The Rationale for the Combination of the Monoclonal Antibody NEO-201 with Immune Checkpoint Inhibitors (ICI)

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PRECISION BIOLOGICS, INC.

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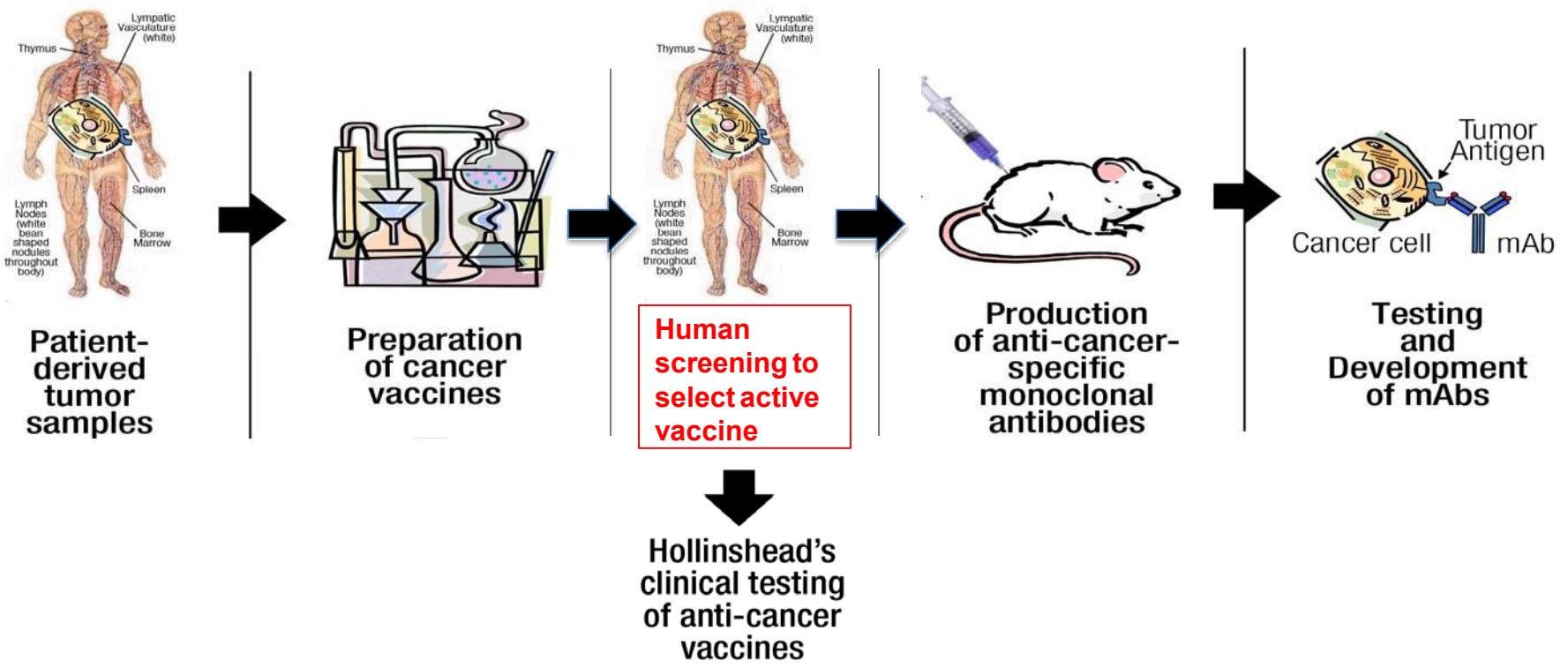
OUTLINES

- **Background of NEO-201**
- **NEO-201 target epitope**
- **NEO-201 mechanisms of action**
- **Results from Phase I 1st in human NEO-201 study**
- **Rationale to combine NEO-201 with Immune Checkpoint Inhibitors (ICIs)**
- **Preliminary results from Phase 2 combination study with Pembrolizumab in patients refractory to ICIs**

Only Human Derived and Human Tested Neo-Epitope Platform to Create Novel Therapeutics



- Only platform that has discovered functional neo-antigens that have been tested for immunogenic responses
- Validated targets with anti-tumor activity

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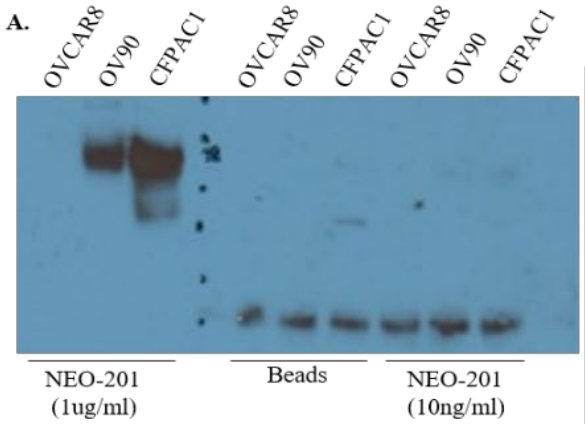
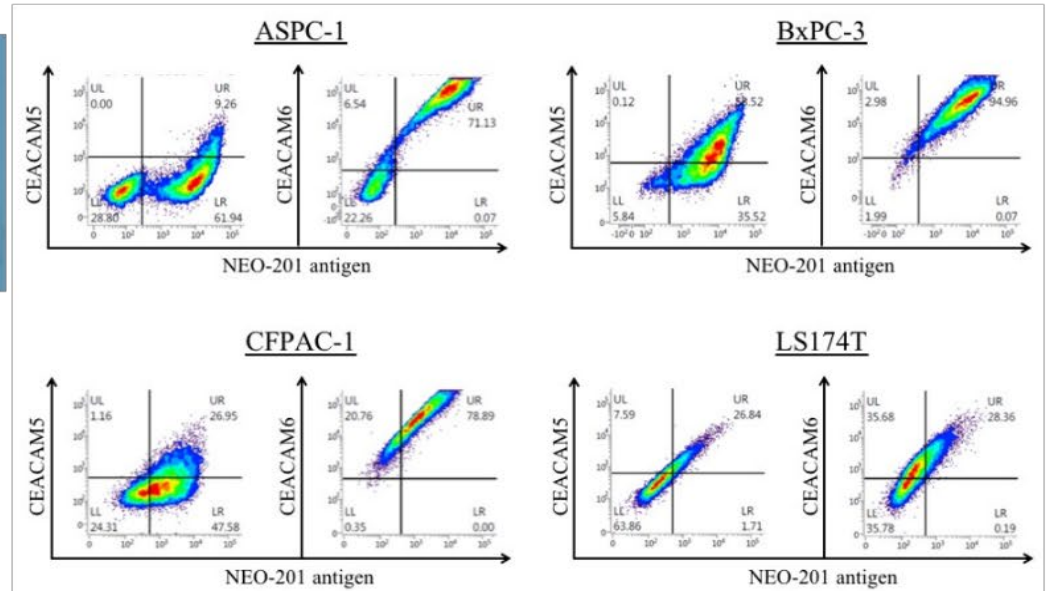
NEO-201 Binds to tumor associated form of CEACAM-5 and CEACAM-6



PEPTIDE	CFPAC1	OV90	#AAs	MW	Calc pi
CEACAM-6	4	3	344	37.2	5.82
CEACAM-5	1	2	702	76.7	5.69
CEACAM-7	1	2	265	29.4	5.54



CELL LINE (CANCER TYPE)	MARKER	% POSITIVE	MFI
ASPC-1 (pancreas)	NEO-201	**75.86**	**9,078**
	CEACAM5	20.04	869
	CEACAM6	**77.87**	**52,138**
BxPC-3 (pancreas)	NEO-201	**97.41**	**5,259**
	CEACAM5	**79.54**	**711**
	CEACAM6	**98.45**	**18,690**
CFPAC-1 (pancreas)	NEO-201	**85.21**	**1,728**
	CEACAM5	25.83	1,108
	CEACAM6	**96.50**	**27,792**
LS174T (colon)	NEO-201	29.15	858
	CEACAM5	36.34	1,030
	CEACAM6	**63.41**	**1,462**

From these screening the Carcinoembryonic Antigen-Related Cell Adhesion Molecule (CEACAM)5, also known as CEA, and CEACAM 6 were identified as the most likely targets of NEO-201

Zeligs et al. ***Frontiers in Oncology*, 2020**

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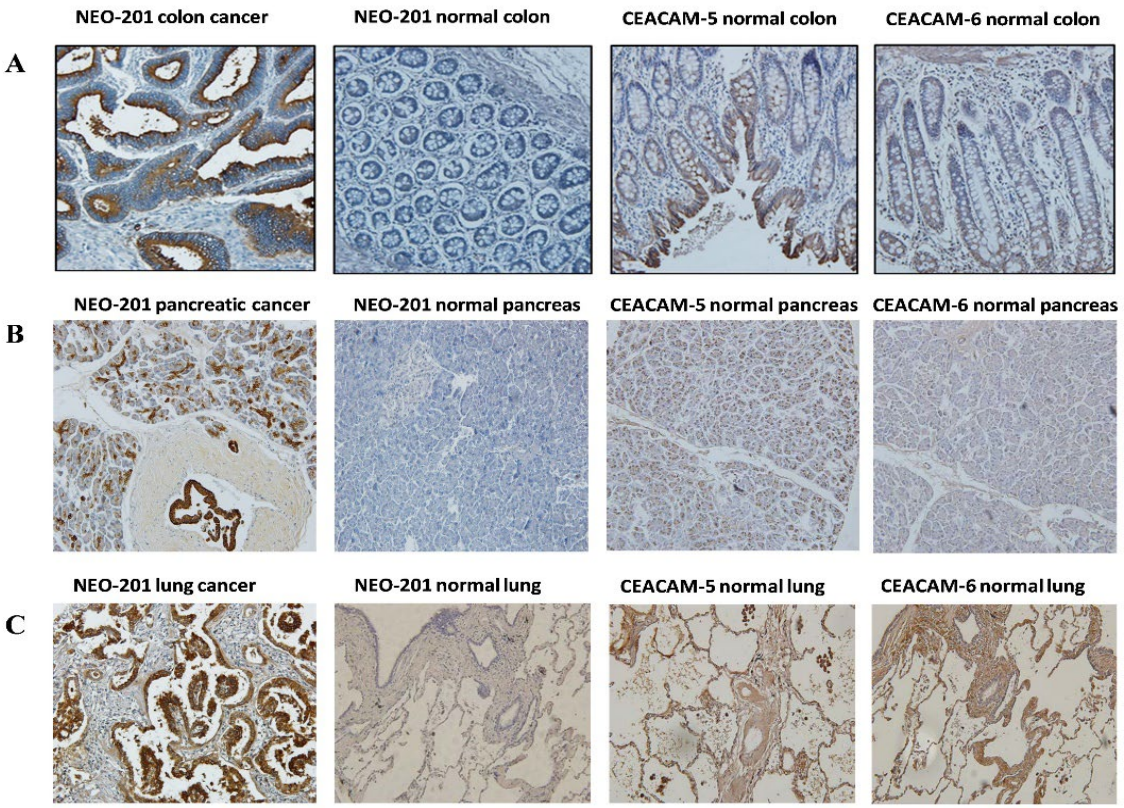
IHC Confirmed that NEO-201 Reactivity is Specifically Tumor-Associated



Tsang *et al.* ***Cancers*, 2022**

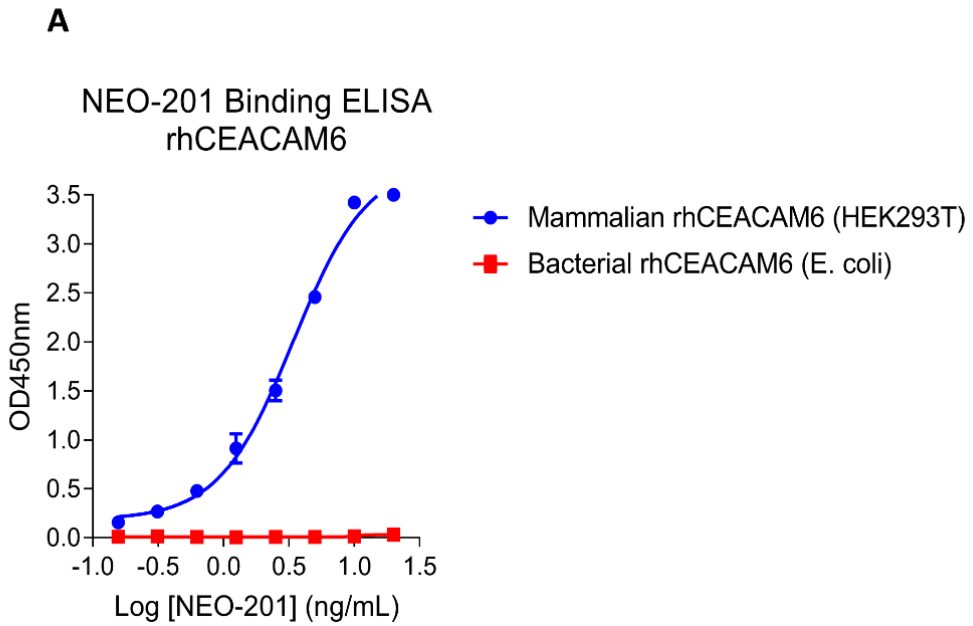
- **NEO-201 does not react against normal epithelial tissue CEACAM-5/6 positive.**
  - Majority of normal tissues stained CEACAM5^{+} and/or CEACAM6^{+}
- NEO-201 reacts against tumor tissue CEACAM-5/6 positive.
  - Majority of sampled tumors stained "triple positive" – NEO-201^{+} CEACAM5^{+} CEACAM6^{+}

