Mechanisms of action of a neoantigen-targeting antibody NEO-201

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PRECISION BIOLOGICS, INC.

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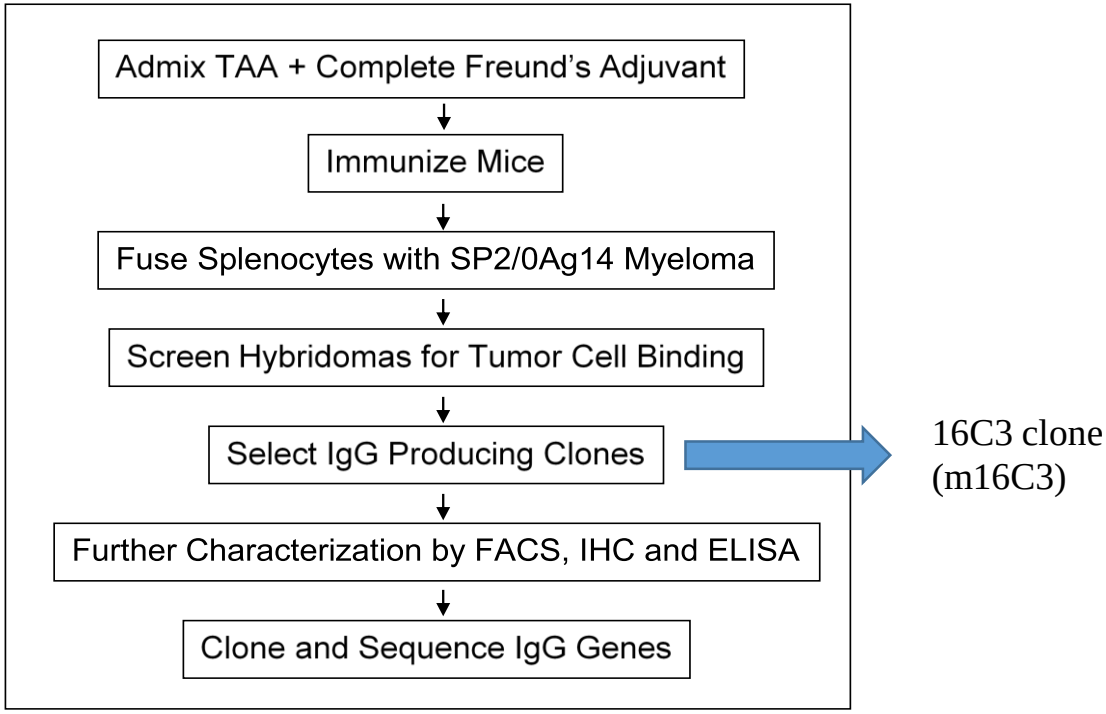
BACKGROUND ON NEO-201

Generation of NEO-201 monoclonal antibody.

NEO-201 is a novel humanized IgG1 mAb that was generated from tumor-associated antigens (TAAs) derived from tumor membrane fractions pooled from surgically resected specimens from 79 patients with colon cancer



ELISA and FACS: m16C3 strongly binds to colorectal adenocarcinoma cell line **L174T** and pancreatic adenocarcinoma cell line **CFPAC-1**.

The m16C3 protein sequence was humanized and designated as **NEO-201**.

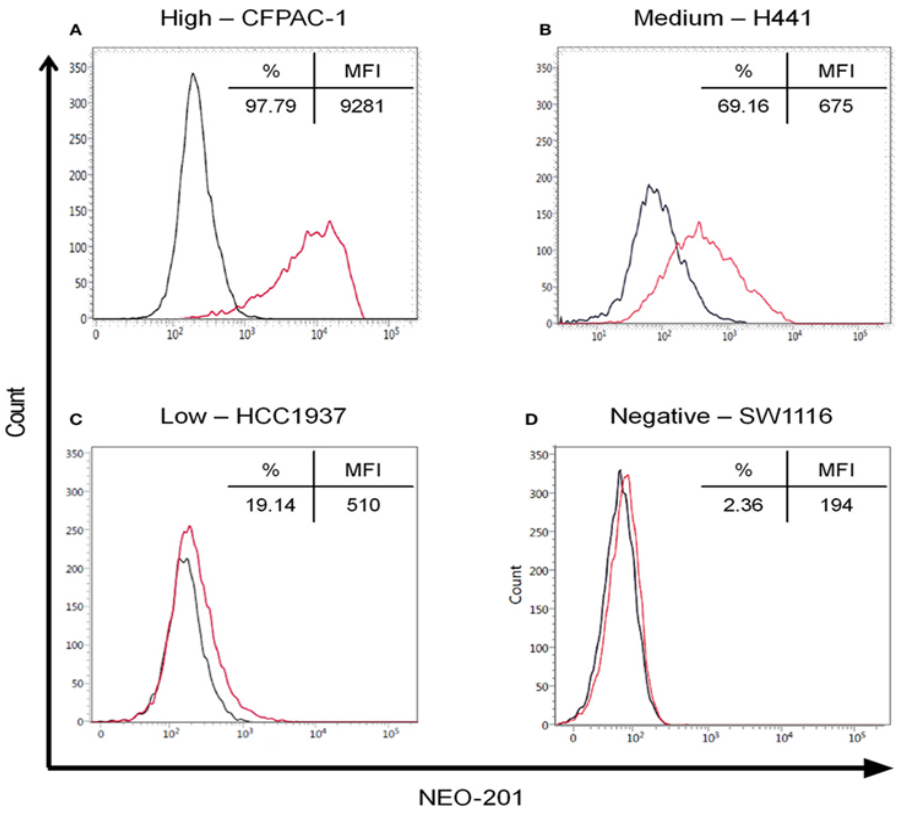
NEO-201 binds to various human carcinoma cell lines

Flow cytometry analysis of NEO-201 binding to tumor cell lines derived from various types of solid tumors.

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 positive cell lines appear in bold text.

NEO-201 positivity was defined as % positive >10%

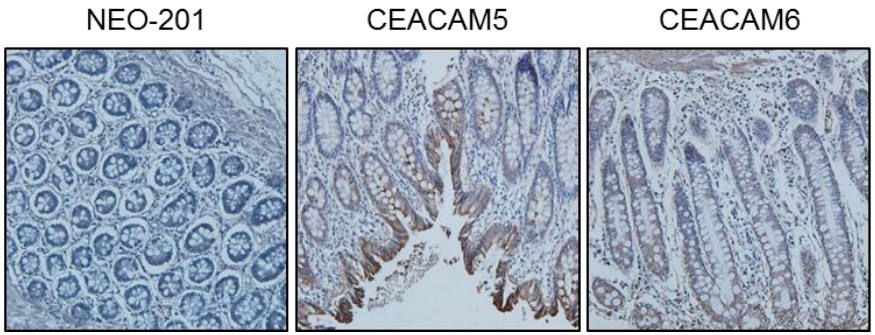


Positivity was determined using fluorescence minus one (FMO) controls. Positive cell lines were ranked according to their quantified expression level (% positive × MFI), and then sorted into groups of low (<200), medium (200-1000), and high (<1000) expression

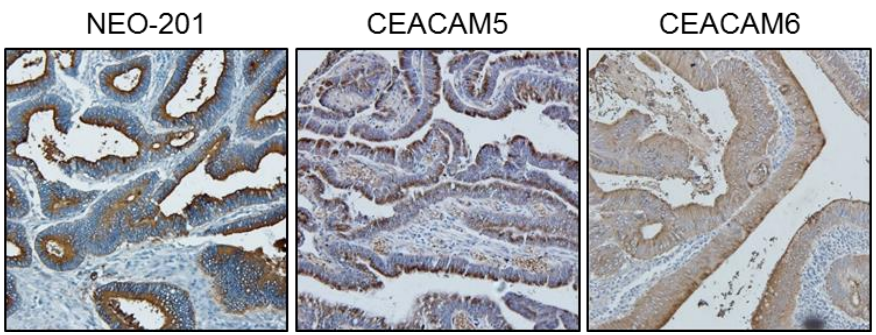
Fantini *et al.* ***Frontiers in Immunology*, 2018**

NEO-201 reactivity is tumor-associated

Normal Colon Tissue



Tumor Tissue



- NEO-201 does not react against normal epithelial tissue CEACAM5/6 positive.
  - Majority of normal tissues stained CEACAM5^{+} and/or CEACAM6^{+}
  - Colon (29/31, 94%), pancreatic (26/28, 93%), lung (30/32, 94%)

- NEO-201 reacts against tumor tissue CEACAM5/6 positive.
  - Majority of sampled tumors stained “triple positive” – NEO-201^{+} CEACAM5^{+} CEACAM6^{+}
  - Colon (28/32, 88%), pancreatic (23/30, 77%), lung (16/32, 50%)

MECHANISMS OF ACTION

1) **NEO-201 mediates Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) against human tumor cell lines**

