Indirect mechanisms of action of a novel IgG1 monoclonal antibody, NEO-201, that enhance immune killing of tumor

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Introduction

NEO-201 is an IgG1 mAb targeting variants of CEACAM5/6 that demonstrates tumor sensitivity and specificity. Functional analysis revealed that NEO-201 is capable of engaging innate immune effector mechanism including ADCC and CDC to directly kill tumor cells expressing its target. Previous studies demonstrated safety/tolerability in non-human primates, and an ongoing clinical trial at the NCI is currently exploring its dosing and safety. We have explored indirect mechanisms of its action that may enhance immune tumor killing. 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. Phenotypic and functional analyis on human regulatory T cells (Tregs) *in vitro* also showed that NEO-201 can target and eliminate Tregs, suggesting that this tumor-targeting mAb may also mediate the down regulation of the Treg-mediated immunosuppression of anticancer immunity.

Experimental Design

Flow cytometry analsyis and CDC assays were performed to evaluate the ability of NEO-201 to target and eliminate human Tregs *in vitro*.
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 from healthy donors . Phenotypic analysis was conducted by flow cytometry for the following markers: CD4, CD25, CD127, FoxP3, CD15s, CD45RA, NEO-201 antigen, CEACAM5 and CEACAM6.
In addition, *in vitro* functional assays, using various human tumor cell lines as target cells and NK-92 cells (CEACAM1+/CD16-) as effectors, were conducted to assess the ability of NEO-201 to enhance antitumor cytotoxicity of NK-92 cells by blocking the interaction between CEACAM5 on tumor cells and CEACAM1 on NK cells.

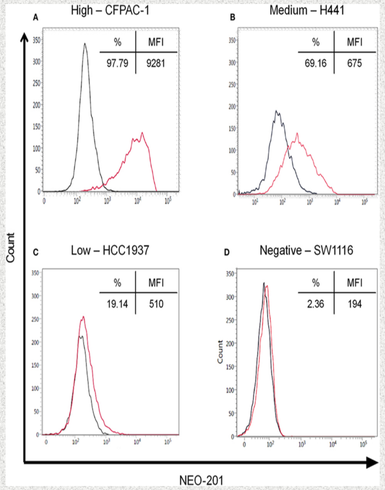
Results

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%.

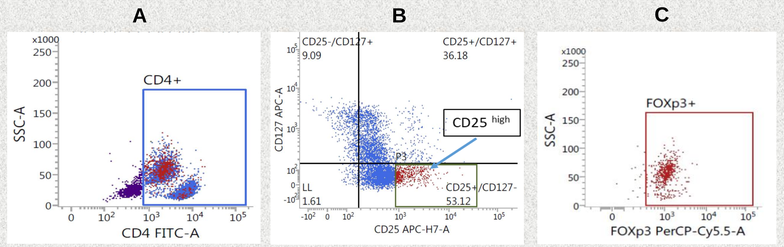


Positivity was determined using fluorescence minus one (FMO) controls. Positive cell lines were ranked according to their quantified expression level (% positive × MFI), and then sorted into groups of low (<200), medium (200-1000), and high (<1000) expression.

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

Phenotypic analysis of isolated Tregs as determined by flow cytometry

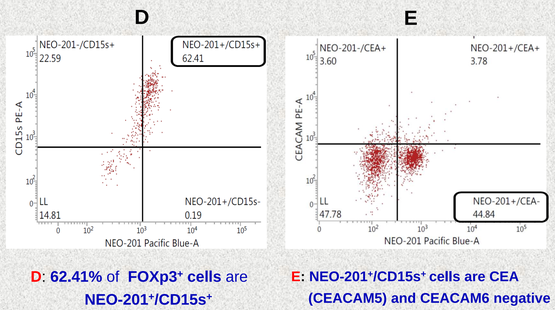
EasySep™ Human CD4$^+$CD127^{low}CD25$^+$ Regulatory T Cell Isolation kit (Healthy donor 1)