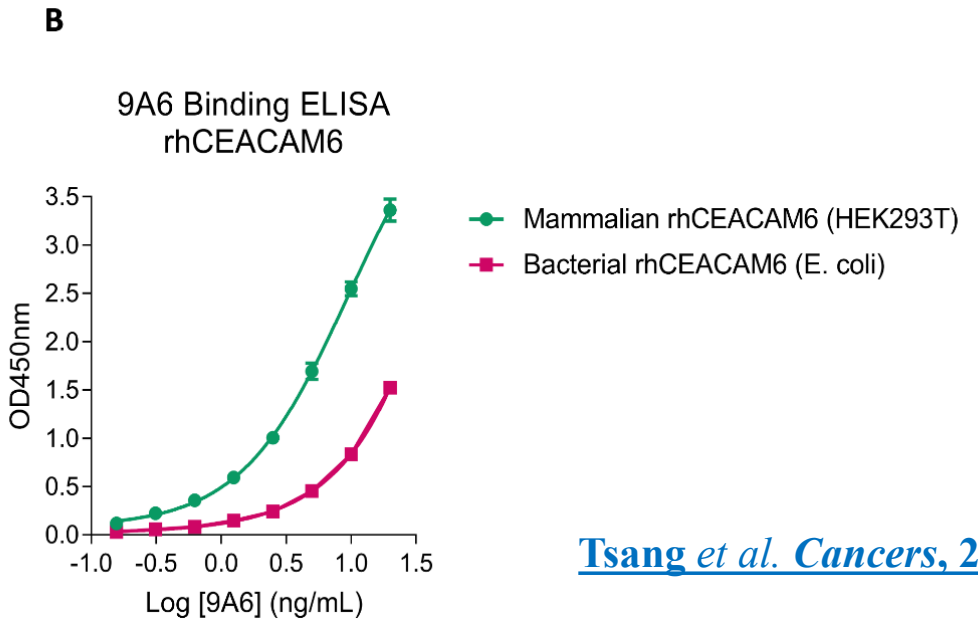
It is possible that a difference in glycosylation pattern explains the specific binding of NEO-201 to specific tumor-associated CEACAM-5 and CEACAM-6 variants but not to those expressed on healthy tissues as shown by the immunohistochemistry analysis.

NEO-201 Binding to O-glycans

Rationale to Study O-GLYCAN Binding:

NEO-201 binds to mammalian expressed rhCEACAM6 but not bacterial expressed rhCEACMA6 by ELISA





Tsang *et al.* ***Cancers*, 2022**

Post-translation modification (glycosylation) is made by **mammalian cells** on CEACAM-6;
Bacterial cells do not make post-translational modifications to be added on protein structures

NEO-201 Binding to O-glycans

Role of O-Glycans in Tumor Cells

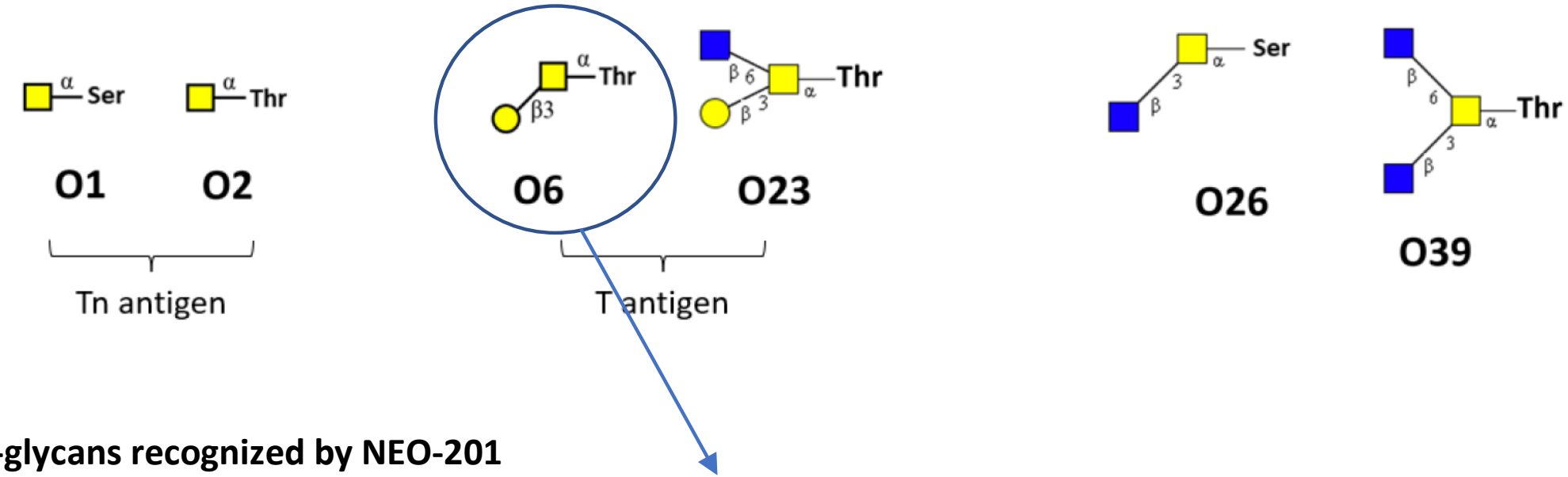
Tumor cells are characterized by the expression of truncated *O*-glycans, such as Tn antigen and T antigen, on the cell surface. These truncated structures are expressed by multiple tumor types, such as:

- **Solid tumors of epithelial origin:** breast cancers, ovarian cancers, gastric cancers, and colon cancers.

- **Liquid tumors**: AML and multiple myeloma

Truncated *O*-glycans, in general support tumor progression and their presence is strongly correlated to poor prognosis.

NEO-201 strongly binds to core 1 O-glycans



O-glycans recognized by NEO-201

O6, CORE-1 strongest binding

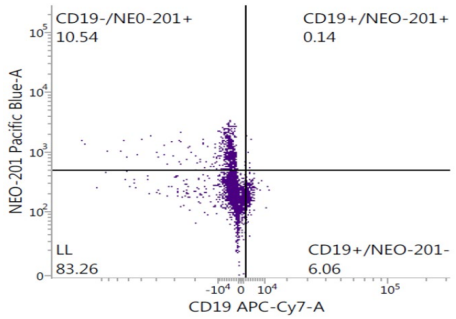
Tsang *et al.* ***Cancers*****, 2022**

The O-glycan array analysis consisting of 94 different O-glycans demonstrated that NEO-201 interact with different O-glycans.

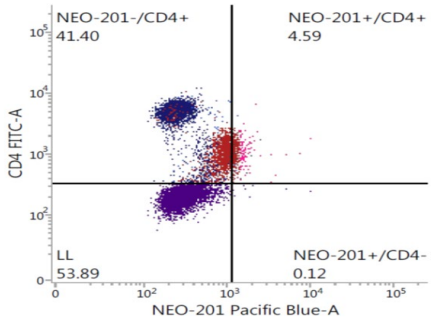
Binding to Core-1 O-glycans is the strongest observed

NEO-201 Targets Neutrophils and a Small Population of CD4+ T-cells in Human PBMCs From Healthy Donors

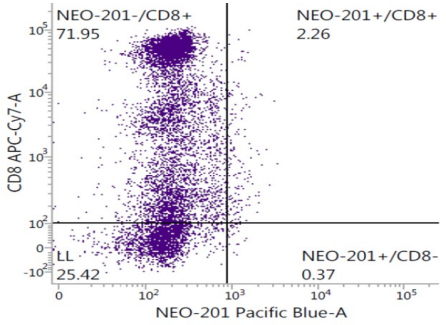
B cells



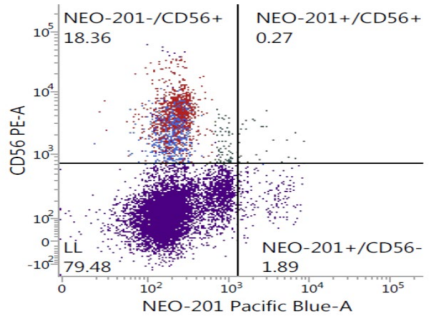
CD4⁺ T cells



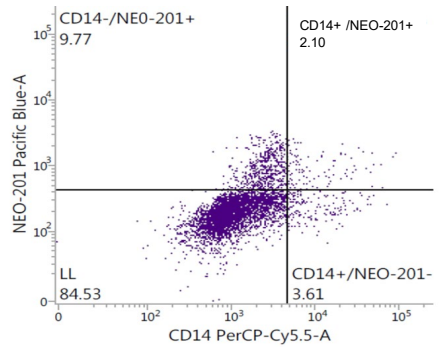
CD8⁺ T cells



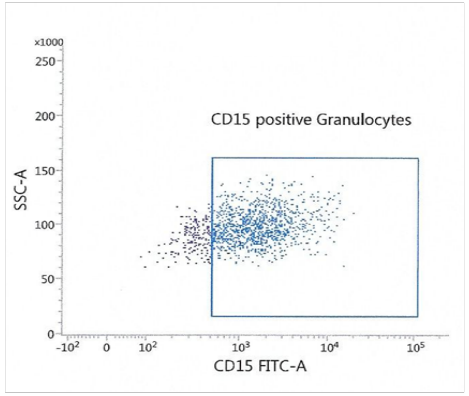
NK cells



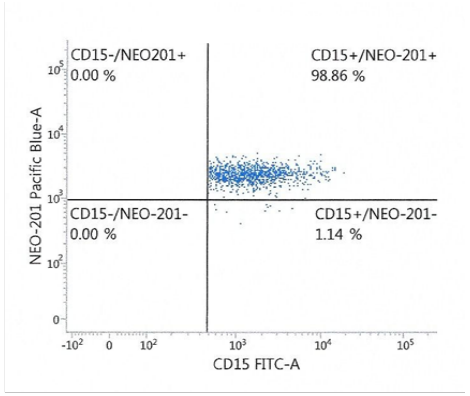
Monocytes



Granulocytes



CD15⁺ granulocytes



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

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

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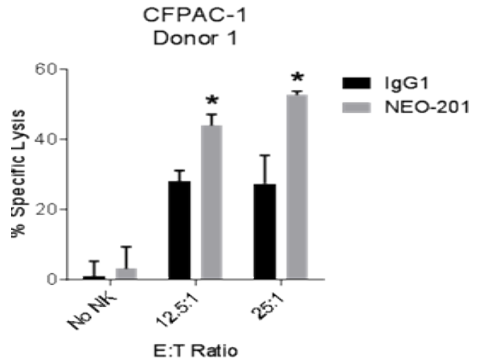
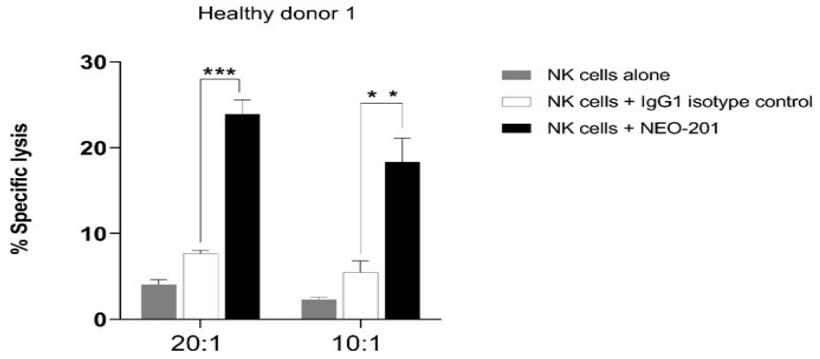
NEO-201 kills cells expressing truncated Core-1 O-glycans through antibody dependent cell-mediated cytotoxicity (ADCC)