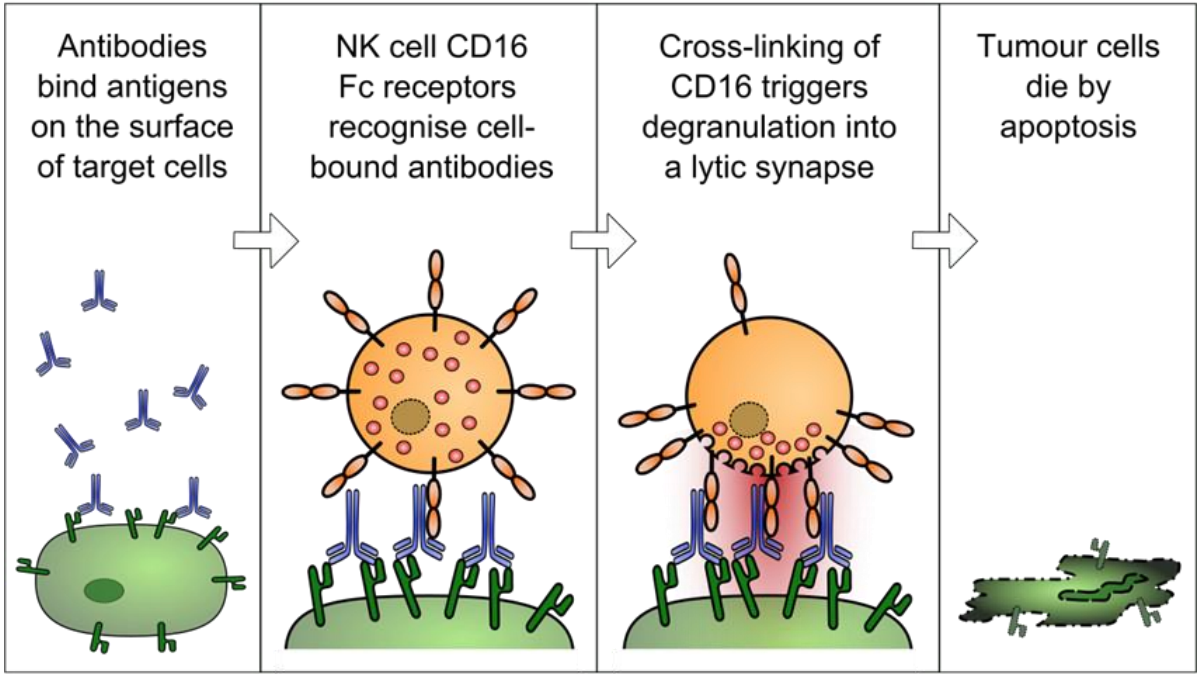
2) **NEO-201 mediates Complement-Dependent Cytotoxicity (CDC) against human tumor cell lines**

3) **NEO-201 Enhances Natural Killer Cell Cytotoxicity Against Tumor Cells Through Blockade of the Inhibitory CEACAM5/CEACAM1 Immune Checkpoint Pathway**

4) **NEO-201 targets and eliminates human immunosuppressive regulatory T cells (Tregs)**

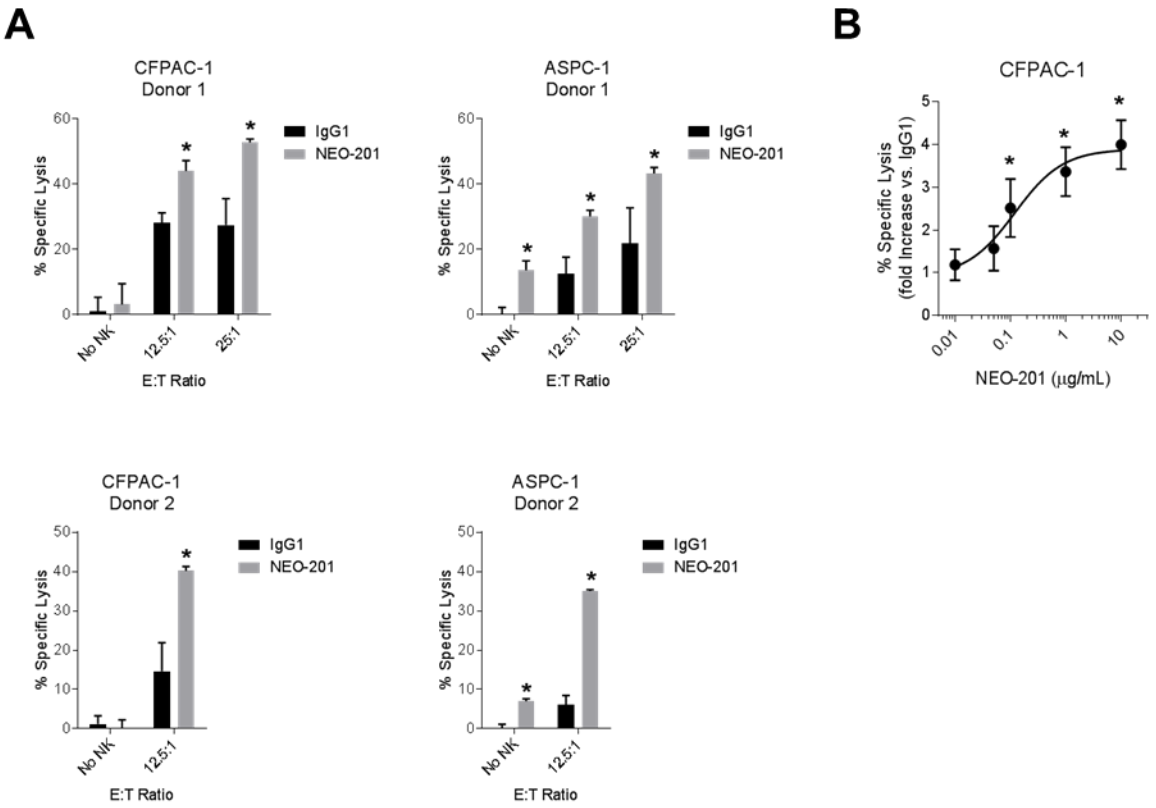
MECHANISMS OF ACTION

1) **NEO-201 mediates Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) against human tumor cell lines**



https://www.genscript.com/ADCC-and-CDC-assay-services.html

NEO-201 mediates ADCC against human tumor cell lines

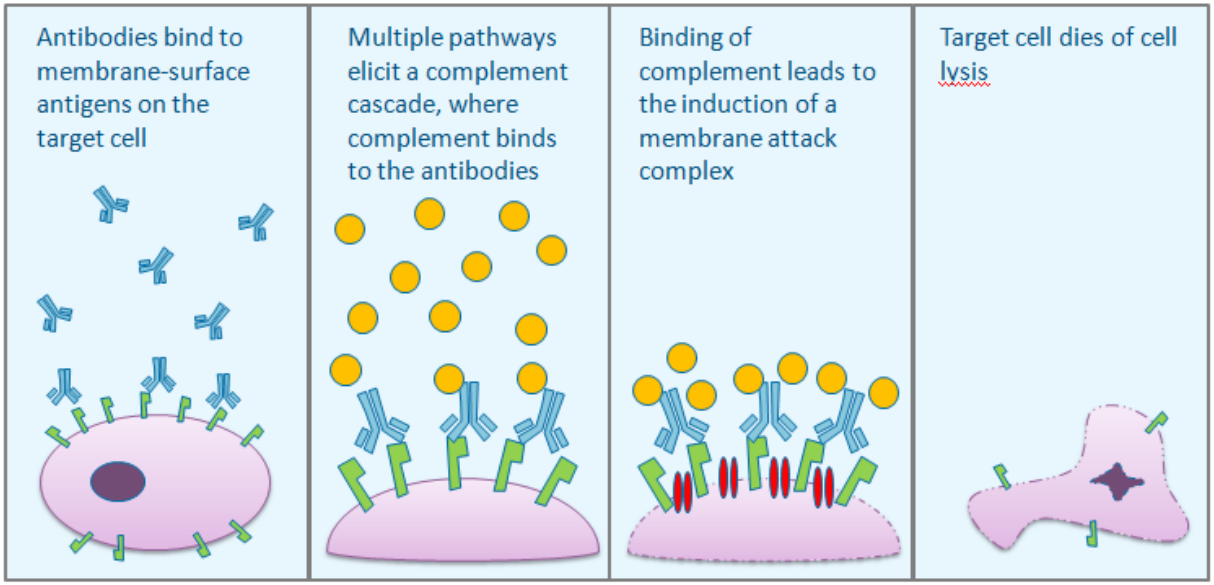


- (**A**) ADCC activity using CFPAC-1 or ASPC-1 cells as target cells. Cells were treated with 10µg/mL of NEO-201 or human IgG1 (negative control). Purified NK cells from two healthy donors were used as effector cells at the indicated E:T ratios. (**B**) ADCC assay using CFPAC-1 cells treated with increasing doses of NEO-201. NK cells were used at an E:T ratio of 12.5:1.
- * statistically significant ($p < 0.05$) by T-test.

