7	58	93
M4150952	5.4	3	78	79

- 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=9 patients found to harbor TILs that express exhaustion markers including PD-1.

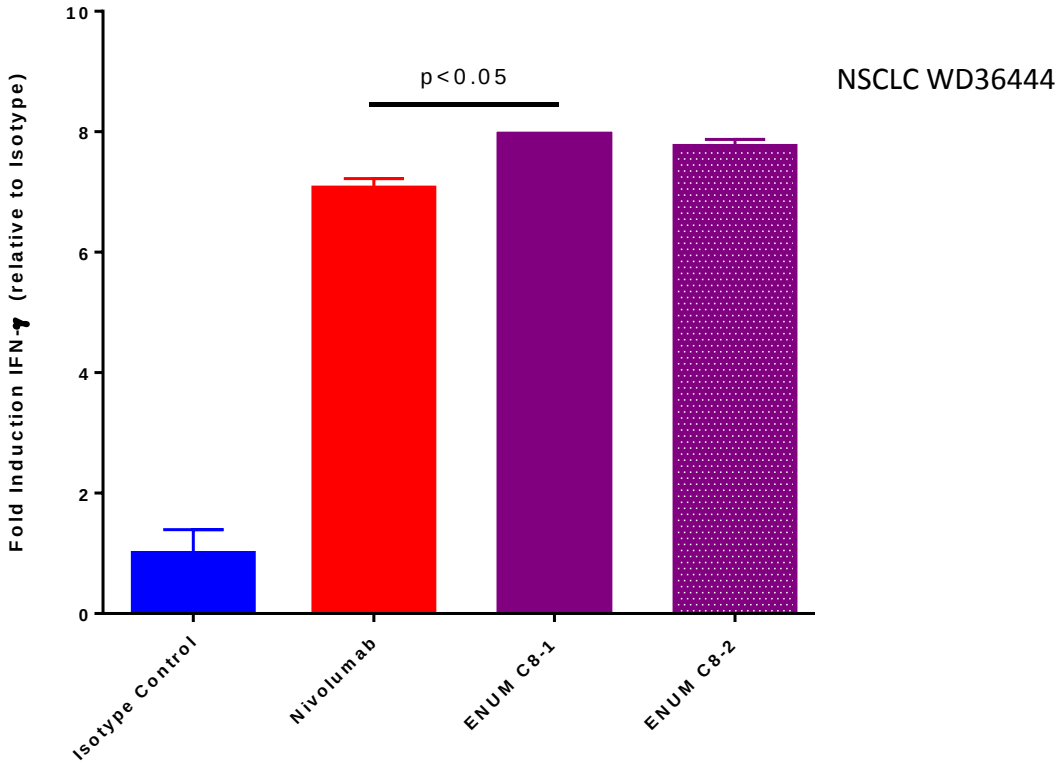
1. What is the activity of the T cells following activation?

2. Is cellular activity modified by the addition of an anti-PD1 antibody?

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

Ex Vivo Reversal of TIL Exhaustion: PD-1 Blockade Observed Restoring Function to T cells Derived From Human Lung Biopsy

ENUM 244C8 elicits increased activity compared to nivolumab



Functional modification of TILs with PD-1 blockade. Tumor cells containing 14% lymphocytes were activated in the presence of anti-CD3+anti-CD28 and the indicated antibodies ENUMC8-1, ENUMC8-2, isotype control human IgG4 S228P (Bio-Rad), and Nivolumab S228P (Invivogen) used at 20 ug/mL. Supernatants were collected 24 hours after activation and IFN-γ was measured by ELISA (Biolegend). Data is presented as fold induction IFN-γ relative to isotype control treated sample. ENUMC8-1 and ENUMC8-2 are humanized variants of ENUM 244C8. p<0.05 for ENUMC8-1 and ENUMC8-2.

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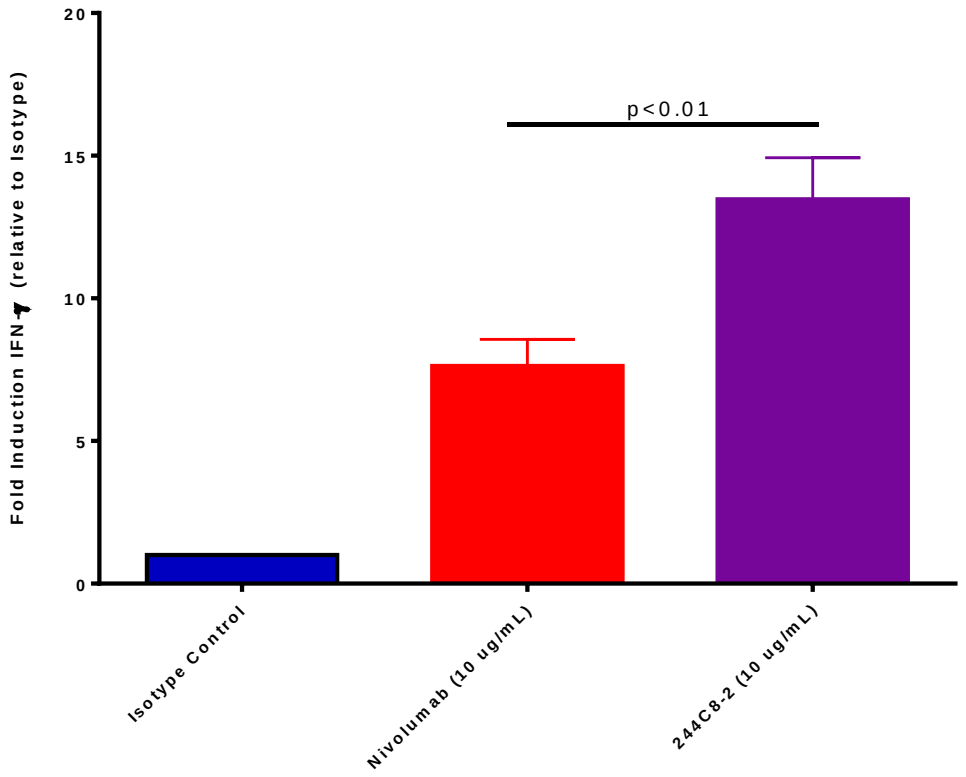
Ex Vivo Reversal of TIL Exhaustion: PD-1 Blockade Observed Restoring Function to T cells Derived From Human Lung Biopsy (Continued)

