An anti-carcinoma monoclonal antibody (mAb) NEO-201 can also target and eliminate human immunosuppressive regulatory T cells (Tregs)

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Introduction

NEO-201 is an IgG1 mAb reactive against many different carcinomas, but not reactive against most normal tissues. Functional analysis revealed that NEO-201 is capable of engaging innate immune effector mechanisms to kill tumor cells, such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). In addition, NEO-201 can block the interaction between CEACAM5 expressed on tumor cells and CEACAM1 expressed on NK cells to reverse CEACAM1-dependent inhibition of NK cytotoxicity.

Previous studies showed that NEO-201 attenuates growth of human tumor xenografts in mice and demonstrates safety/tolerability in non-human primates with a transient decrease in neutrophils being the only adverse effect observed. A clinical trial evaluating NEO-201 in adults with chemo–resistant solid tumors is ongoing at the NIH clinical Center.

Preclinical evaluation showed that NEO-201 reacts against human regulatory T cells (Tregs) and here we further investigated the phenotypic and functional effects of NEO-201 on human Tregs *in vitro*

Experimental Design

Peripheral blood mononuclear cellS (PBMCs) were collected from 5 healthy donors and used for phenotypic and functional analysis.

EasySep™ Human $CD4^+CD127^{low}CD25^+$ Regulatory T Cell Isolation kit and EasySep™ Human Biotin NEO-201^+ Selection Kit (biotin-labeled NEO-201 mAb) were used to isolate Tregs from PBMCs. Phenotypic analysis was conducted by flow cytometry for the following markers: CD4, CD25, CD127, FoxP3, CD15s, CD45RA, CCR4, NEO-201 antigen, CEACAM5 and CEACAM6. The ability of NEO-201 to mediate killing of opsonized Tregs was evaluated using a CDC assay.

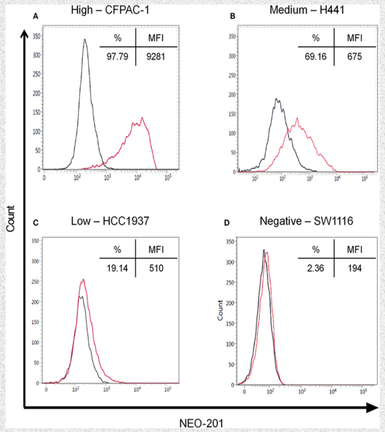
Results

1. NEO-201 binds to various human carcinoma cell lines

Tumor Cell Line Flow Cytometry

CELL LINE	TUMOR TYPE	% POSITIVE	MFI
COLO 205	**Colon**	**10.33**	**245**
HT-29	**Colon**	**38.40**	**352**
LS174T	**Colon**	**46.46**	**345**
SW1116	Colon	2.36	194
SW1463	Colon	1.23	278
SW480	Colon	1.70	575
ASPC-1	**Pancreatic**	**79.26**	**8927**
BxPC-3	**Pancreatic**	**97.25**	**2584**
CAPAN-2	**Pancreatic**	**29.69**	**327**
CFPAC-1	**Pancreatic**	**97.79**	**9281**
PANC-1	Pancreatic	3.29	289
H441	**NSCLC (adenocarcinoma)**	**69.16**	**675**
H522	NSCLC (adenocarcinoma)	1.38	238
HCC4006	**NSCLC (adenocarcinoma)**	**99.27**	**9899**
HCC827	**NSCLC (adenocarcinoma)**	**77.46**	**692**
SK-LU-1	NSCLC (adenocarcinoma)	1.77	685
CALU-1	NSCLC (squamous)	4.22	571
H1703	NSCLC (squamous)	4.16	111
H226	NSCLC (squamous)	4.83	209
H520	**NSCLC (squamous)**	**61.78**	**443**
AU-565	**Breast (HER2+)**	**50.04**	**227**
BT-474	**Breast (PR+/HER2+)**	**68.79**	**591**
HCC1500	Breast (ER+/PR+)	1.53	597
SK-BR-3	Breast (HER2+)	1.61	329
T-47D	Breast (ER+/PR+)	8.00	161
ZR-75-1	**Breast (ER+/PR+/HER2+)**	**68.80**	**550**
BT-549	Breast (ER-/PR-/HER2-)	1.47	477
HCC1937	**Breast (ER-/PR-/HER2-)**	**19.14**	**510**
HCC38	Breast (ER-/PR-/HER2-)	2.15	226
MDA-MB-468	Breast (ER-/PR-/HER2-)	6.33	344

NEO-201 is reactive against a broad range of *in vitro* cultured tumor cell lines. NEO-201 positive cell lines appear in bold text. NEO-201 positivity was defined as % positive >10%.