Fantini *et al.* ***Frontiers in Immunology*, 2018**

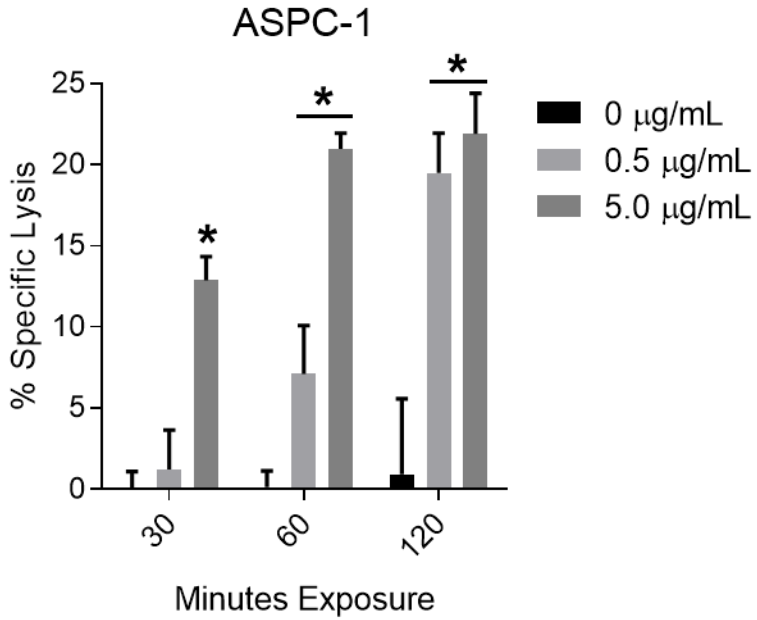
MECHANISMS OF ACTION:

2) NEO-201 mediates Complement-Dependent Cytotoxicity (CDC) against human tumor cell lines



https://www.genscript.com/ADCC-and-CDC-assay-services.html

NEO-201 mediates CDC against human tumor cell lines

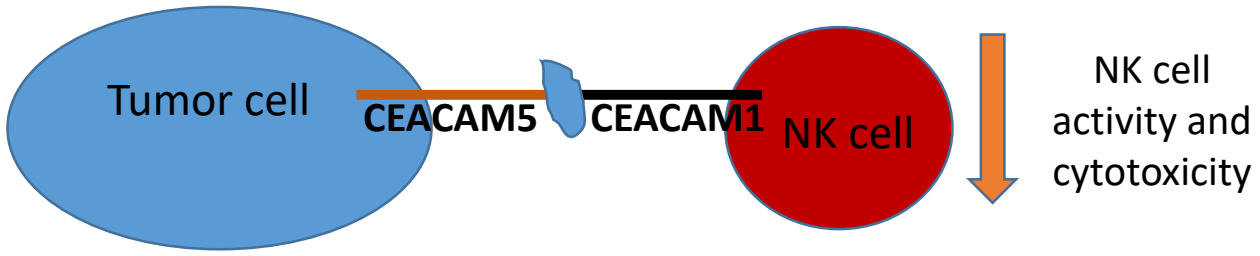


- CDC assay using ASPC-1 cells treated with rabbit complement (1:8 dilution) and the indicated doses of NEO-201 for the indicated durations.
- * statistically significant ($p < 0.05$) by T-test.

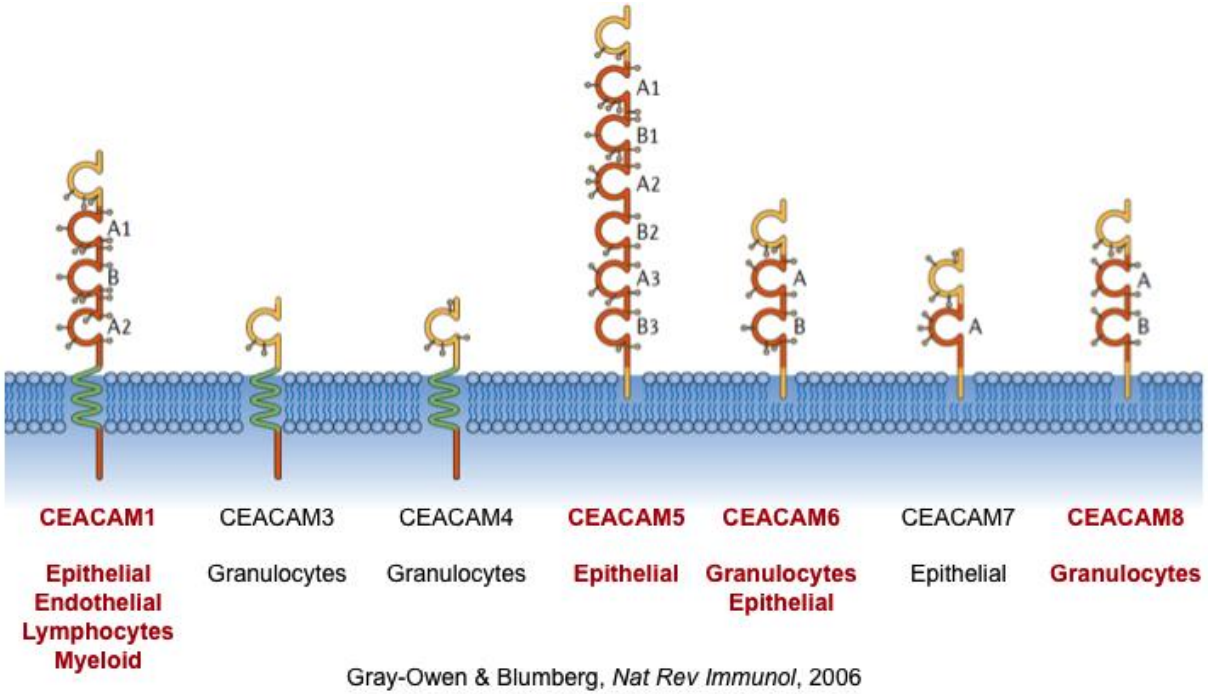
Fantini *et al.* ***Frontiers in Immunology*****, 2018**

MECHANISMS OF ACTION:

3) NEO-201 Enhances Natural Killer Cell Cytotoxicity Against Tumor Cells Through Blockade of the Inhibitory CEACAM5/CEACAM1 Immune Checkpoint Pathway



BIOLOGY OF CEACAM FAMILY MEMBERS



Gray-Owen & Blumberg, *Nat Rev Immunol*, 2006

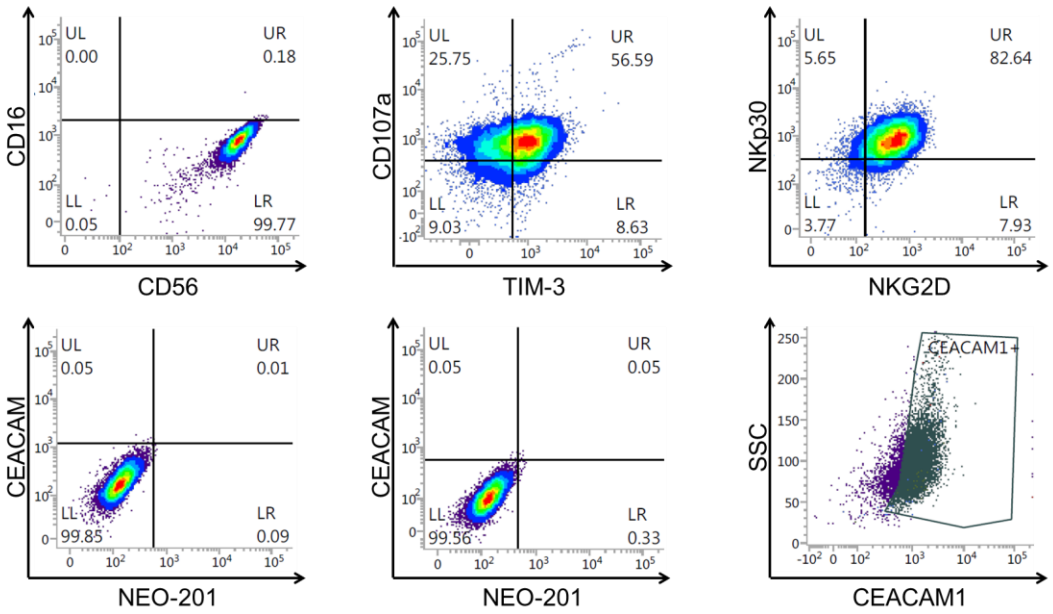
- CEACAM1 is a type-1 transmembrane protein containing an extra-cellular N-terminal variable domain followed by up to three constant C2-like immunoglobulin domains.

- CEACAM1 is the only member of CEACAM family to possess an immunoreceptor tyrosine-based inhibitory motif (ITIM). Phosphorylation of ITIMs in immune and epithelial cells inhibits signaling by binding a variety of effector proteins that down-regulate cell signaling.