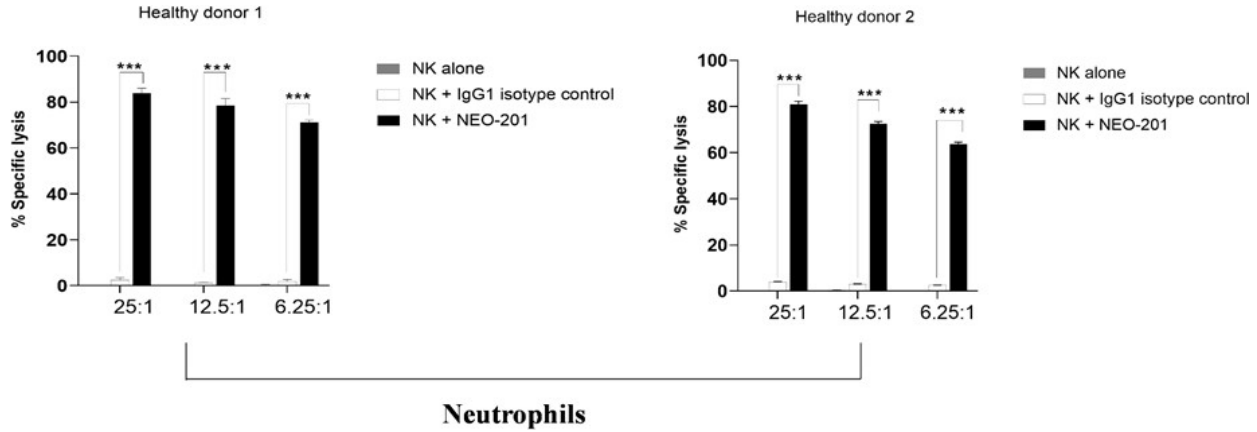
CFPAC-1 (Pancreatic cell line): expresses mainly Core-1 O-glycans



HL-60 (AML cell line): expresses mostly extended Core-1 O-glycans



CD15+ Granulocytes (Neutrophils): express Core-1 O-glycans



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

Tsang *et al.* ***Cancers,*** **2022**

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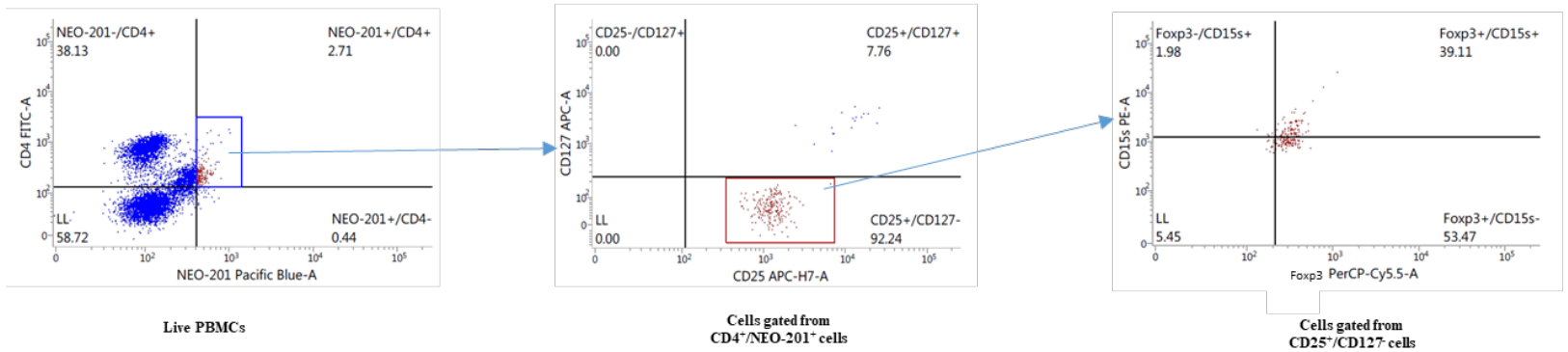
NEO-201 targets human immunosuppressive Tregs in human PBMCs from healthy donors and cancer patients

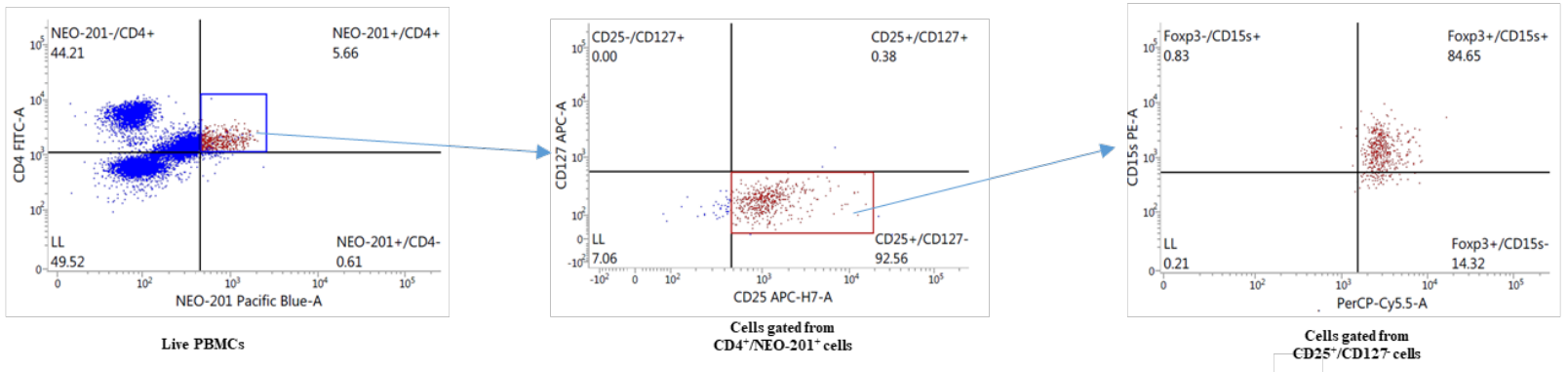
Healthy donor #2: PBMCs

A



Cancer patient #5: PBMCs

B



NEO-201+/CD4+ population express Tregs markers

Tregs recognized by NEO-201 are CD4+/CD25+/CD127-/Foxp3+ cells

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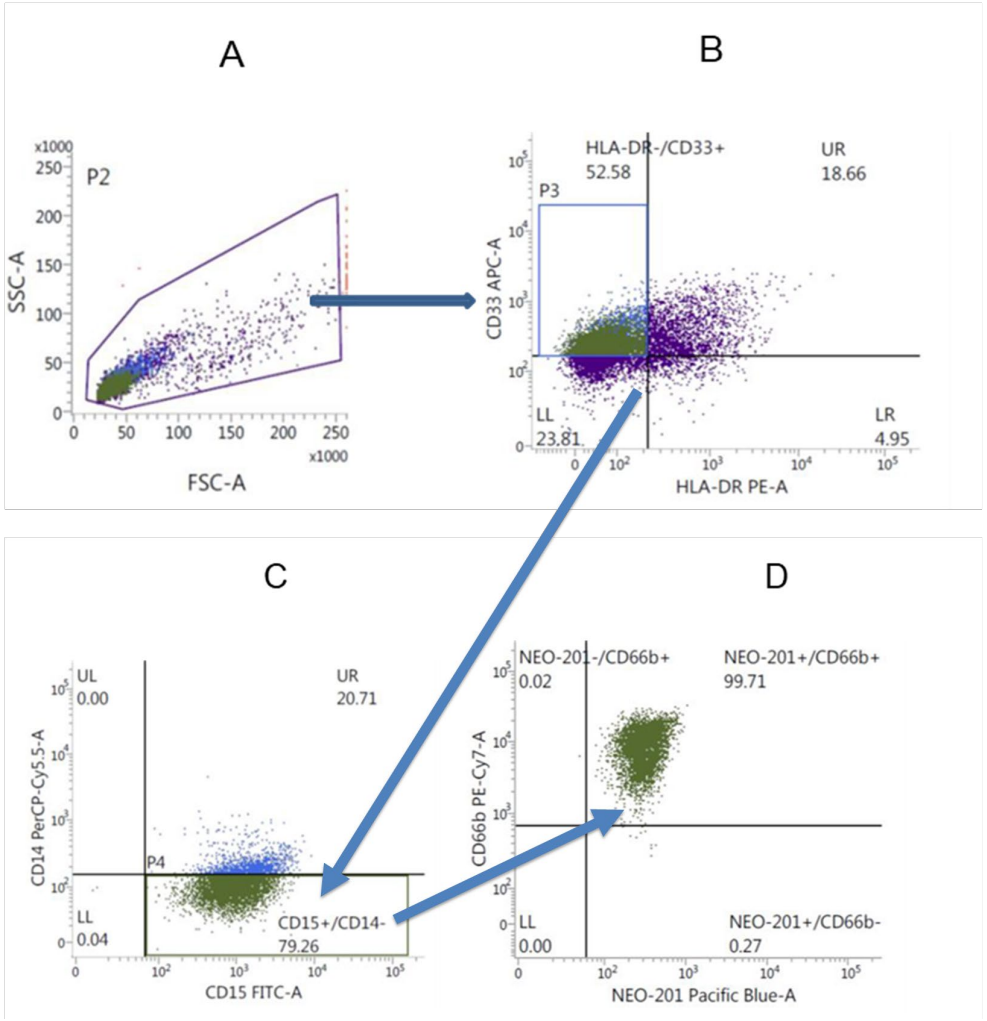
NEO-201 targets granulocytic myeloid-derived suppressor cells (gMDSCs)



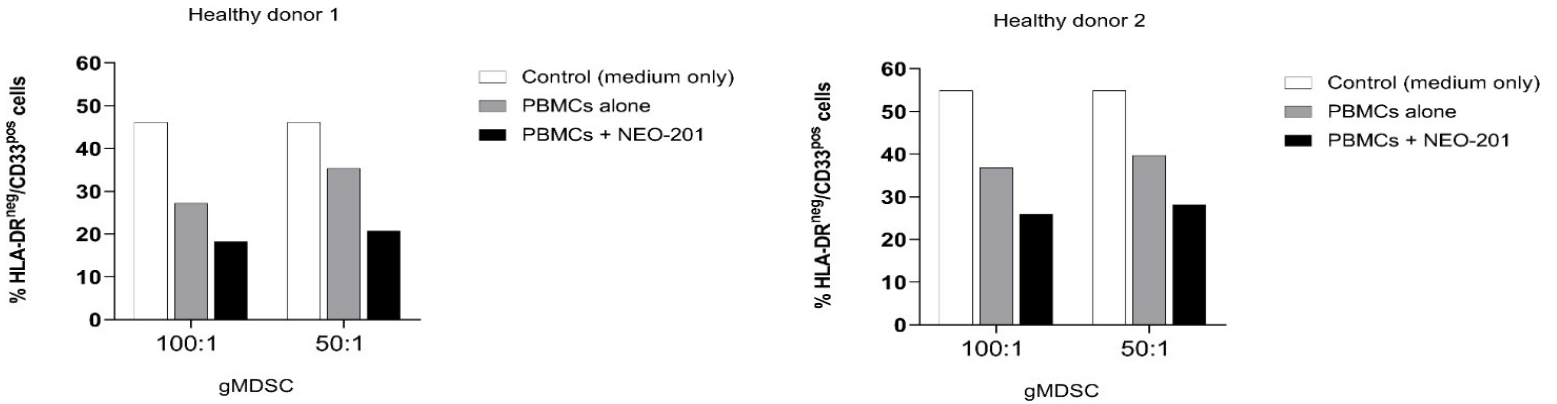
gMDSCs generated from Neutrophils

- Neutrophils were isolated using EasySep™ direct human neutrophil isolation kit.
- Isolated neutrophils were cultured in complete RPMI1640 supplemented with human GM-CSF (10ng/ml) and human IL-6 (10ng/ml).
- After 7 days, cells were then profiled by flow cytometry
- **NEO-201 recognizes CD33+/HLA-DR$_{neg}$/CD15+ /CD14-/CD66b+ cells**