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$^+$**

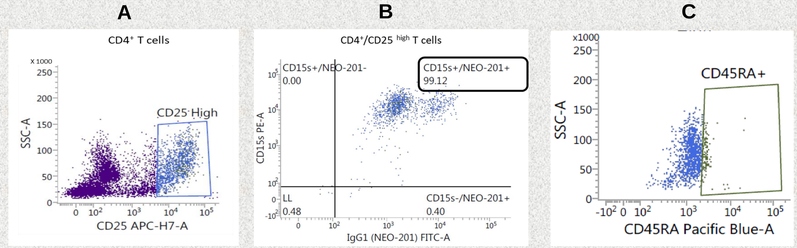


D: **62.41%** of **FOXp3$^+$ cells** are **NEO-201$^+$/CD15s$^+$**

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**

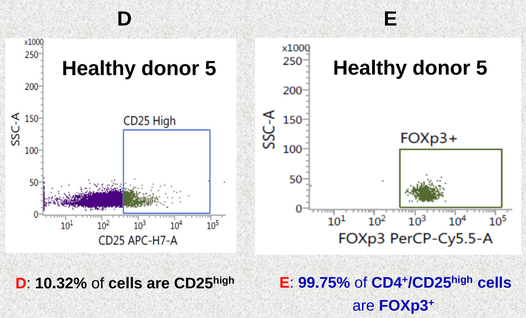
EasySep™ Human Biotin NEO-201$^+$ Selection Kit (Healthy donor 4)



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**



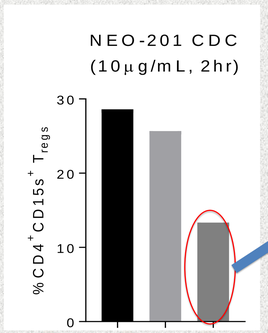
D: **10.32%** of **cells are CD25^{high}**

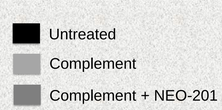
E: **99.75%** of **CD4$^+$/CD25^{high} cells** are **FOXp3$^+$**

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

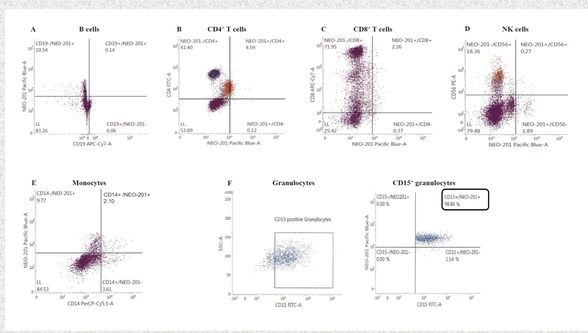




50 % killing compared to untreated cells

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

4. NEO-201 binds only to granulocytes in human hematopoietic cells



NEO-201 doesn't react with other hematopoietic subsets (B cells, CD4+ T cells, CD8+ T cells, NK cells, monocytes)

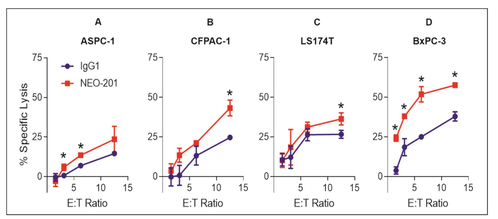
5. NEO-201 enhances NK-92 cell cytotoxicity against CEACAM5$^+$ / NEO-201$^+$ tumor cells

Tumor Cell Line Flow Cytometry

Cell line	*CEACAM1$^+$*	*CEACAM5$^+$/NEO-201$^+$*
	% positive (MFI)	
ASPC-1	**61.15** (707)	9.26 (869/9078)
BxPC-3	2.45 (1471)	**58.52** (1447/6420)
CFPAC-1	**18.67** (1938)	**26.95** (1108/1728)
LS174T	2.43 (3287)	**26.84** (1030/858)

ASPC-1, BxPC-3, CFPAC-1 are human pancreatic carcinoma cell lines.
LS174T is a human colorectal carcinoma cell line.
Positive marker expression appears in bold text, where positivity was defined as % positive >10 %.

NK-92 killing assay



NEO-201 and human IgG1 (negative control) were used at a concentration of **10µg/mL**. **NK-92 cells** were used as effector cells at the following **E:T ratios: 1.56:1, 3.12:1, 6.25:1, 12.5:1.** Asterisks denote statistical significance of NK-92 + NEO-201 vs NK-92 + IgG *p < 0.05.

Conclusion

NEO-201 mediates direct killing of tumor cells expressing its target through both ADCC and CDC. This study demonstrates that NEO-201 can mediate immune killing through additional mechanisms including blocking the interaction between CEACAM5 on tumor cells and CEACAM1 on NK cells to reverse CEACAM1-dependent inhibition of NK cytotoxicity as well as to recognize and eliminate human Tregs. Ongoing studies are looking at leveraging this phenomenon by combining NEO-201 with checkpoint inhibitors.