- CEACAM1 is also a potent inhibitor of natural killer (NK) cell function; binding between CEACAM1 on NK cells and CEACAM1 or CEACAM5 on tumor cells inhibits activation signaling by NKG2D, which prevents NK cell cytolysis and permits tumor cells to evade NK killing.

The NK-92 cell line is a CEACAM1^{+} model for non-ADCC natural killer cell cytotoxicity

NK-92 Cell Line Phenotype Analysis Flow Cytometry



NK-92 cells are an immortalized IL-2-dependent human natural killer cell line that lacks ADCC activity.

NK-92 cells express typical NK cell markers except CD16 (no ADCC):

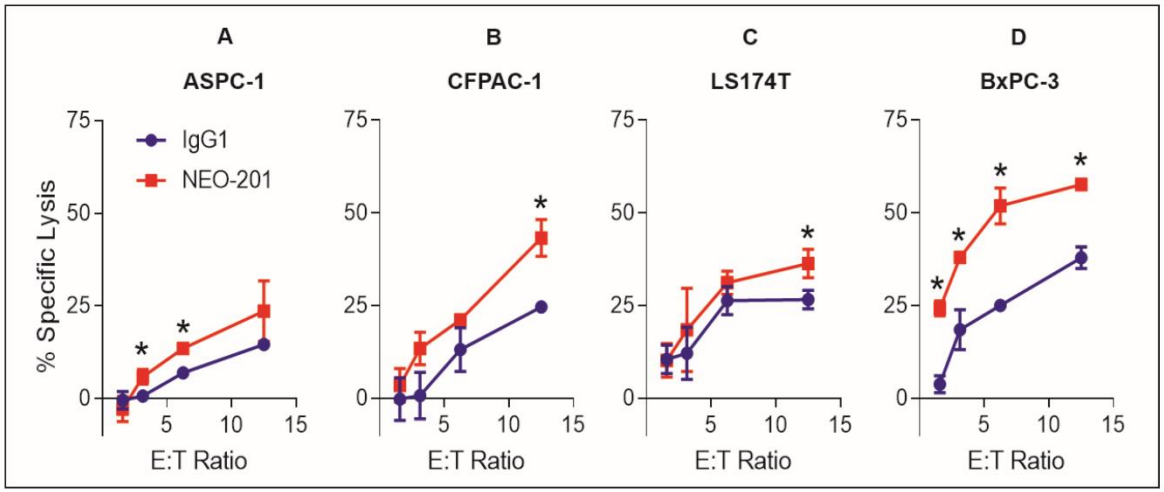
CD56^{+}	NK lineage marker
CD16^{neg}	ADCC function
CD107a^{+}	Degranulation marker
TIM-3^{+}	Inhibitory receptor
NKp30^{+}	Cytotoxicity receptor
NKG2D^{+}	Cytotoxicity receptor
CEACAM1^{+}	**Inhibitory receptor**

No reactivity with CEACAM5, CEACAM6, or NEO-201 mAb.

Fantini *et al.* ***Cancer Biother Radiopharm*, 2020**

NEO-201 enhances NK-92 cell cytotoxicity against CEACAM5+/NEO-201+ tumor cells

Cell line	CEACAM1^{+}	CEACAM5^{+}/NEO-201^{+}
	% positive (MFI)	
ASPC-1	**61.15** (707)	9.26 (869/9,078)
BxPC-3	2.45 (1,471)	**58.52** (1,447/6,420)
CFPAC-1	**18.67** (1,938)	**26.95** (1,108/1,728)
LS174T	2.43 (3,287)	**26.84** (1,030/858)



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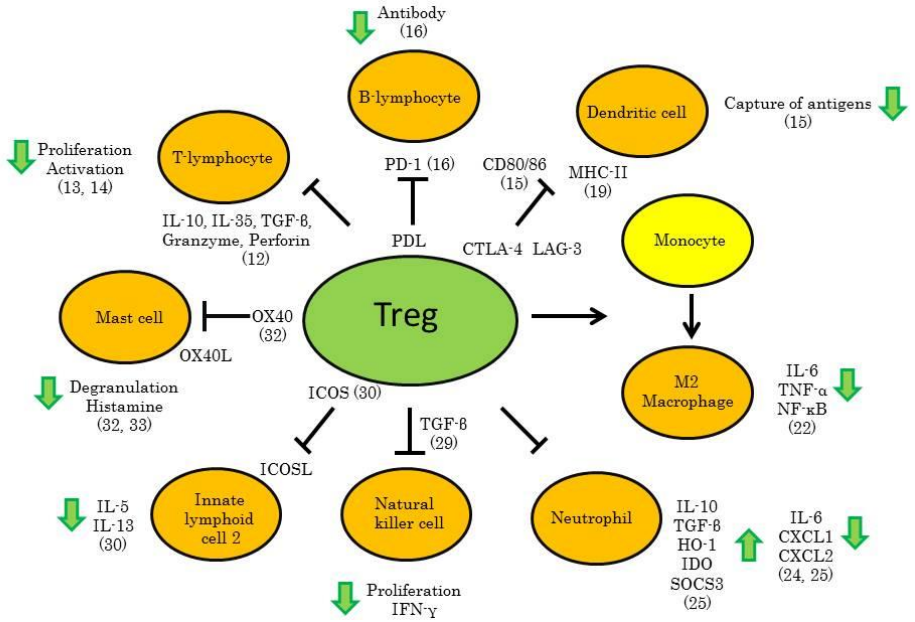
MECHANISMS OF ACTION:

4) NEO-201 targets and eliminates human immunosuppressive regulatory T cells (Tregs)

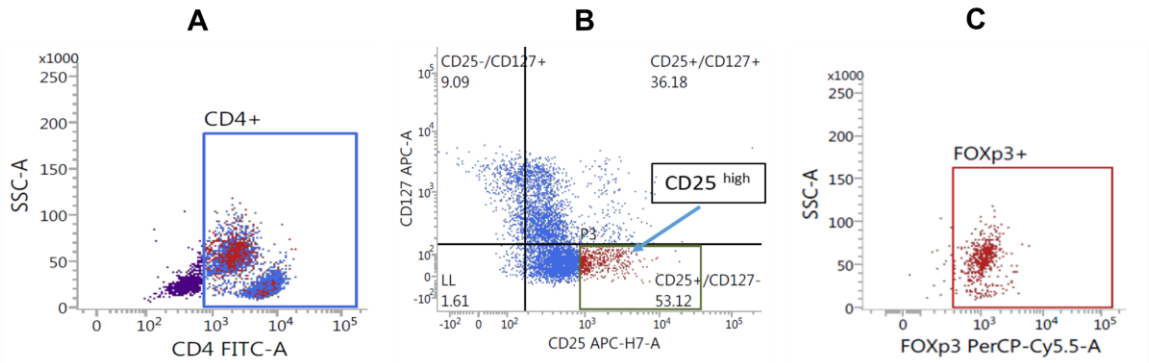


Seike M. *J Dermatological Research*, 2019

NEO-201 targets human immunosuppressive regulatory T cells (Tregs)

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

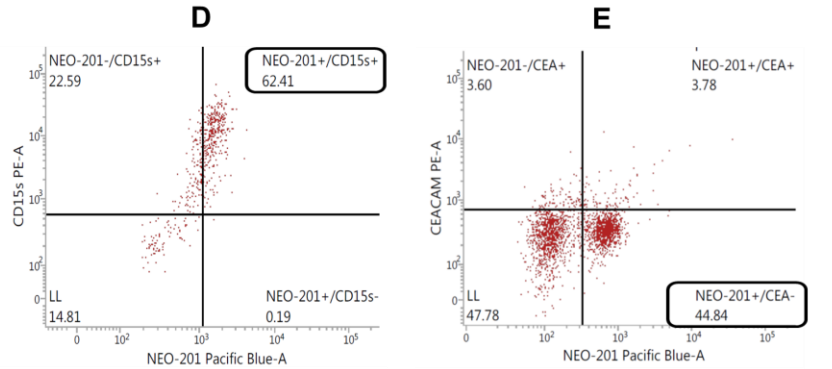
(Healthy donor 1)



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

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

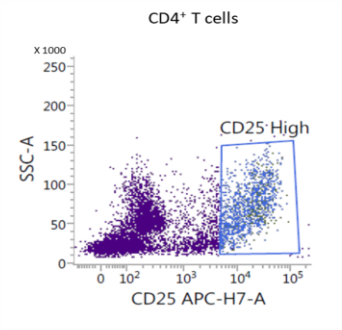
Fantini *et al.* ***SITC annual meeting*, 2019**

NEO-201 targets human immunosuppressive regulatory T cells (Tregs)

EasySep™ Human Biotin NEO-201⁺ Selection Kit

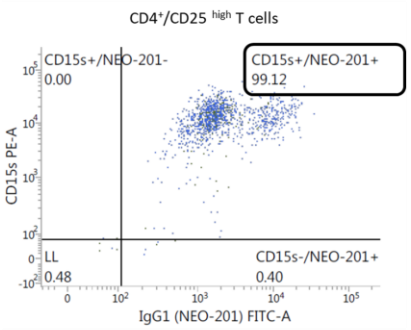
(Healthy donor 4)