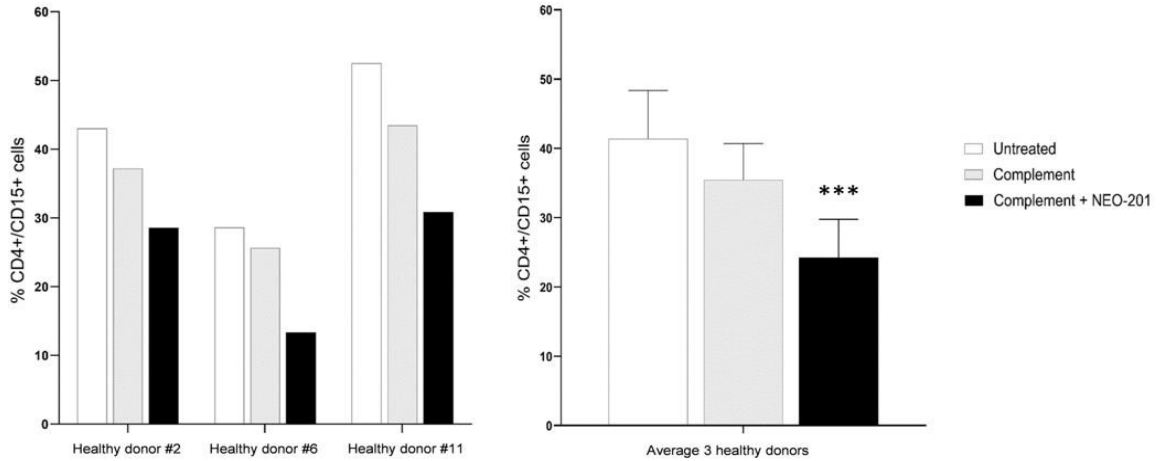
NEO-201 mediates elimination of immunosuppressive gMDSCs & Tregs

Immunosuppressive cells induced by tumors play an important role in inhibiting anti-cancer immune therapies from being effective*

NEO-201 eliminates gMDSCs via ADCC



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



*Constantinidou et al. Pharmacol. Ther. 2019, 194, 84–106.

*Woods et al. Clin. Cancer Res. 2018, 24, 6236–6247.

*Maj et al. Nat. Immunol. 2017, 18, 1332–1341.

*Kamada et al. Proc. Natl. Acad. Sci. USA 2019, 116, 9999–10008

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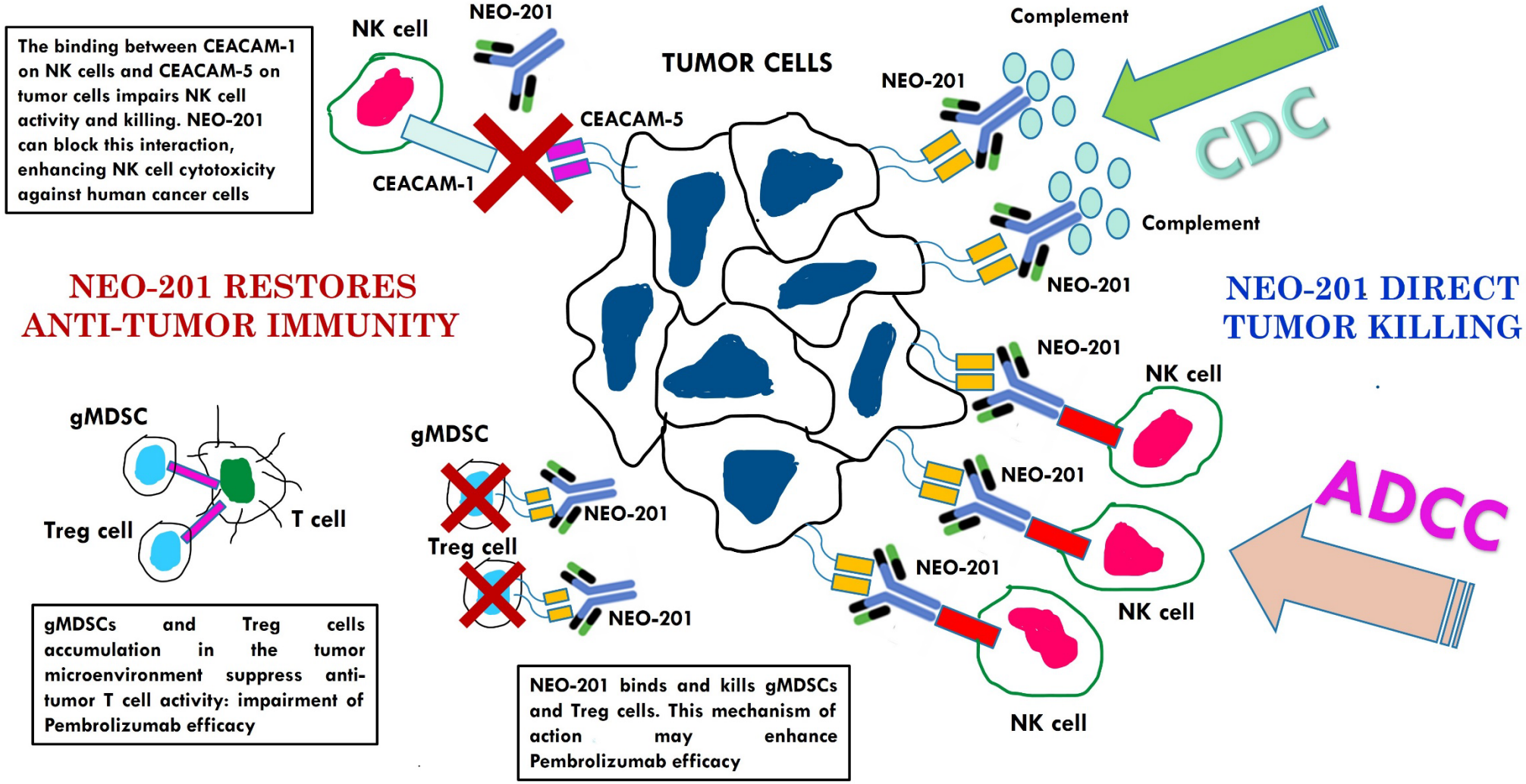
Mechanisms of action of NEO-201

The binding between CEACAM-1 on NK cells and CEACAM-5 on tumor cells impairs NK cell activity and killing. NEO-201 can block this interaction, enhancing NK cell cytotoxicity against human cancer cells



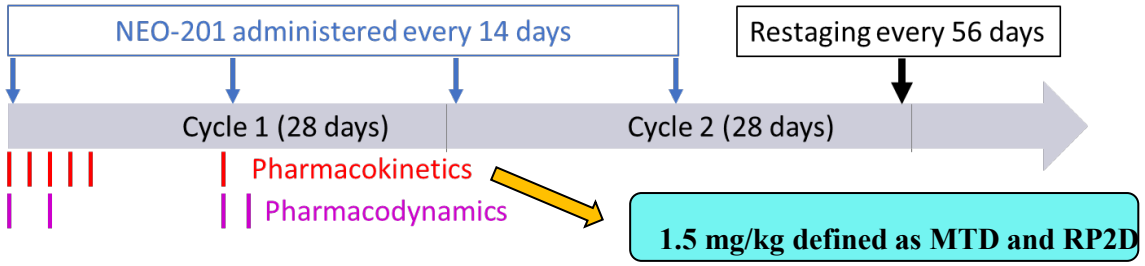
gMDSCs and Treg cells accumulation in the tumor microenvironment suppress anti-tumor T cell activity: impairment of Pembrolizumab efficacy

NEO-201 binds and kills gMDSCs and Treg cells. This mechanism of action may enhance Pembrolizumab efficacy

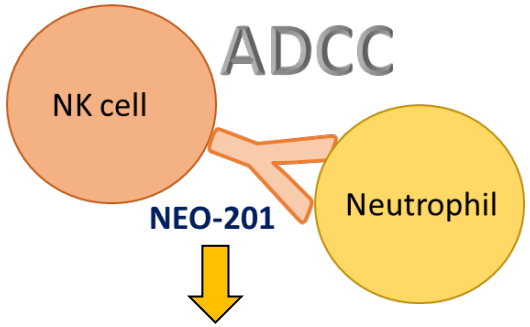
Completed Phase 1 at NCI, NIH, USA

Study design

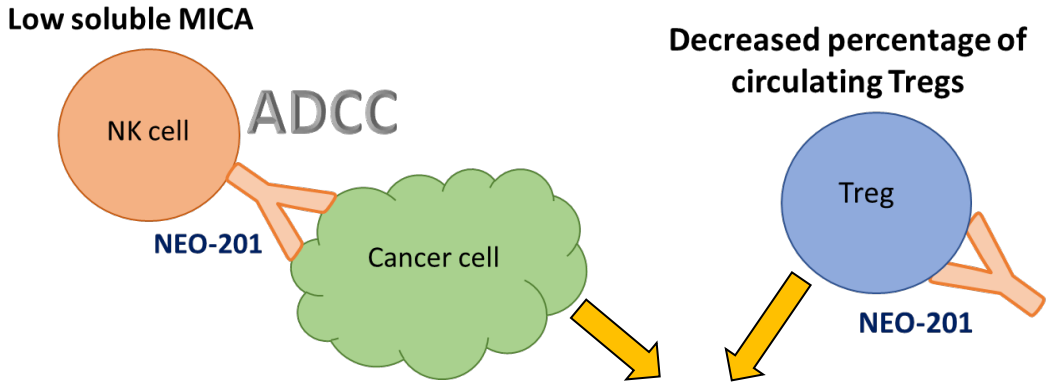
Dose Escalation Schedule		
Cohort	Dose (mg/kg)	Number Patients
DL 1	1	4
DL1.5	1.5	6
DL 2	2	7



Outcomes



Main toxicity: transient neutropenia in all patients mitigated with filgrastim after 5-6 days from NEO-201 infusion



Best response: stable disease (>56 days) in 4 patients with colorectal cancer

All patients with SD had mutations in RAS genes. Three patients harbored KRAS gene mutations, one patient had mutation in NRAS gene (one patient showed both KRAS and NRAS mutated)

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Lesson Learned from Phase 1 Clinical Trial

Cole et al. J Exp Clin Cancer Res (2023) 42:76
https://doi.org/10.1186/s13046-023-02649-6

Journal of Experimental & Clinical Cancer Research

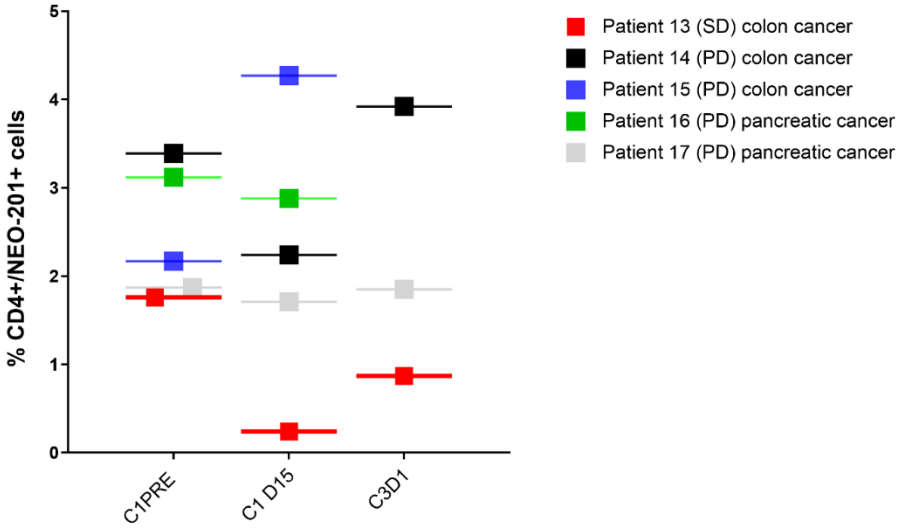
RESEARCH Open Access

First-in-human phase 1 clinical trial of anti-core 1 O-glycans targeting monoclonal antibody NEO-201 in treatment-refractory solid tumors

Christopher B. Cole[1†], Maria Pia Morelli[1†], Massimo Fantini[2*], Markku Miettinen[3], Patricia Fetsch[3], Cody Peer[4], William D. Figg[4], Tyler Yin[4], Nicole Houston[1], Ann McCoy[1], Stanley Lipkowitz[1], Alexandra Zimmer[1], Jung-min Lee[1], Miroslava Pavelova[1], Erin N. Villanueva[1], Kathryn Trewhitt[1], B. Brooke Solarz[1], Maria Fergusson[1], Sharon A. Mavroukakis[2], Anjum Zaki[2], Kwong Y. Tsang[2], Philip M. Arlen[2] and Christina M. Annunziata[1*]