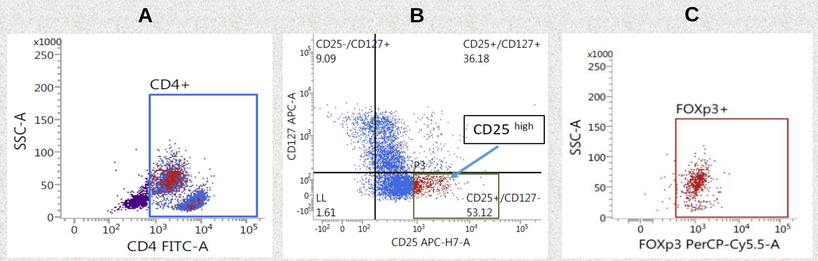
Positivity was determined using fluorescence minus one (FMO) controls. Positive cell lines were ranked according to their quantified expression level (% positive × MFI), and then sorted into groups of low (<200), medium (200-1000), and high (<1000) expression.

2. NEO-201 binds to human regulatory T cells (Tregs)

Phenotypic analysis of isolated Tregs as determined by flow cytometry

EasySep™ Human $CD4^+CD127^{low}CD25^+$ Regulatory T Cell Isolation kit

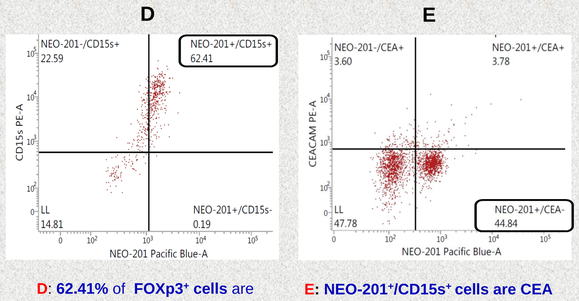
(Healthy donor 1)



A: **86.27 %** of **isolated cells** are **$CD4^+$**

B: **10.15%** of **$CD4^+$ cells** are **$CD25^{high}CD127^-$**

C: **97.30%** of **$CD4^+/CD25^{high}/CD127^-$ cells** are **$FOXp3^+$**



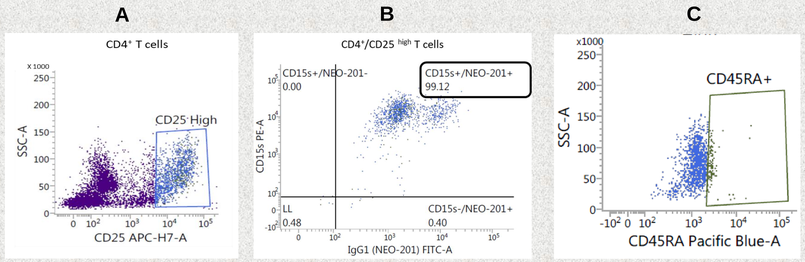
D: **62.41%** of **$FOXp3^+$ cells** are **NEO-$201^+/CD15s^+$**

E: Cells were stained with **PE Mouse Anti-Human CD66 antibody** (Clone B1.1/CD66) which recognizes *CEACAM1* ***CEACAM6***, *CEACAM3* and ***CEACAM5***

E: NEO-$201^+/CD15s^+$ cells are CEA (CEACAM5) and CEACAM6 negative

EasySep™ Human Biotin NEO-201^+ Selection Kit

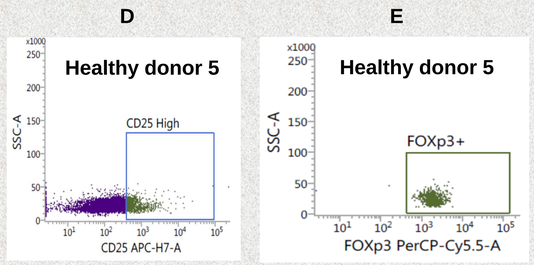
(Healthy donor 4)