A



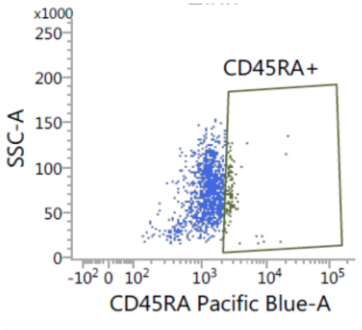
A: **15.69%** of **cells are CD25^{high}**

B



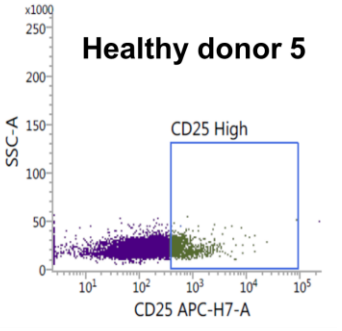
B: **99.12%** of **CD25^{high} cells** are **NEO 201⁺/ CD15s⁺**

C



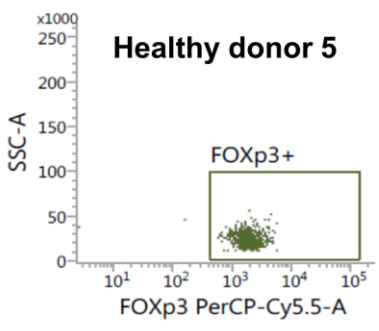
C: **NEO 201⁺/CD15s⁺ cells are CD45RA negative**

D



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

E



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

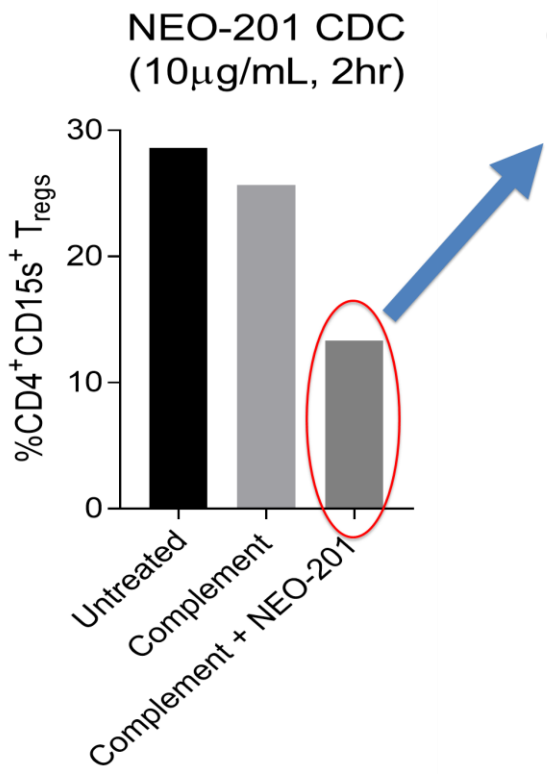
2 STEP human regulatory T cells isolation:

1) **CD4⁺ T cells were isolated from whole PBMCs**

2) **CD4⁺ T cells were bounded to biotinylated NEO-201 to isolate NEO-201⁺ Tregs**

Fantini *et al.* ***SITC annual meeting*, 2019**

NEO-201 eliminates human immunosuppressive regulatory T cells (Tregs) through CDC



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

50 % killing compared to untreated cells

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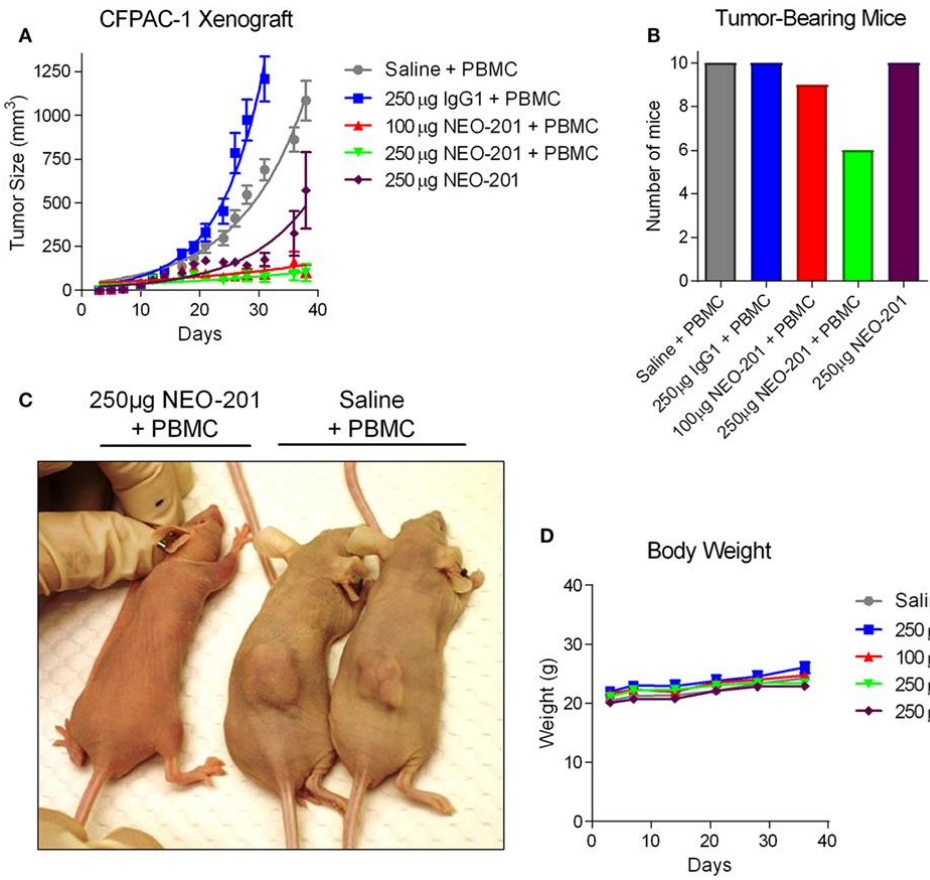
Antitumor efficacy of NEO-201 in CFPAC-1 tumor xenografts



- Tumors were established in 6-week old female athymic NU/NU nude mice by implanting tumor cells subcutaneously in the right flank of the mice.
- Once tumors reached ~100 mm3 in size, mice were then injected intraperitoneally with:

1. vehicle alone (saline solution) + PBMCs
2. human IgG1 (250 µg) + PBMCs
3. NEO-201 (100 and 250 µg) + PBMCs
4. NEO-201 (250 µg) alone

NEO-201 + PBMCs induced a substantial reduction in tumor growth at both dose levels compared to either the saline + PBMCs or human IgG + PBMCs control groups

Fantini *et al.* ***Frontiers in Immunology*****, 2018**

CONCLUSIONS

- This study demonstrates that NEO-201 has several mechanisms of action. NEO-201 is able to mediate both ADCC and CDC.
- In addition, NEO-201 can block the interaction between tumor cell CEACAM5 and NK cell CEACAM1 to reverse CEACAM1-dependent inhibition of NK cytotoxicity.
- These results suggest that NEO-201 may potentially reverse CEACAM1-dependent immunosuppression of NK cells in patients whose tumors express the NEO-201-reactive variant of CEACAM5.
- NEO-201 can also target and eliminate human immunosuppressive regulatory T cells (Tregs).
- Additional mechanisms are under investigation.