STUDY PUBLISHED 3-29-23

Cole et al. JECCR, 2023



PATIENT 13 (SD): reduction of 86.4% and 50.6% of circulating $CD4^+/NEO\text{-}201^+$ Tregs at C1D15 and C3D1 respectively

Conversely, no reduction of circulating $CD4^+/NEO\text{-}201^+$ Tregs was observed in the other 4 patients with PD

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Rationale for Combination of NEO-201 and Pembrolizumab

- The low response rates and resistance to PD-1/PD-L1 blockade may be due to the action of **Regulatory T (Tregs) cells and gMDSCs** in the TME. Tregs cells have been demonstrated to **play a role in increase resistance to PD-1/PD-L1 blockade**

- **Our preliminary data showed that NEO-201 can target and mediate elimination of Tregs cells and gMDSCs through CDC and ADCC respectively.**

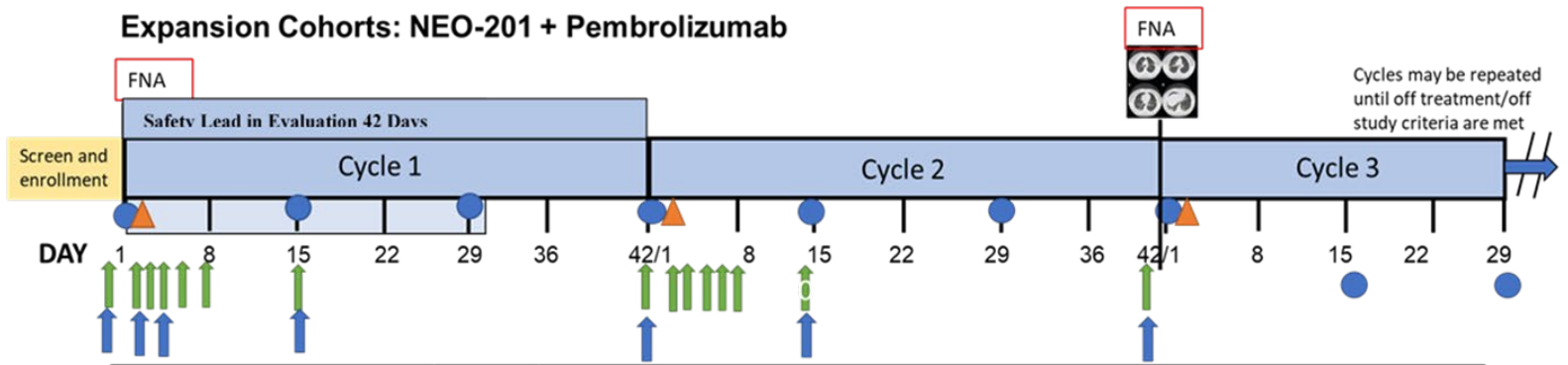
- Based on this data we **hypothesize that combining NEO201 with PEMBROLIZUMAB may overcome resistance to PD-1/PD-L1 checkpoints inhibitors by depleting immune-suppressive cells (Tregs and gMDSCs) and enhancing immune system mediated anti-tumor activity in subjects for whom pembrolizumab is currently indicated**

NEO-201 + Pembrolizumab Phase 2 Clinical Trial

Objectives

- Determine Objective Response Rate (ORR = CR, PR, SD) as determined by RECIST v1.1 guidelines and progression free survival (PFS) in four cohorts of subjects (subjects with NSCLC, HNSSC, uterine and cervical cancers) receiving NEO-201 at the RP2D in combination pembrolizumab at the FDA approved adult dose
- Assess immunogenicity of NEO-201 in adults with relapsed or chemo-resistant solid tumors participating in the dose escalation cohort and in the first 10 subjects receiving combination therapy.

Study Schema



- Subjects receive NEO-201 at RP2D (1.5 mg/kg) IV every 2 weeks on Days 1, 15 and 29 of a 42-day cycle.
- Pembrolizumab is administered at 400 mg IV every 6 weeks on Day 2 of every 42-day cycle.

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NEO-201 and Pembrolizumab phase 2 clinical trial: preliminary data

To date 7 subjects have been considered evaluable for efficacy and completed first stage restaging scans.

Disease Cohort	Enrolled	Time on Study	Response
NSCLC	**1**	**3 months**	**Stable disease (SD)**
Cervical	**1**	**8 months +**	**Stable disease (SD)**
HNSCC	**1**	**11 months +**	**Stable disease (SD)**
HNSCC	1	3 months	Progressive disease (PD
Endometrial	3	3 months	Progressive disease (PD)

3 of 7 evaluable patients have shown SD

NEO-201 + Pembrolizumab: Immune Monitoring

3 patients with SD and 4 patients with PD at the first radiological assessment

Evaluation of percentage of Tregs and gMDCSs from PBMCs pre and post treatment by flow cytometry in 7 patients that underwent the first radiological assessment:

Time points analyzed:

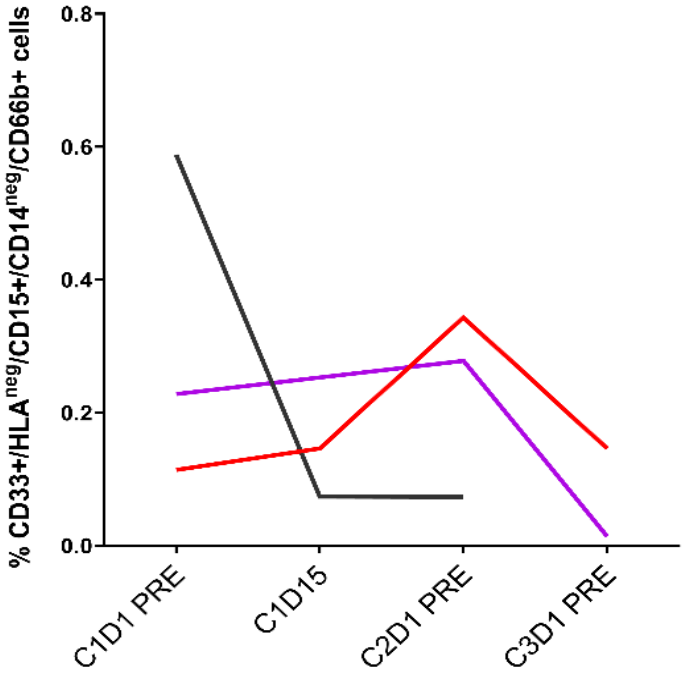
- before to start the treatment with NEO-201 (C1D1 PRE)
- after 14 days of first infusion with NEO-201 (C1D15),
- before to cycle 2 (C2D1 PRE; 42 days after first infusion)
- before of cycle 3 (C3D1 PRE; 84 days after first infusion)

Comparison of Levels of gMDSCs and Tregs Pre and Post Treatment in Patients with SD

Patients that showed a decrease of both gMDSCs and Tregs achieved the longest stabilization of the disease (patient 1 and 7)

gMDSCs:

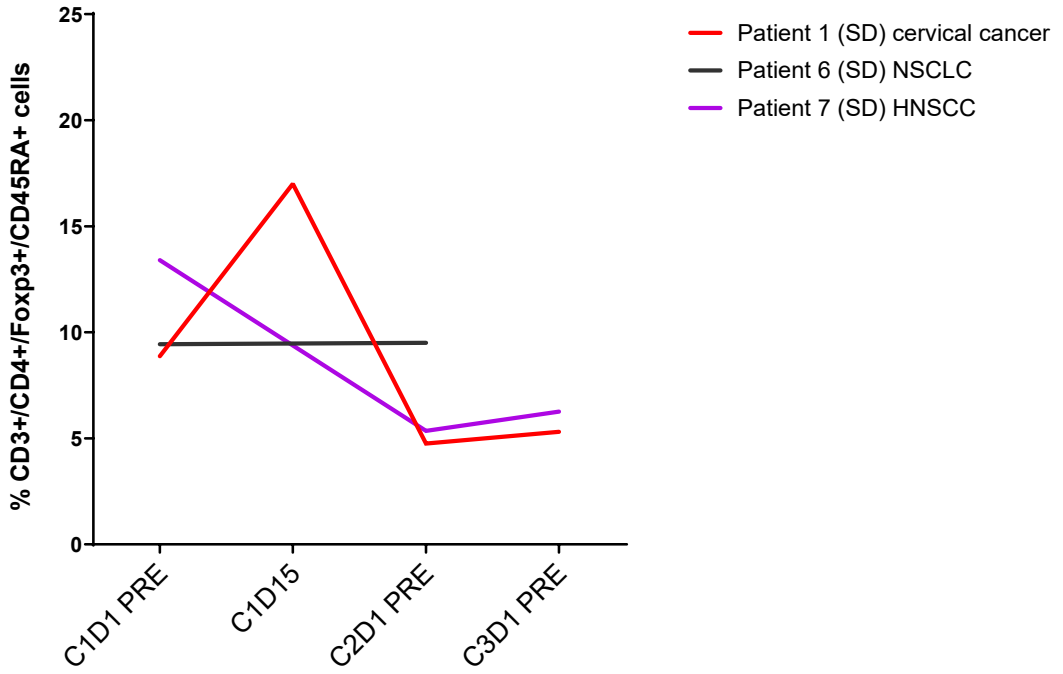
general downtreand of circulating gMDSCs



gMDSC population, within alive PBMCs, was defined as HLA-DR^{neg}/CD33^{+}/CD15^{+}/ CD14^{neg}/CD66b^{+} cells.

Tregs:

general downtrend of circulating Tregs



Tregs population, within CD4+/CD3+ cells in alive PBMCs, was defined as CD3+/CD4+/Foxp3+/CD45RA+ cells

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Patients with Stable Disease (SD)

Cervical cancer patient:

- 50 years old with chemo-resistant metastatic mucinous carcinoma.
- 80% tumor tissue positive for NEO-201 staining in IHC with 3+ intensity.
- Stable disease for more than 8 months after first infusion with both antibodies
- Patient qualified for debulking surgery requiring exiting the study

Head and neck squamous cell carcinoma patient:

- 72 years old with moderately differentiated carcinoma.
- 10-15% tumor tissue positive for NEO-201 staining in IHC with 3+ intensity.
- Stable disease for more than 11 months after first infusion with both antibodies

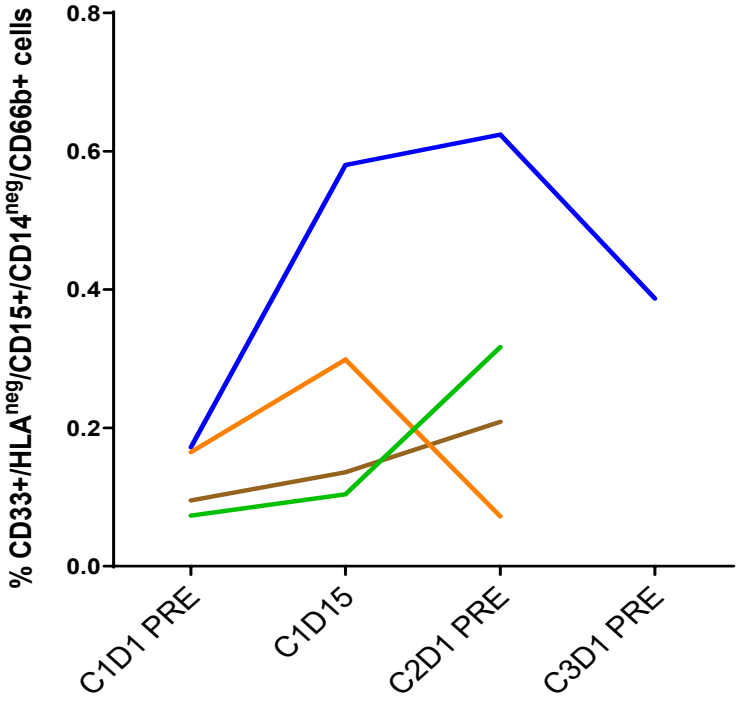
NSCLC patient:

- 69 years old with metastatic lung adenocarcinoma
- 90% tumor positive for NEO-201 staining in IHC with 3+ intensity
- Stable disease by RECIST on Day 84.
- Patient was taken off study per treating physician's decision.