ENUM 244C8 elicits increased activity compared to nivolumab



Functional modification of NSCLC Tumor infiltrating lymphocytes with PD-1 blockade. 3e5 WD36686 tumor cells containing 17% lymphocytes were activated in the presence of anti-CD3+anti-CD28 and the indicated antibodies ENUMC8-2, Isotype control human IgG4 S228P (Bio-Rad), and Nivolumab S228P (Invivogen) used at 10 ug/mL. Tissue culture supernatants were collected 24 hours after activation and IFN-γ was measured by ELISA (Biolegend). Data is presented as fold induction IFN-γ relative to Isotype control treated sample.

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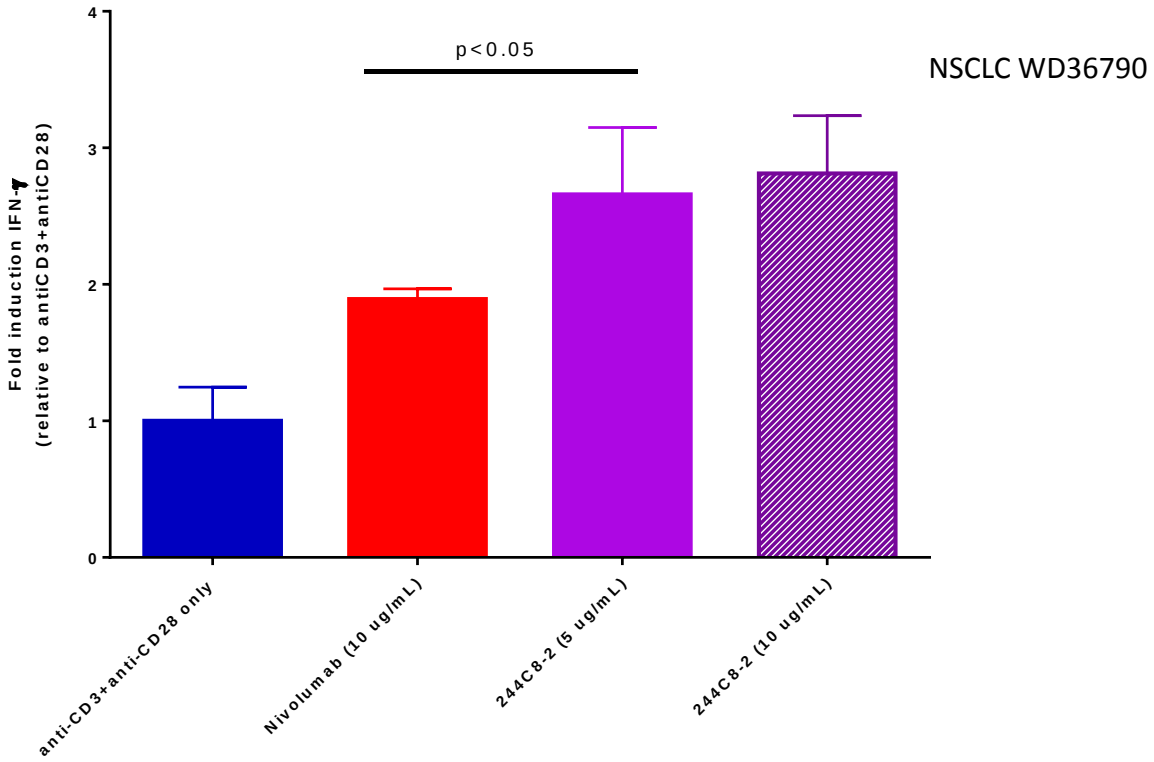
Ex Vivo Reversal of TIL Exhaustion: PD-1 Blockade Observed Restoring Function to T cells Derived From Human Lung Biopsy (Continued)

ENUM 244C8 elicits increased activity compared to nivolumab



Functional modification of Tumor infiltrating lymphocytes with PD-1 blockade. 3e5 WD36790 tumor cells containing 10 % lymphocytes were activated in the presence of anti-CD3+anti-CD28 and the indicated antibodies: 244C8-2 (at two different concentrations), Nivolumab S228P (Invivogen). Tissue culture supernatants were collected 24 hours after activation and IFN-γ was measured by ELISA (Biolegend). Data is presented as fold induction IFN-γ relative to anti-CD3+anti-CD28 only. p<0.05 for ENUMC8-2 at both 5 ug/mL and 10 ug/mL.

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Conclusions

- ENUM 244C8 is a humanized anti-PD-1 antibody that binds PD-1 but does not compete with PD-L1 and thus represents a pharmacologically novel class of anti-PD-1 therapeutic.
- Following incubation with anti-CD3/anti-CD28 antibodies activation of NSCLC tumor-derived T cells was limited and addition of anti-PD-1 antibodies was required to enhance IFN-γ secretion.
- ENUM 244C8 can augment *ex vivo* IFN-γ secretion by PD-1^{+} TILs to a greater level than nivolumab.
- Enumeral's platform enables generation of broad antibody diversity that can translate into functionally distinct and potentially improved therapeutic candidates.

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