ACKNOWLEDGMENTS

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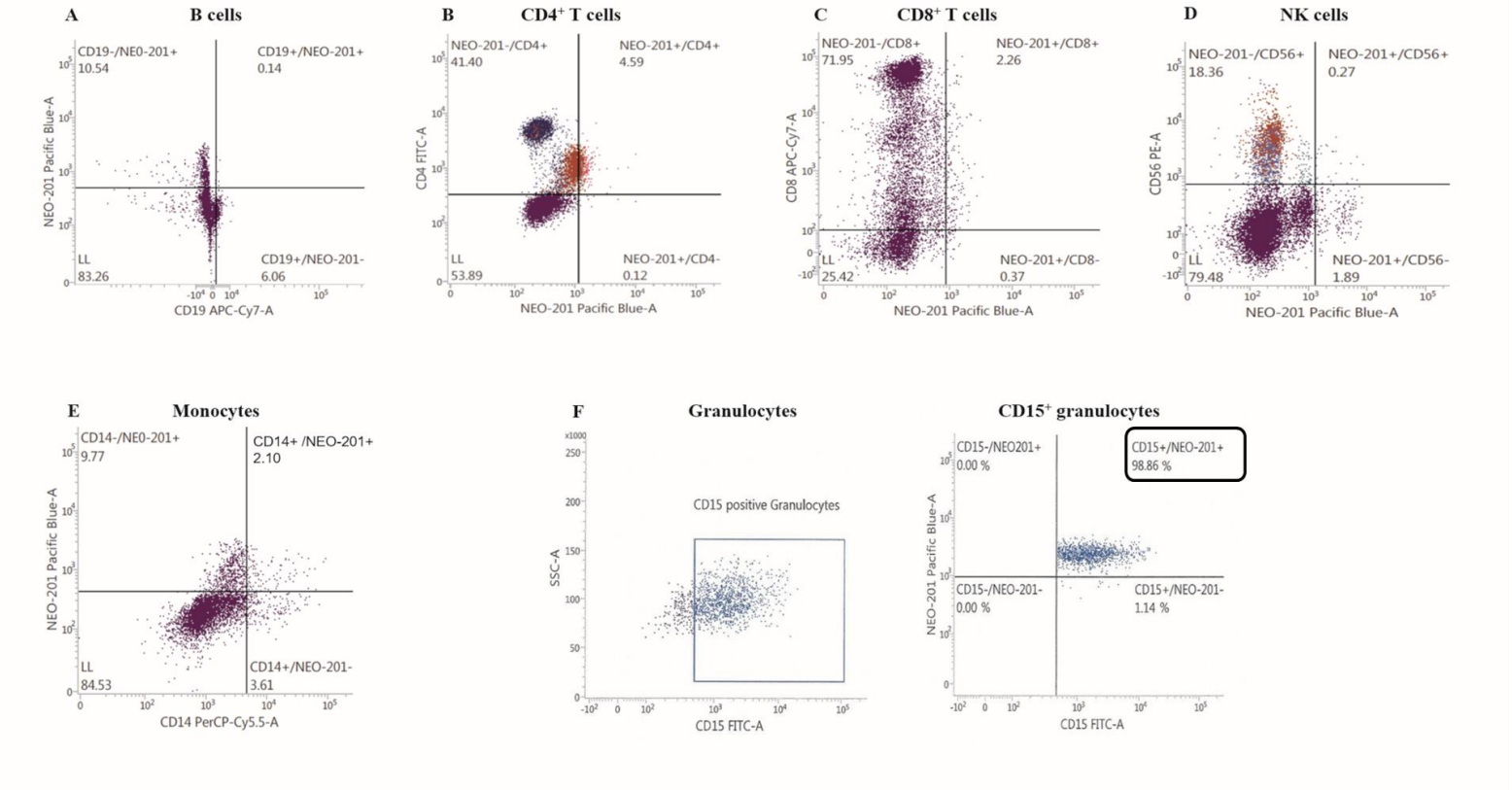
- Dr. Philip M. Arlen,M.D., CEO, Precision Biologics, Inc.
- Dr. Christina M. Annunziata, M.D., Ph.D., Head of Translational Genomics Section, Women's Malignancies Branch, NCI, NIH
- Dr. M.Pia Morelli, M.D., Ph.D., Women's Malignancies Branch, NCI, NIH

BACKUP SLIDES

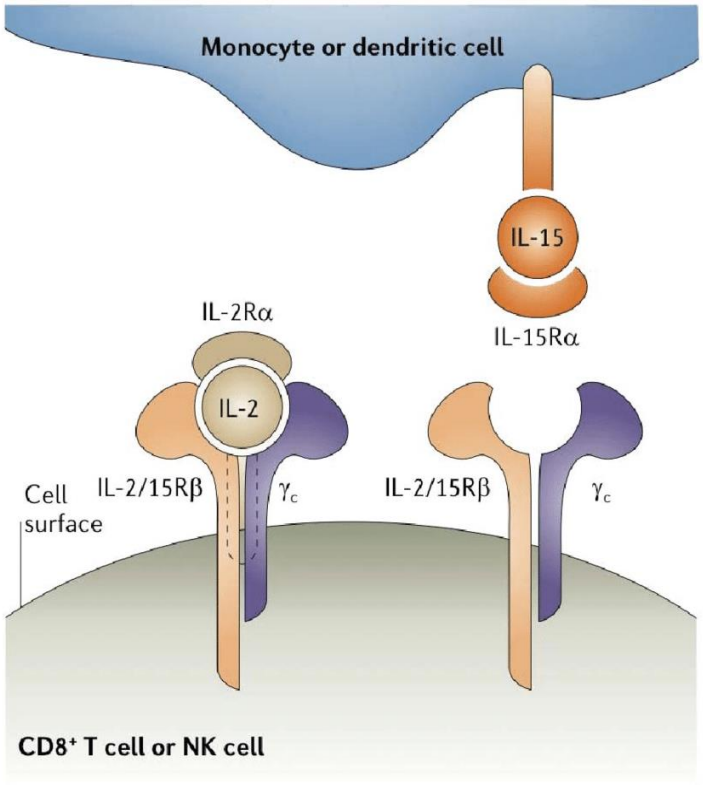
NEO-201 binds only to granulocytes in human hematopoietic cells



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

Fantini *et al.* ***Cancer Biother Radiopharm*, 2020**

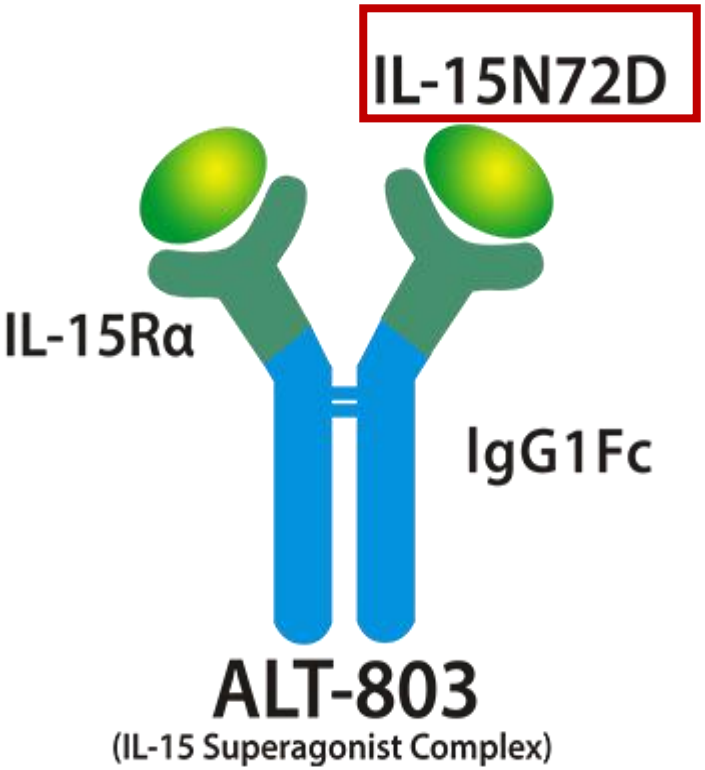
NK cell antitumor activity can be modulated by IL-15



Cancers **2011**, *3*, 3856-3893

- The cytokine interleukin-15 (IL-15) plays a crucial role in the immune system by affecting NK cell development, proliferation, cytotoxicity, and cytokine production.
- IL-15 binds to the IL-15Rα present on the surface of monocytes or dendritic cells and is presented to NK and CD8+ T cells where it forms a complex with IL-15Rβ to activate several intracellular signaling pathways.

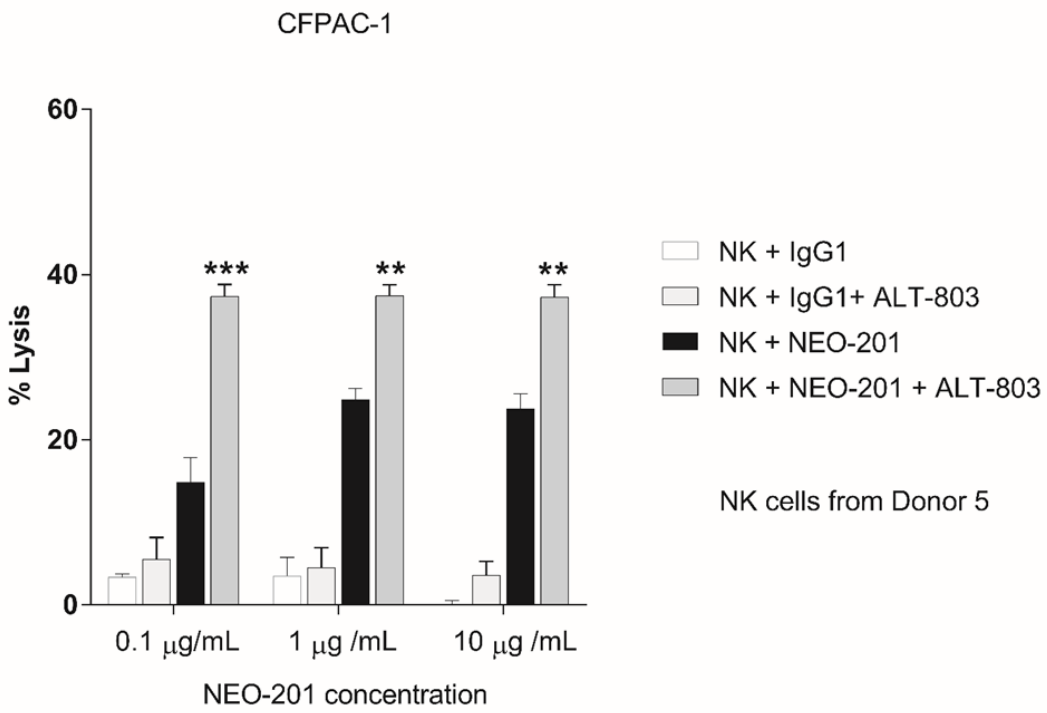
IL-15 superagonist technology: ALT-803



https://altorbioscience.com

- ALT-803 consists of an IL-15 variant **(IL-15N72D)** bound to an IL-15 receptor α/IgG1 Fc fusion protein, resulting in improved stability, longer persistence in lymphoid tissues and enhanced anti-tumor activity compared to native IL-15 *in vivo*.
- Induces NK cells and CD8+ T-cell proliferation.
- ALT-803 was found to enhance ADCC against a wide range of human carcinoma cells *in vitro*.
- ALT-803 also exerted a significant anti-tumor activity in murine breast, colon and melanoma tumor-bearing mice.