Comparison of Levels of gMDSCs and Tregs Pre and Post Treatment in Patients with PD

Uptrend of gMDSCs and/or Tregs correlated with progression of the disease

gMDSCs:
general uptrend of circulating gMDSCs



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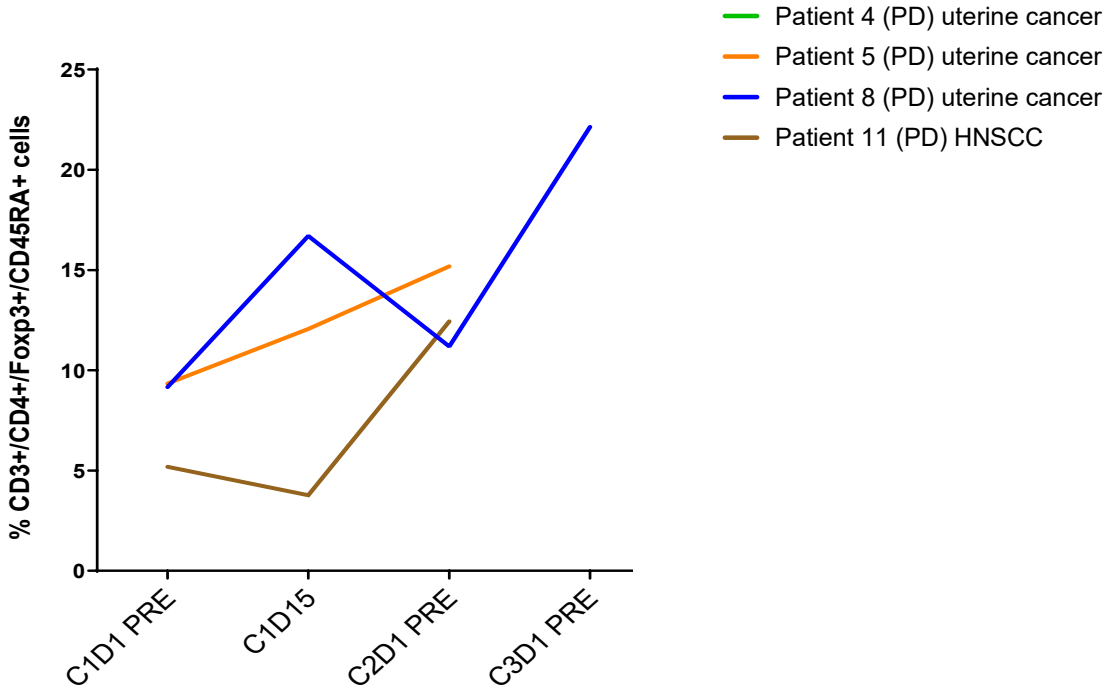
Tregs:
general uptrend of circulating Tregs



Tregs population, within CD4+/CD3+ cells in alive PBMCs, was defined as CD3+/CD4+/Foxp3+/CD45RA+ cells

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Conclusions

- NEO-201 target and kills specifically cells expressing core 1 O-glycans
- NEO-201 does not target and destroy healthy tissues
- **NEO-201 has several mechanisms of action**. NEO-201 is able to mediate both ADCC and CDC.
- NEO-201 can also target and eliminate human Tregs and gMDSCs
- Preliminary results from Phase 2 clinical trial combining NEO-201 + Pembrolizumab showed that a decrease of both circulating Tregs and gMDSCs in patients with durable SD is suggestive of good prognosis for treatment with NEO-201 in combination with ICIs.

APPENDIX

PRECISION BIOLOGICS

List of publications: NEO-201

1. Fantini, M. et. al. Preclinical characterization of a novel monoclonal antibody NEO-201 for the treatment of human carcinomas. *Front Immunol* **2018**, *8*, 1899. https://pubmed.ncbi.nlm.nih.gov/29354121/
2. Fantini, M. et al. An IL-15 Superagonist, ALT-803, Enhances Antibody-Dependent Cell-Mediated Cytotoxicity Elicited by the Monoclonal Antibody NEO-201 Against Human Carcinoma Cells. *Cancer Biother Radiopharm* **2019**, *34*, 147-159. https://pubmed.ncbi.nlm.nih.gov/30601063/
3. Fantini, M. et al. The Monoclonal Antibody NEO-201 Enhances Natural Killer Cell Cytotoxicity Against Tumor Cells Through Blockade of the Inhibitory CEACAM5/CEACAM1 Immune Checkpoint Pathway. *Cancer Biother Radiopharm* **2020**, *35,* 190-198. https://pubmed.ncbi.nlm.nih.gov/31928422/
4. Zeligs, K.P. et al. Evaluation of the anti-tumor activity of the humanized monoclonal antibody NEO-201 in preclinical models of ovarian cancer. *Front Oncol* **2020**, *10,* 805. https://pubmed.ncbi.nlm.nih.gov/32637350/
6. Tsang, K.Y. et al. Development and Characterization of an Anti-Cancer Monoclonal Antibody for Treatment of Human Carcinomas. *Cancers* **2022**, *14*, 3037. https://www.mdpi.com/2072-6694/14/13/3037
7. Tsang, K.Y. et al. Identification of the O-Glycan Epitope Targeted by the Anti-Human Carcinoma Monoclonal Antibody (mAb) NEO-201. *Cancers* **2022**, *14*, 4999. https://www.mdpi.com/2072-6694/14/20/4999
8. Cole, C.B. et al. First-in-human phase 1 clinical trial of anti-core 1 O-glycans targeting monoclonal antibody NEO-201 in treatment-refractory solid tumors. *J Exp Clin Cancer Res* **2023,** 42(1):76. https://jeccr.biomedcentral.com/articles/10.1186/s13046-023-02649-6

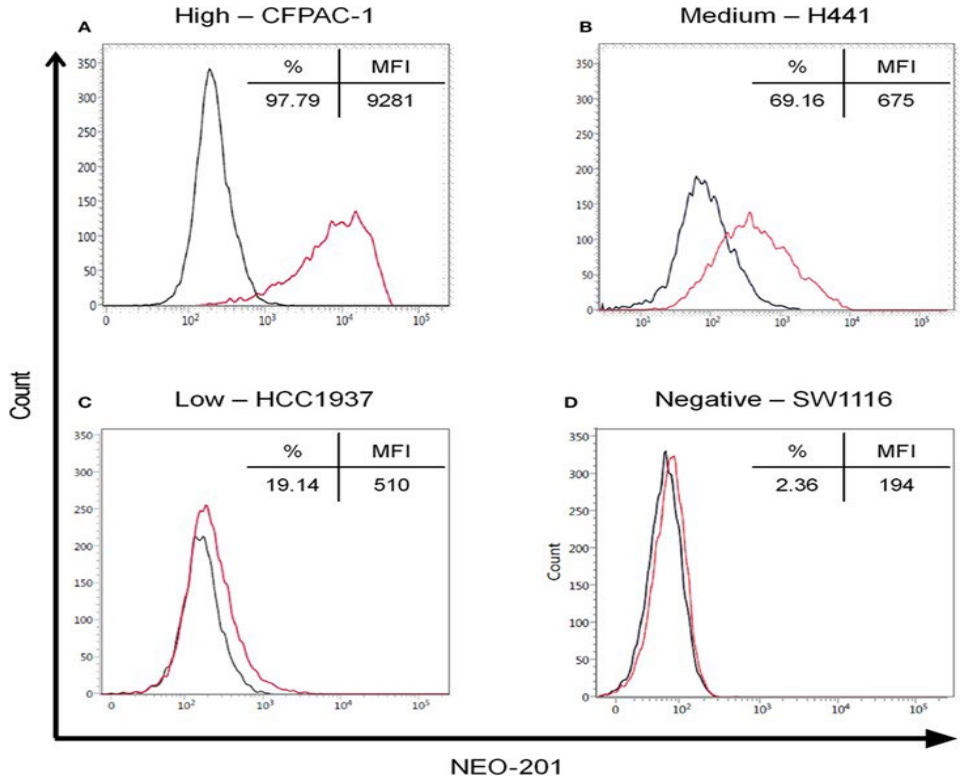
PRECISION BIOLOGICS

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%

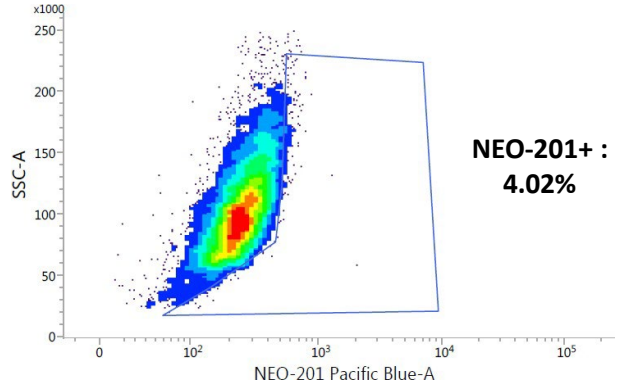


Positivity was determined using fluorescence minus one (FMO) controls. Positive cell lines were ranked according to their quantified expression level (% positive × MFI), and then sorted into groups of low (<200), medium (200-1000), and high (<1000) expression

Fantini et al. Frontiers in Immunology, 2018

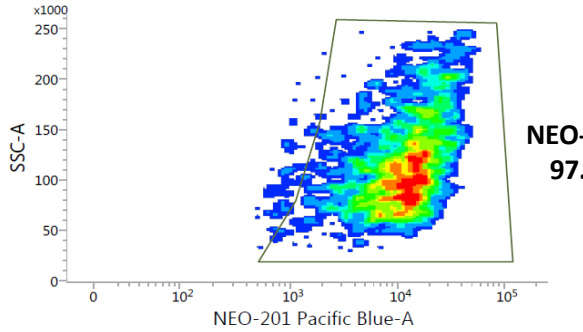
PRECISION BIOLOGICS

NEO-201 binds to truncated Core-1 O-glycans expressed by human solid and blood tumors and by neutrophils



NEO-201+ : 4.02%

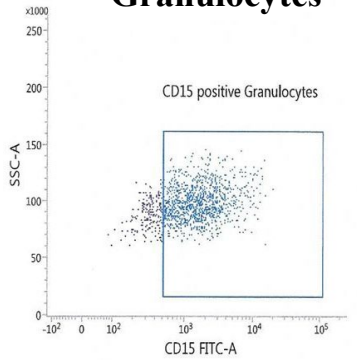
K562 (CML cell line): expresses only Core-2 O-glycan, thus is negative for NEO-201 binding



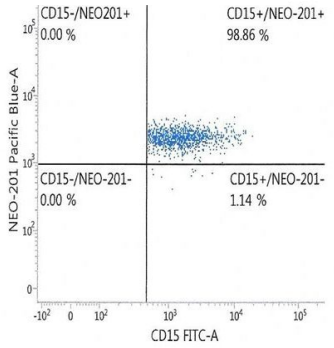
NEO-201+ : 97.20%

CFPAC-1 (Pancreatic cell line): expresses mainly Core-1 O-glycans

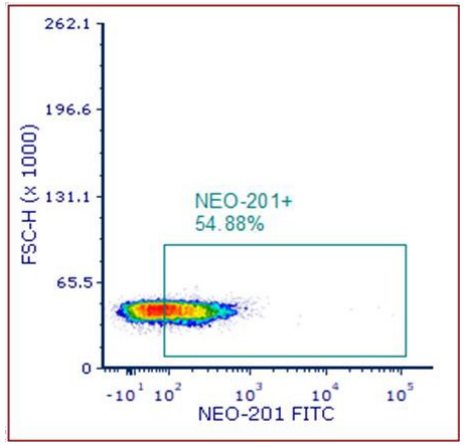
Granulocytes



$CD15^+$ granulocytes



CD15+ Granulocytes (Neutrophils): expresses Core-1 O-glycan

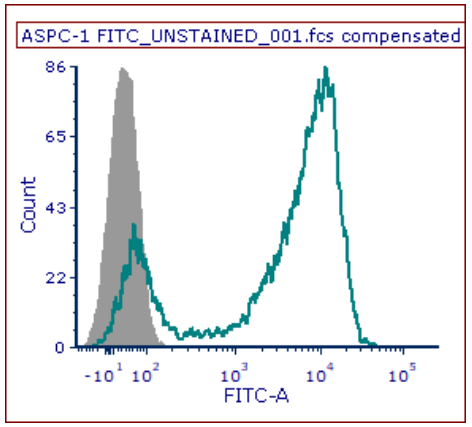


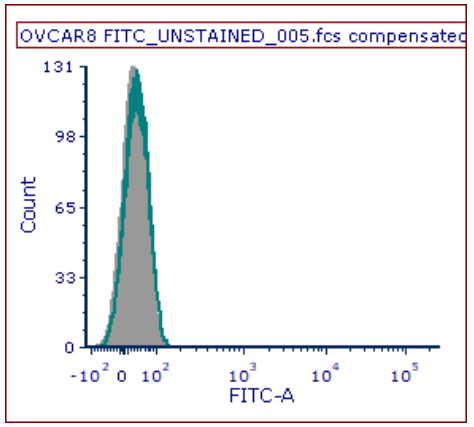
HL-60 (AML cell line): expresses mostly extended Core-1 O-glycans