A: **15.69%** of **cells are $CD25^{high}$**

B: **99.12%** of **$CD25^{high}$ cells** are **NEO 201^+/ $CD15s^+$**

C: **NEO $201^+/CD15s^+$ cells are CD45RA negative**



D: **10.32%** of **cells are $CD25^{high}$**

E: **99.75%** of **$CD4^+/CD25^{high}$ cells** are **$FOXp3^+$**

2 STEP human regulatory T cells isolation:

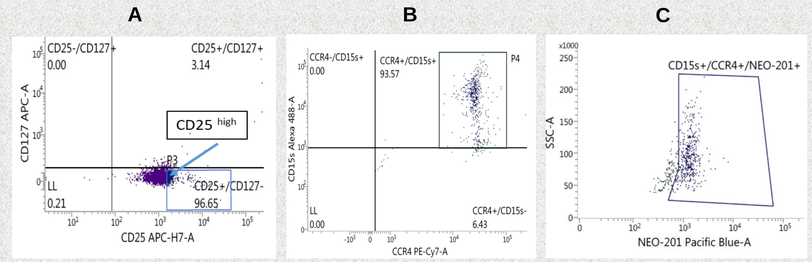
1) **$CD4^+$ T cells were isolated from whole PBMCs**
2) **$CD4^+$ T cells were bounded to biotinylated NEO-201 to isolate NEO-201^+ Tregs**

3. NEO-201^+ Tregs are $CCR4^+$

Phenotypic analysis of isolated Tregs as determined by flow cytometry

EasySep™ Human $CD4^+CD127^{low}CD25^+$ Regulatory T Cell Isolation kit

(Healthy donor 2)



A: **20.65%** of **$CD4^+$ cells** are **$CD25^{high}CD127^-$**

B: **93.57%** of **$CD25^{high}CD127^-$/ $FOXp3^+$ cells are $CCR4^+CD15s^+$**

C: **80%** of **$CCR4^+/CD15s^+$ cells** are **NEO-201**

98.12% of **$CD25^{high}CD127^-$ cells are $FOXp3^+$ (plot not shown)**

4. The percentage of NEO-$201^+/CD15s^+$ cells in the isolated Tregs ranges from 60%-80%

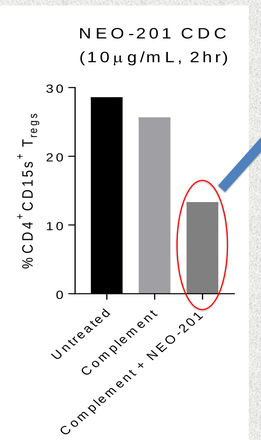
EasySep™ Human $CD4^+CD127^{low}CD25^+$ Regulatory T Cell Isolation kit

DONOR	% of NEO-$201^+/CD15s^+$ cells in $CD4^+/CD25^{high}/CD127^-/FOXp3^+$ cells
Healthy Donor 1	62.41
Healthy Donor 2	78.09
Healthy Donor 3	60.93
RANGE	**60.93-78.09**

EasySep™ Human Biotin NEO-201^+ Selection Kit

DONOR	% of NEO-$201^+/CD15s^+$ cells in $CD4^+/CD25^{high}/CD127^-/FOXp3^+$ cells
Healthy Donor 4	50.76
Healthy Donor 5	99.12
RANGE	**50.76-99.12**

5. NEO-201 mediates CDC activity against isolated $CD4^+/CD15s^+$/NEO-201^+ Tregs.



Human Tregs were isolated using the EasySep™ Human $CD4^+CD127^{low}CD25^+$ Regulatory T Cell Isolation kit

50 % killing compared to untreated cells

Conclusion

This study demonstrates that NEO-201 reacts against human Tregs and can be used as a novel marker to identify and to purify Tregs. NEO-201 mAb could also eliminate NEO-201^+ Tregs by CDC *in vivo*. This study demonstrates for the first time that this tumor-targeting mAb may also mediate through a novel mechanism down regulating Treg-mediated immunosuppression of anticancer immunity.