Enhanced ADCC against human carcinoma cell lines can be mediated by lower concentration of NEO-201 when NK cells are treated with ALT-803

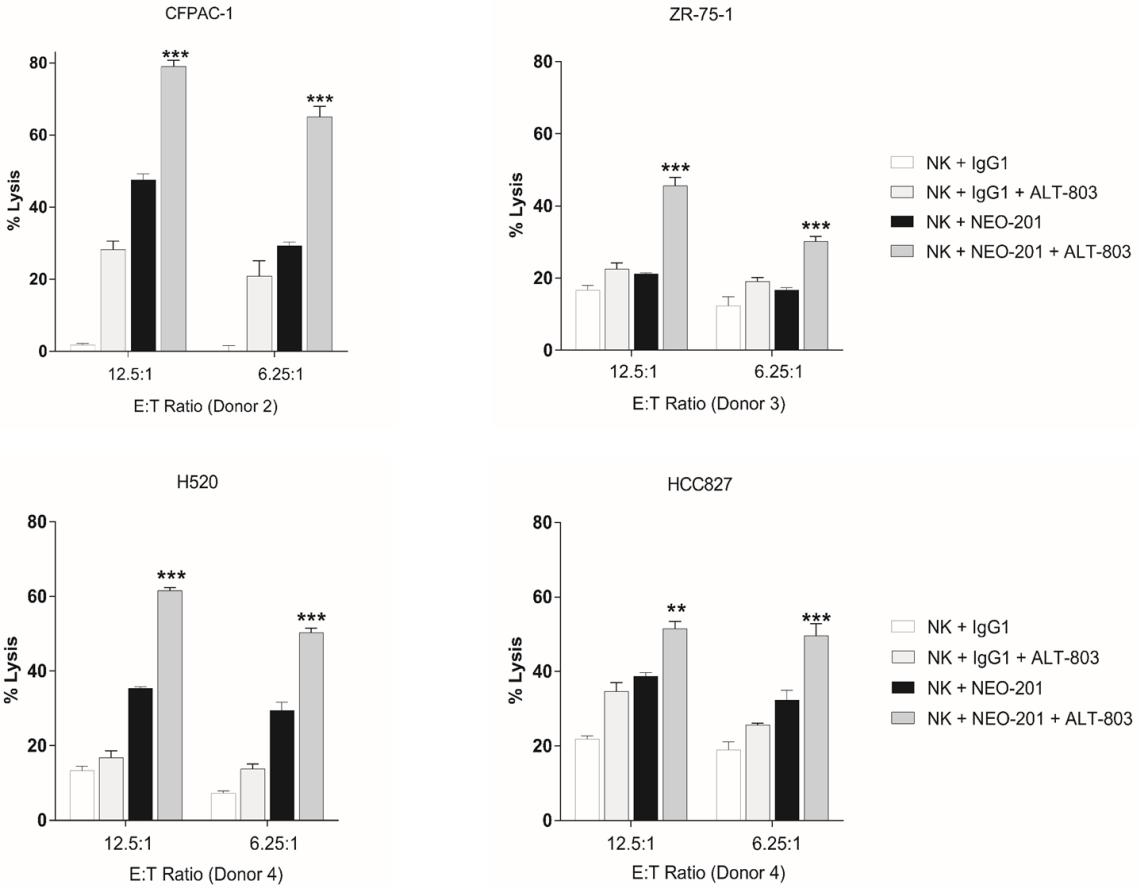


Treatment of NK cells with ALT-803 could decrease the dose of NEO-201 needed to achieve its clinical efficacy if used in combination therapy

Carcinoma cells were used as target cells in the presence of 10µg/mL of NEO-201 or human IgG1 (negative control) in the ADCC assay. Purified NK cells from a healthy donor were treated with ALT-803 (25 ng/mL) or vehicle control for 48h prior to be used as effector cells at at an E:T ratio of 12.5:1

Fantini *et al.* ***Cancer Biother Radiopharm*, 2019**

ALT-803 enhances ADCC mediated by NEO-201 against additional human carcinoma cell lines



ALT-803 significantly enhanced NEO-201-mediated ADCC at both E:T ratios in all cell lines as compared to untreated cells

Carcinoma cells were used as target cells in the presence of 10µg/mL of NEO-201 or human IgG1 (negative control) in the ADCC assay. Purified NK cells from three healthy donors were treated with ALT-803 (25 ng/mL) or vehicle control for 48h prior to be used as effector cells at the indicated E:T ratios

Fantini *et al*. ***Cancer Biother Radiopharm*, 2019**

ALT-803 modulates the phenotype of human healthy donor NK cells towards a more active cytotoxic function

% positive (MFI) (Donor 7)

Treatment	$CD16^-/CD56^+$	$CD16^+/CD56^+$	*$TIM\text{-}3^+$	*$NKG2D^+$	*$TIM\text{-}3^+/NKG2D^+$	*$CD107a^+$	*Granzyme B^+	*$PD\text{-}1^+$	*$CD158d^+$
Untreated	9.51 (52/3,457)	89.27 (7,434/1,048)	42.87 (843)	87.99 (317)	43.95 (799/307)	99.99 (887)	98.84 (474)	12.82 (907)	14.58 (167)
ALT-803	9.03 (84/**15,419**)	90.97 (6,915/**3,605**)	**81.56** (**2,972**)	78.33 (**590**)	**74.68** (**3,011/500**)	99.91 (**2,711**)	99.88 (**1,346**)	16.18 (1,272)	17.68 (**1,064**)

% positive (MFI) (Donor 8)

Treatment	$CD16^-/CD56^+$	$CD16^+/CD56^+$	*$TIM\text{-}3^+$	*$NKG2D^+$	*$TIM\text{-}3^+/NKG2D^+$	*$CD107a^+$	*Granzyme B^+	*$PD\text{-}1^+$	*$CD158d^+$
Untreated	12.27 (83/908)	87.73 (6,425/3,043)	33.92 (827)	79.46 (465)	24.85 (853/511)	99.95 (13,775)	99.75 (1,157)	5.36 (211)	12.72 (227)
ALT-803	13.23 (91/757)	86.77 (5,848/**7,810**)	**92.14** **(2,462)**	**95.40** **(1,152)**	**85.30** **(2,517/1,190)**	100 (10,365)	100 (**2,457**)	3.87 (229)	11.00 (219)

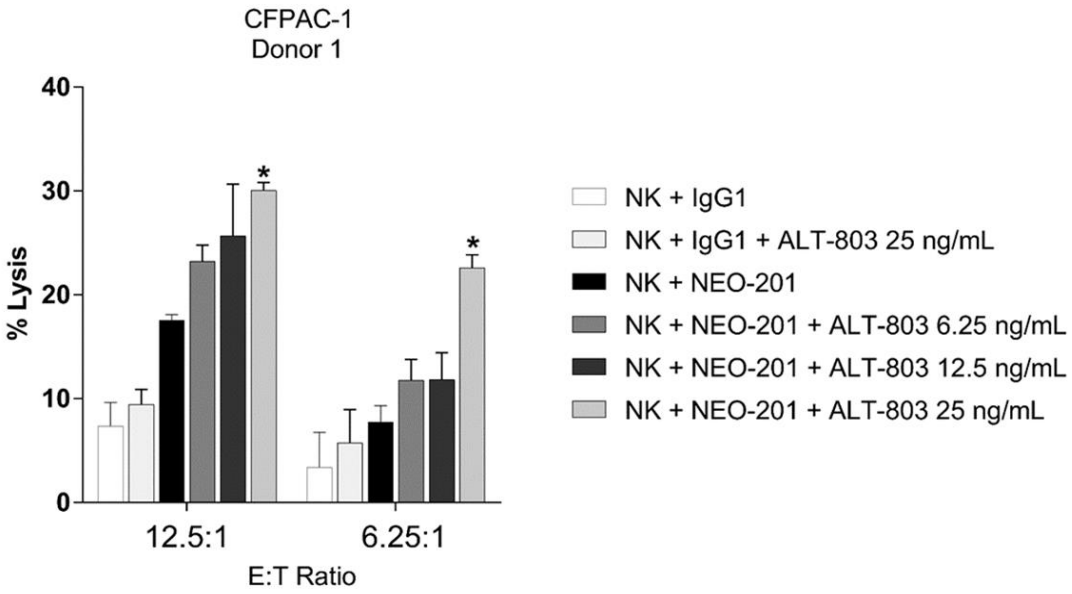
NK cells were treated with ALT-803 (25 ng/mL) or vehicle control (untreated) for 48h, prior to be analyzed by flow cytometry

Values in bold represent an increase equal or above 40% in protein levels and/or MFI in treated cells compared to untreated cells.