PRECISION BIOLOGICS

Tumor cells negative for NEO-201 binding are not killed by NEO-201

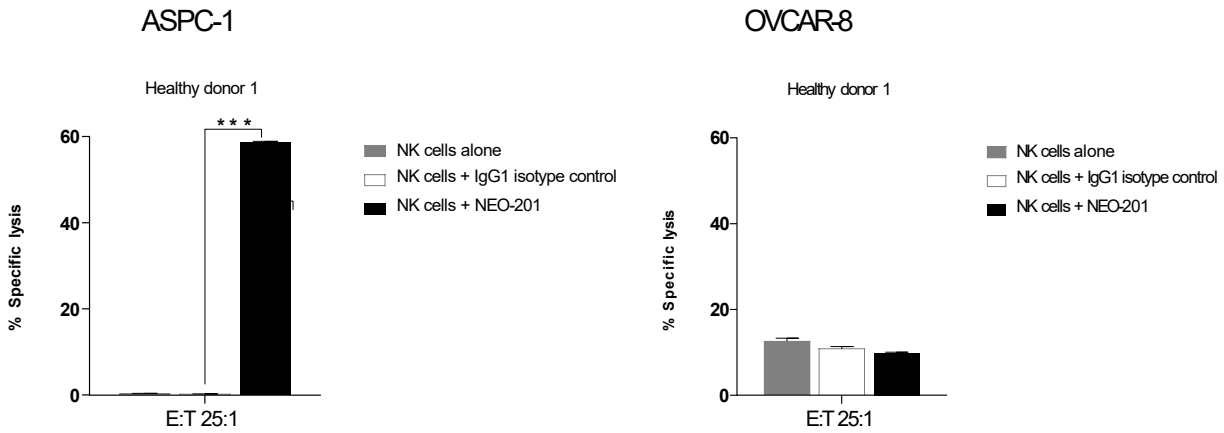
NEO-201 binding to tumor cells: flow cytometry analysis





	% NEO-201 POSITIVE	
	ASPC-1	OVCAR8
UNSTAINED	0	1
NEO-201 FITC	**81**	**1**

ADCC killing assay

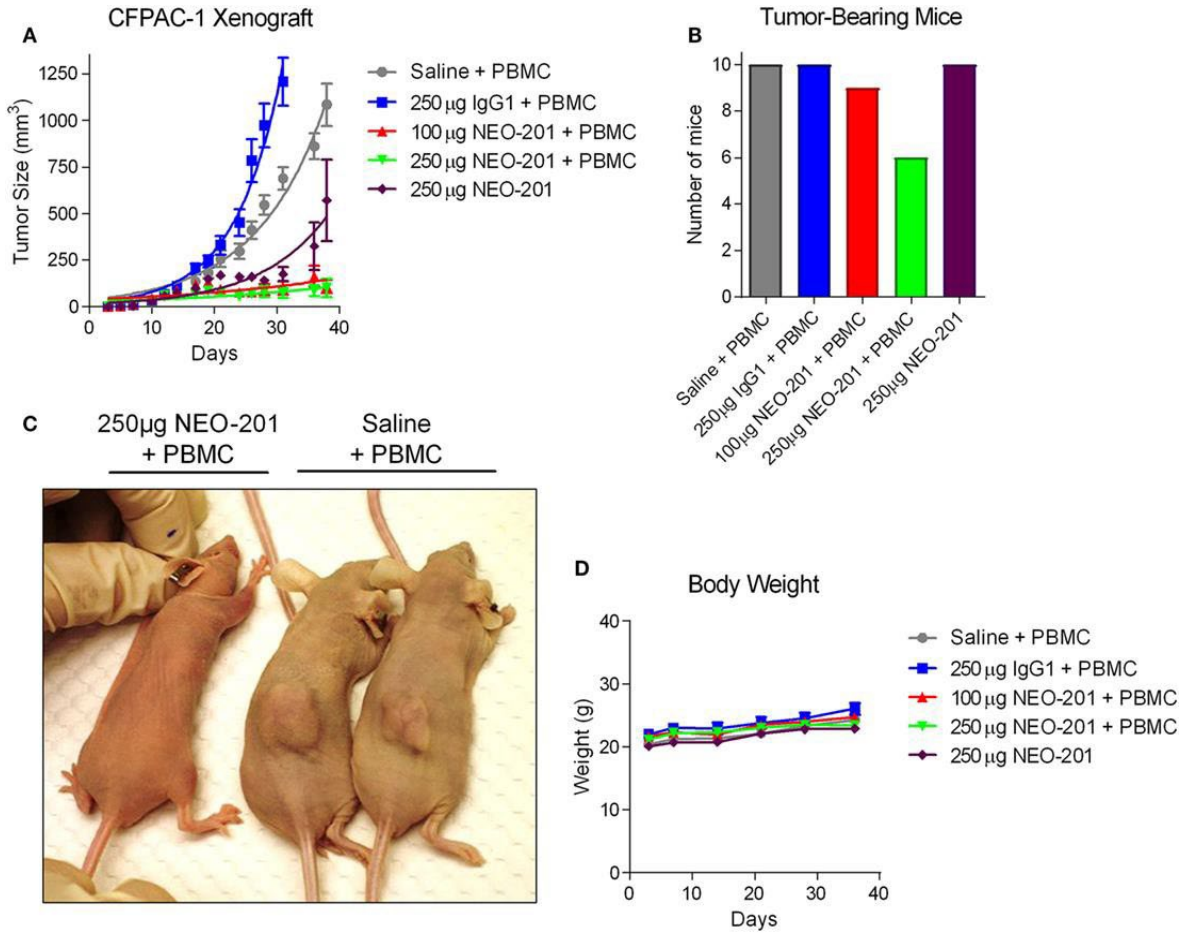


ADCC activity using ASPC-1 (pancreatic carcinoma) or OVCAR-8 (ovarian carcinoma) cells as target cells. Cells were treated with 10µg/mL of NEO- 201 or human IgG1 (negative control). Purified NK cells from one healthy donors were used as effector cells at an E:T ratio of 25:1.

***statistically significant ($p < 0.001$) by T-test.

PRECISION BIOLOGICS

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.

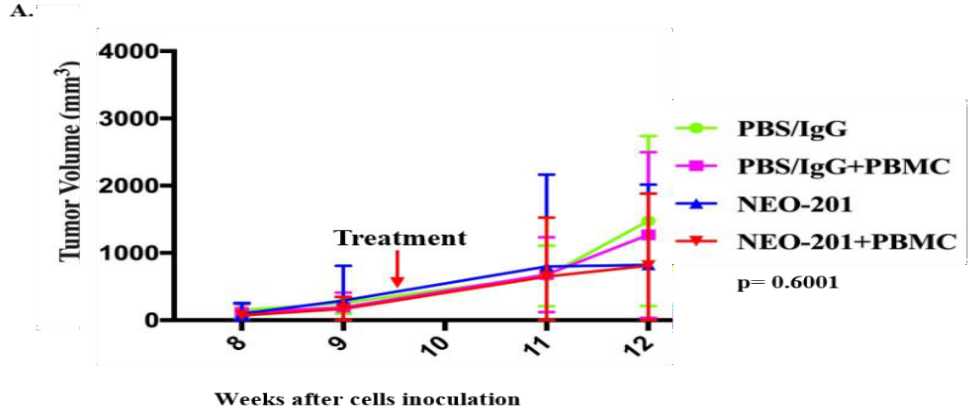
Once tumors reached ~100 mm^3 in size, mice were then injected intraperitoneally with:

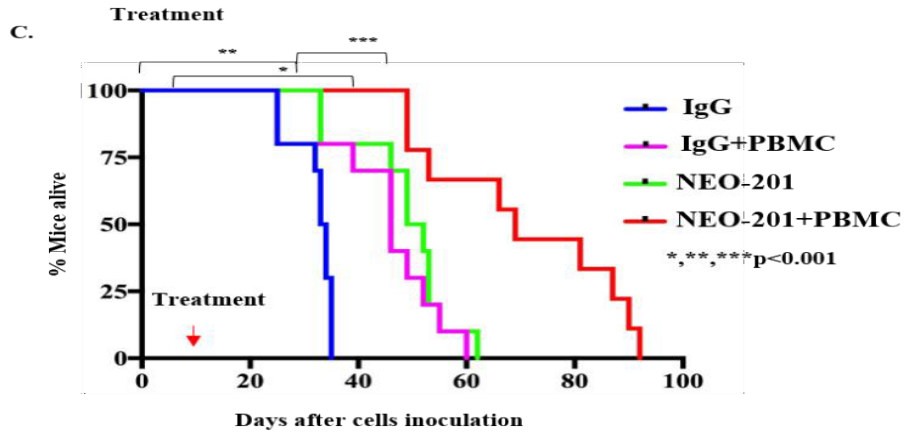
1. vehicle alone (saline solution) + PBMCs
2. human IgG1 (250 µg) + PBMCs
3. NEO-201 (100 and 250 µg) + PBMCs
4. NEO-201 (250 µg) alone

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

Antitumor efficacy of NEO-201 in ovarian cancer orthotopic tumor model





Tumors were established in 6-week old female athymic NU/NU nude mice by implanting OV-90 tumor cells into the right ovarian bursa (A) or into peritoneal cavity to mimic disseminated ovarian cancer (C).

Once tumors reached ~100 mm³ in size, mice were then injected intraperitoneally with:

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

NEO-201 + PBMCs induced a substantial reduction in tumor growth and prolonged the survival of treated mice compared to the human IgG + PBMCs control group.

Zeligs et al. Frontiers in Oncology, 2020

Phase 1 Dose Escalation of NEO-201 Alone: Completed

Gender	Male: 6 (35%)	Female: 11 (65%)			
Age (years)	30-50: 5 (29%)	51-60: 4 (24%)	61-70: 5 (29%)	71-80: 2 (12%)	> 80: 1 (6%)
Cancer Type	Colorectal: 11 (65%)	Pancreatic: 4 (24%)	Breast: 2 (12%)		
Race	White: 15 (88%)	African American: 2 (12%)			
Ethnicity	Non-Hispanic: 17 (100%)				
Performance Status	ECOG 0: 6 (35%)	ECOG 1: 9 (53%)	ECOG 2: 2 (12%)		

Seventeen (17) patients with advanced solid tumors were enrolled on a first-in-human, dose escalation clinical study of NEO-201 with 3 dose levels ranging from 1 mg/kg to 2 mg/kg

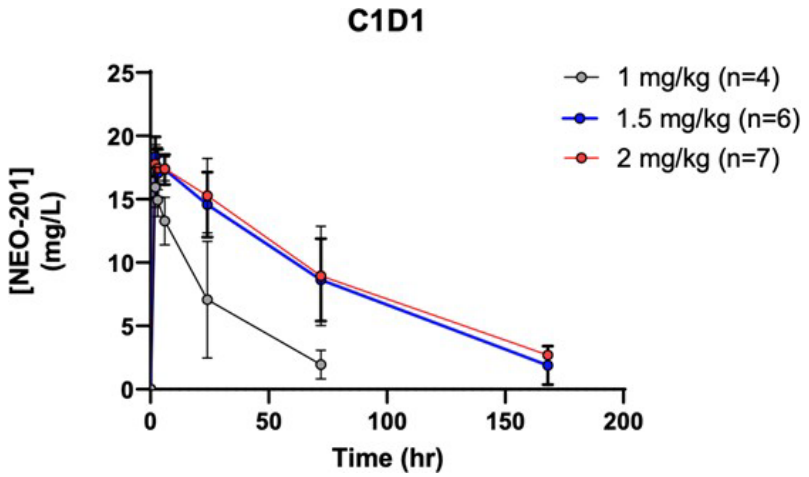
4 received NEO-201 at dose level 1 (1 mg/kg)
7 received NEO-201 at dose level 2 (2 mg/kg)
6 received NEO-201 at dose level 1.5 (1.5 mg/kg)

Cole et al. JECCR, 2023

Drug was administered via IV infusion biweekly.
The primary objective was to assess the safety via dose limiting toxicities (DLTs), determine the maximum tolerated dose (MTD) and the recommended phase 2 dose (RP2D). Based on the toxicity data and the results of interim PK analysis, **the MTD and RP2D was determined to be 1.5mg/kg.**

Phase 1 dose escalation of NEO-201: PK analysis

Cole et al. JECCR, 2023

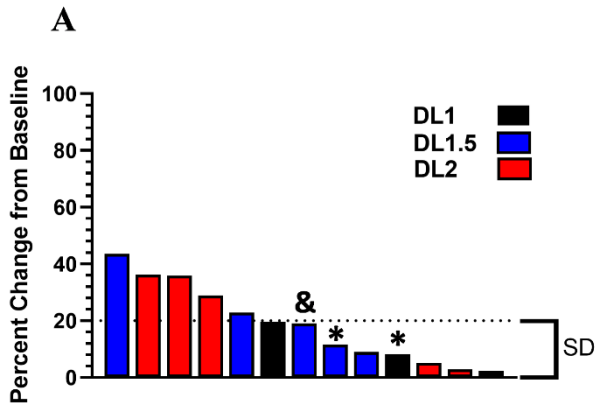


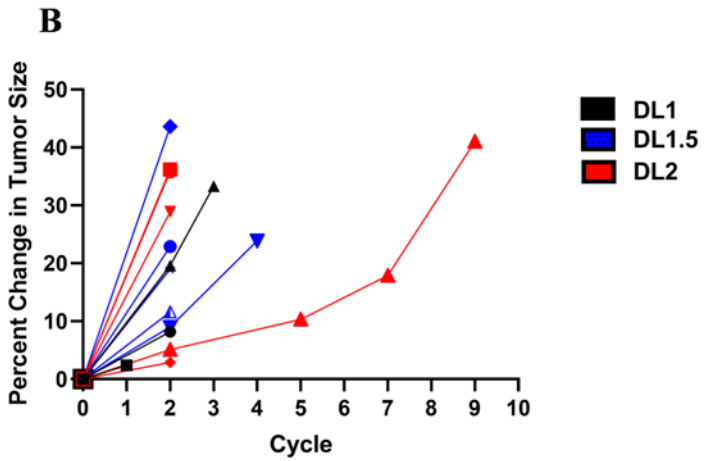
First Dose (C1D1)	n	C_{MAX} (mg/L)	AUC_{LAST} (hr*mg/L)	$T_{1/2}$ (hr)	CL/F (mL/hr)	Vz/F (L)
1.0 mg/kg Q2W	4	16.0 (9.7%)	444 (47%)	19.0 (61%)	0.200 (91%)[1]	3.54 (28%)[1]
2.0 mg/kg Q2W	7	18.2 (5.5%)	998 (42%)	80.0 (115%)[2]	0.100 (11%)[2,3]	5.97 (40%)[2,3]
1.5 mg/kg Q2W	6	18.0 (11%)	1217 (39%)	46.2(46%)[4]	0.070 (33%)[4,5]	4.23 (19%)[4,5]

- **PK analysis showed no difference between 1.5 mg/kg and 2 mg/kg cohorts**
- **1.5 mg/kg and 2 mg/kg cohort: it is very likely that at 2mg/kg, NEO-201 it could be cleared between C1D7 and C1D9.**

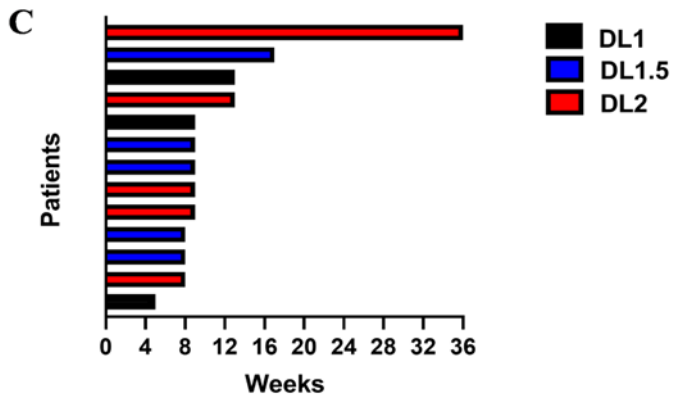
  Transient neutropenia in all patients after 72h from infusion
- **Patients treated with G-CSF starting from C1D5 or C1D6 from infusion**

Lesson Learned from Phase 1 Clinical Trial





[*Cole et al. JECCR, 2023*]



A. **Waterfall plot** indicating best response and best percent change in tumor size for all patients eligible for response evaluation (n=13). Dose level (DL) is indicated as in the legend and the dotted line at 20% indicates the threshold for stable disease (SD) according to RECIST v1.1 criteria.

B. **Spider plot** of percent change in tumor size across all cycles. Each cycle is 28 days in length (4 weeks). Among patients with SD, three patients were restaged after cycle 2. One patient was restaged at the end of cycle 9 (36 weeks of treatment)

C. Swimmer plot of time on study by dose level for all evaluable patients. All 4 patients with SD after at least 4 doses of NEO-201 (8 weeks of treatment) elected to continue receiving therapy.

NEO-201 recognizes naïve Tregs in PBMCs from cancer patients (Phase 2 clinical trial)

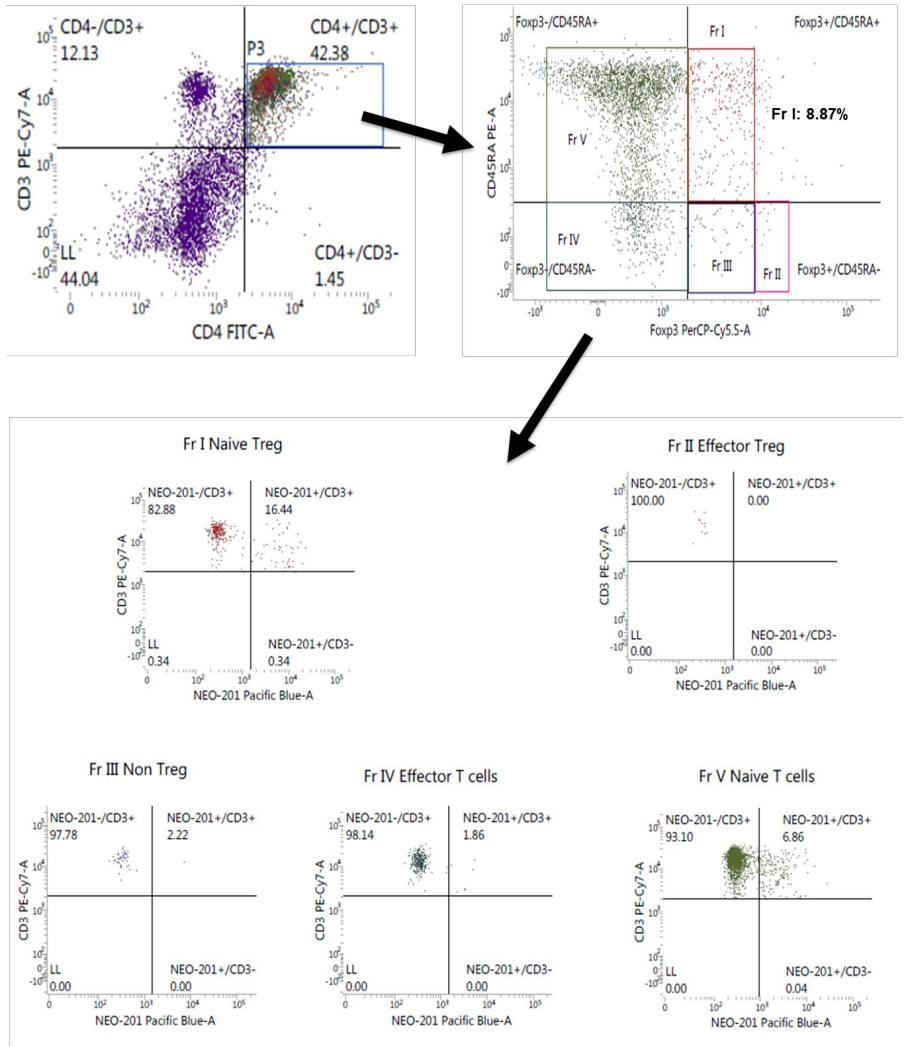


Figure depicts the binding of NEO-201 to naïve Tregs (Pat 1 C1D1 PRE)

Left plot: percentage of CD4+/CD3+ cells in whole viable PBMCs.

Right plot: fractions of CD4+/CD3+ cells based on the CD45RA and Foxp3 status.

Central bottom plots : percentage of NEO-201+/CD3+ cells in each fraction.

Data are presented as percentage of viable cells expressing Treg cell-surface markers. Positivity was determined by using fluorescence-minus-one controls. Analysis was performed using BD FACSuite software.

NEO-201 recognizes gMDSCs in PBMCs from cancer patients (Phase 2 clinical trial)

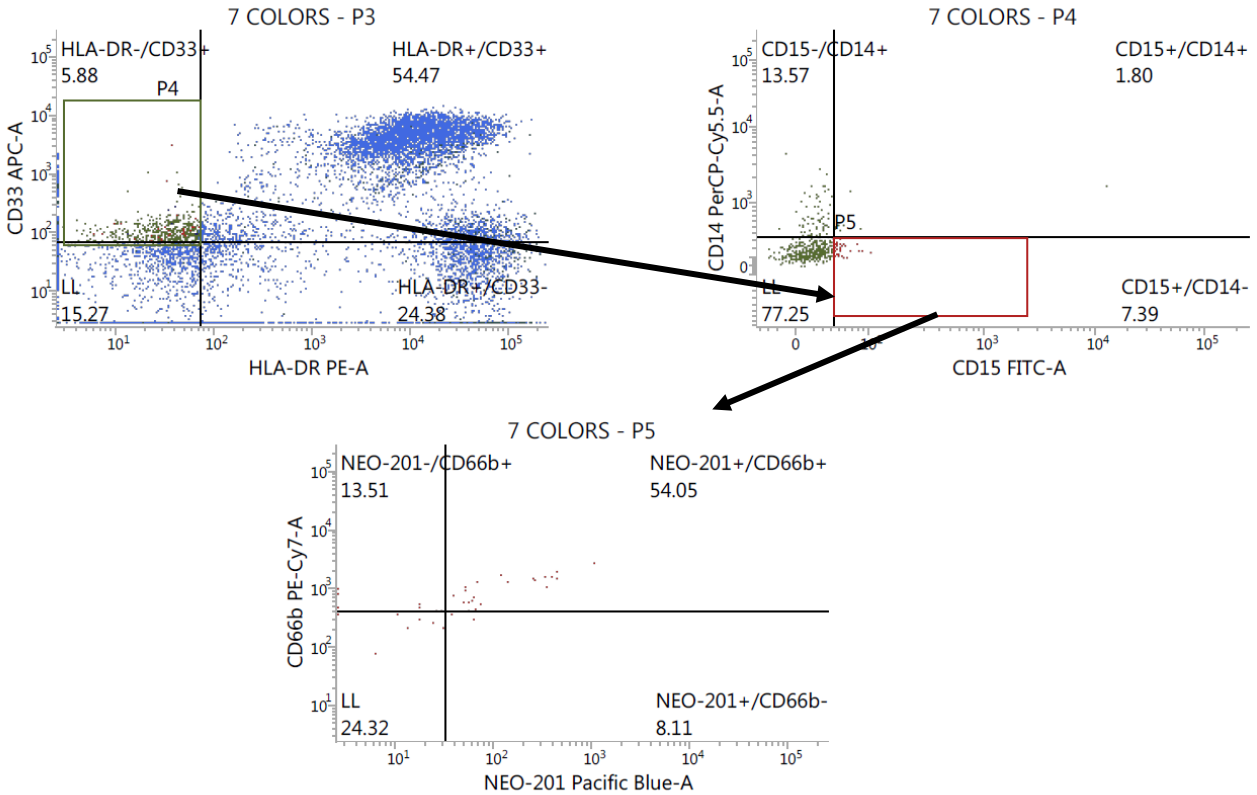


Figure depicts the binding of NEO-201 to gMDSCs (Pat 7 C1D1 PRE)

Left plot: percentage of CD33+/HLA-DRneg cells in whole viable PBMCs.

Right plot: percentage of CD15+/CD14-cells from CD33+/HLA-DRneg cells

bottom plot : percentage of NEO-201+/CD66+ cells from CD33+/HLA-DRneg/CD15+/CD14- cells

Data are presented as percentage of viable cells expressing gMDSCs-surface markers. Positivity was determined by using fluorescence-minus-one controls. Analysis was performed using BD FACSuite software.