*percent of the specific NK marker in the $CD16^+/CD56^+$ population.

Fantini *et al.* ***Cancer Biother Radiopharm*, 2019**

ALT-803 enhance NEO-201-mediated ADCC against highly NEO-201-positive CFPAC-1 cell line in a dose-dependent manner



ALT-803 significantly enhanced NEO-201-mediated ADCC at both E:T ratios only at the highest concentration.

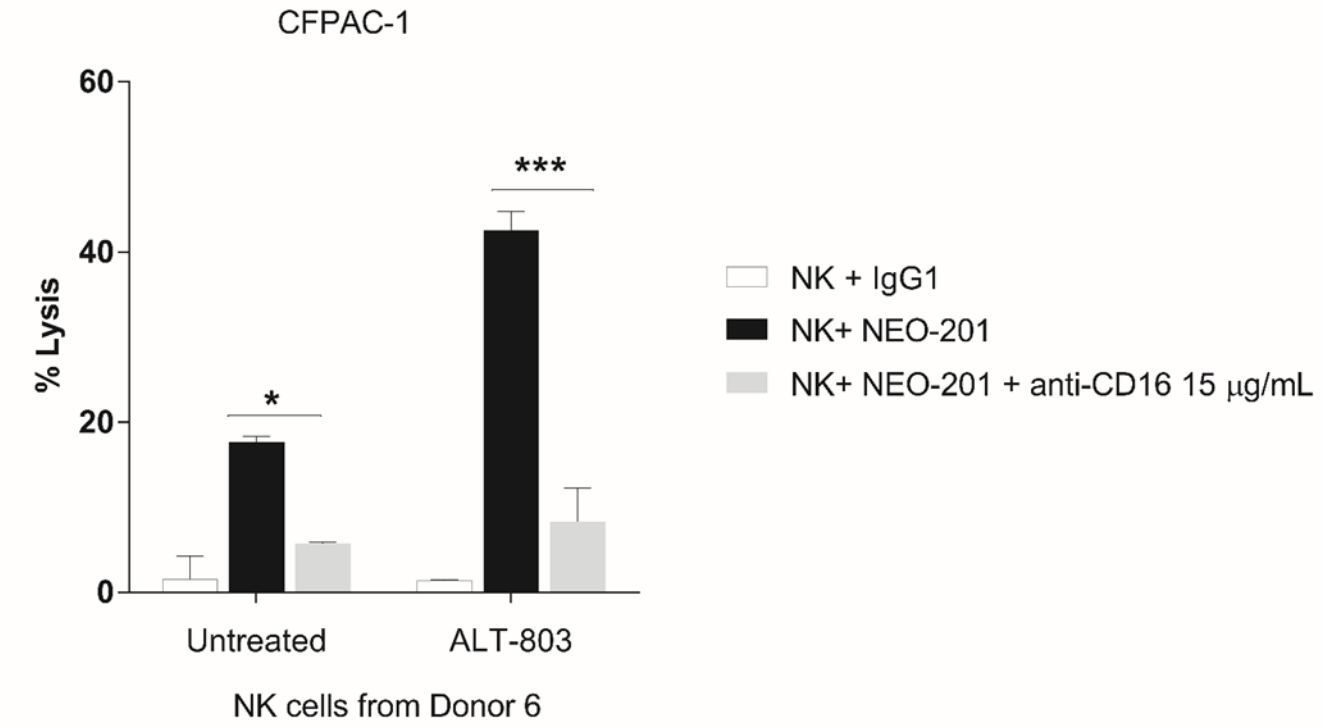
Carcinoma cells were used as target cells in the presence of 10µg/mL of NEO-201 or human IgG1 (negative control) in the ADCC assay. Purified NK cells were exposed to vehicle control or ALT-803 (6.25 to 25 ng/mL) for 48h prior to be used as effector cells at the indicated E:T ratios. Results are presented as mean ± S.E.M. from 3 replicate wells. Asterisks denote statistical significance of NK + NEO-201 + ALT-803 relative to controls (NK + NEO-201; NK + IgG1 + ALT-803) (2-way ANOVA). *$p < 0.05$.

Several ongoing clinical trials are evaluating the safety and efficacy of ALT-803 in combination with conventional cancer treatments

Clinical ID	Phase	Aim	Status	Study Period	Tumor Type	No of patients	Treatment	IL-15 Dose	Principle Investigator	Responsible Party
NCT02384954	I/II	Safety/Efficacy	Recruiting	2015–2023	B Cell Non-Hodgkin Lymphoma (NHL)	Estimated 86	Rituximab + ALT-803 (IV)	NA	H. C. Wong	Altor Bioscience, FL
NCT02465957	II	Safety/Efficacy	Recruiting	2015–2017	Merkel Cell Carcinoma (MCC)	Estimated 24	Activated NK-92 Natural Killer (aNK) Cell Infusions + ALT-803 (SC)	10 µg/kg on the first day of every aNK fusion	Not Provided	NantKwest, Inc., CA
NCT02523469	I/II	Safety	Recruiting	2016–2019	Advanced or Metastatic Non-Small Cell Lung Cancer	Estimated 91	ALT–803 + Nivolumab	6–15 µg/kg	J. Wrangle	Medical University of South Carolina, SC/Altor Bioscience, FL
NCT02559674	I/II	Safety/Efficacy	Recruiting	2016–2022	Advanced Pancreatic Cancer	Estimated 66	Alt-803 (IV) + Gemcitabine (SC) + Nab-Paclitaxel (SC)	NA	H. C. Wong	Altor Bioscience, FL

Immunol Lett. 2017 Oct;190:159-168.

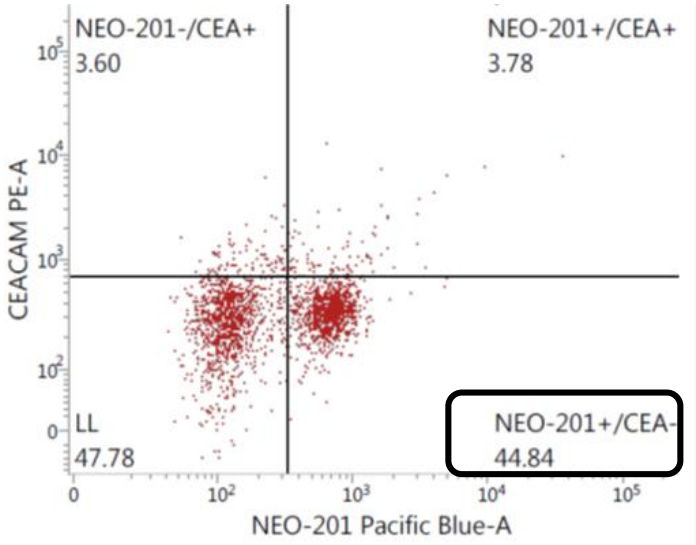
ADCC mediated by NEO-201 can be blocked by the anti- CD16 antibody



CFPAC-1 cells were used as target cells in the presence of 10µg/mL of NEO-201 or human IgG1 (negative control) in the ADCC assay. Purified NK cells from a healthy donor were treated with ALT-803 (25 ng/mL) or vehicle control for 48h prior to be used as effector cells at the E:T ratio of 12.5:1. Where applicable, NK cells were pretreated for 2h with anti-CD16 blocking antibody (15 µg/mL) prior to being used as effectors.

Regulatory T-cells are CEACAM-5 and CEACAM-6 negative as determined by flow cytometry

Phenotypic analysis of isolated T-regs (EasySep™ Human **CD4+CD127lowCD25+ Regulatory T Cell Isolation kit (HD 1)**



44.84% of **CD4$^+$/CD25^{high}/CD127-/FOXp3+ cells** are **NEO-201+ /CEACAM5- and CEACAM6- cells**

Cells were stained with **PE Mouse Anti-Human CD66 antibody** (Clone B1.1/CD66) which recognizes CD66a (*CEACAM1*), **CD66c (*CEACAM6*),** CD66d (*CEACAM3*) and **CD66e (*CEACAM5*)**