Post-treatment neutrophil-to-lymphocyte ratio and gMDSCs as Independent prognostic factors for treatment efficacy with monoclonal antibody NEO-201 and pembrolizumab

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Introduction

NEO-201 is a humanized IgG1 monoclonal antibody (mAb) reactive against multiple human cancers but not against most normal epithelial tissues. NEO-201 can mediate antitumor activity through multiple mechanisms such as antibody-dependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), and blockade of the CEACAM5/CEACAM1 immune checkpoint inhibitory pathway.

NEO-201 binds core 1 O-glycans and showed antibody-dependent cell-mediated cytotoxicity (ADCC) activity against cancer cells expressing core 1 O-glycans [1]. NEO-201 kills cancer cells, neutrophils, and immune suppressor cells (iSCs), including regulatory T cells (Tregs) and granulocytic myeloid-derived suppressor cells (gMDSCs) via ADCC and complement-dependent cytotoxicity [2]. MDSCs have a significant role in dampening the host's immune responses through various ways, including generating arginase 1, releasing reactive oxygen species [3]. Resistance to PD-1/PDL1 blockade may be due to accumulation of iSCs in the tumor microenvironment [3]. Elevated neutrophil-to-lymphocyte ratio(NLR) correlates with poor prognosis [4]. We evaluated post-treatment NLR and depletion of gMDSCs as prognostic markers in patients treated with NEO201 and pembrolizumab.

Methods

PBMCs and serum from cancer patients on the Phase II trial combining NEO-201 with Pembrolizumab(NCT03476681) were used to evaluate the percentage of circulating gMDSCs (flow cytometry) and arginase-1 levels(ELISA). Patients with chemo-resistant solid tumors, who were resistant to prior checkpoint inhibitor therapy, received NEO-201 1.5mg/kg every 2 weeks with pembrolizumab 400mg IV every 6 weeks (1 cycle), and were imaged for response every 2 cycles. gMDSCs percentage in PBMCs and Arginase-1 levels in serum were analyzed before treatment (C1D1), 14 days after first infusion with NEO-201 (C1D15), before cycle 2 (C2D1), and before of cycle 3(C3D1). gMDSC population was defined as HLA-DR neg/CD33+/CD15+/ CD14neg/CD66b+.

1. Schema of Treatment: Expansion Cohorts

Given that NEO-201 has not been previously administered with pembrolizumab, a safety lead-in has been conducted in three subjects who received NEO-201 at 1.5 mg/kg IV every 2 weeks, and pembrolizumab 400 mg IV every 6 weeks. The safety lead-in course was 42 days in length and consisted of 1 dose of pembrolizumab and 3 doses of NEO-201.

Safety lead-in successfully completed. Expansion cohorts currently enrolling

The sample size for the expansion cohorts are calculated based on the desirable ORR (Simon minimax two-stage phase II design) for each cohort (Table 1).

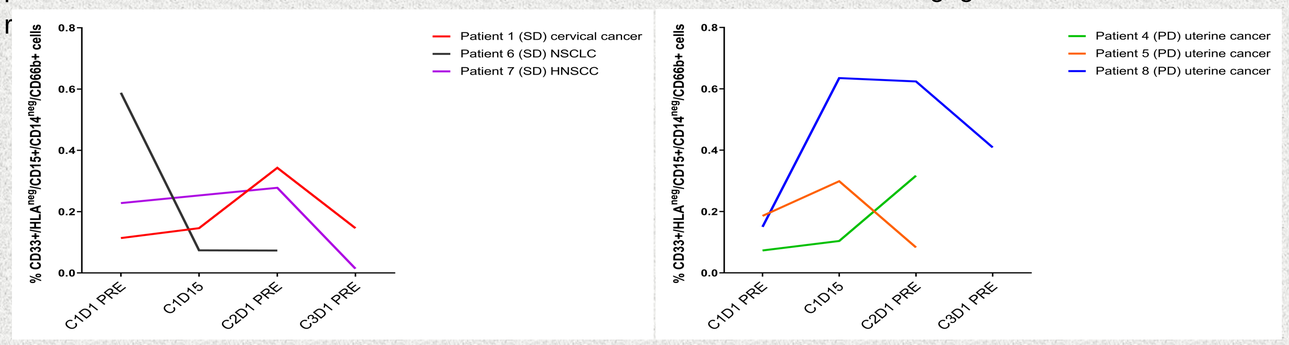
Table 1: Enrollment to two-stage design per expansion cohorts

Disease Cohort	Initial Stage	Acceptable Response Rate % (N/Total)	Second Stage	Sufficiently interesting Response Rate
NSCLC	12	20% (≥1/12)	21	14.3% (≥3/21)
HNSCC	16	25% (≥2/16)	31	19.4% (≥6/31)
Endometrial	16	25% (≥2/16)	31	19.4% (≥6/31)
Cervical	13	25% (≥1/13)	20	15% (≥3/20)

Results

2. Reduction of Quantity of Circulating gMDSCs post Treatment is Associated with Stabilization of the Disease (SD)

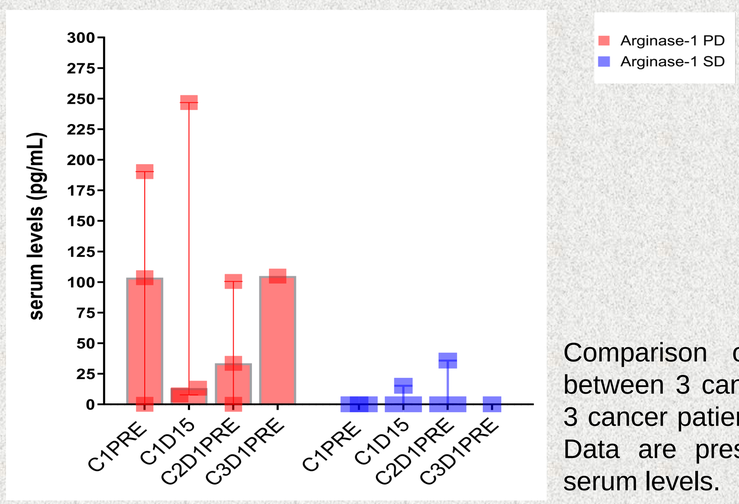
Based on the observation that NEO-201 recognizes and kills gMDSCs via ADCC [2], we compared the percentage of gMDSCs population from PBMCs (HLA-DR^{neg}/CD33^{+}/CD15^{+}/CD14^{neg}/CD66b^{+} cells) of three cancer patients with stable disease (SD) and three cancer patients with progressive disease (PD) pre and post treatment. Then, we correlated the modulation of levels of circulating gMDSCs with the clinical r



Comparison of the percentage of circulating gMDSCs (HLA-DR^{neg}/CD33^{+}/CD15^{+}/CD14^{neg}/CD66b^{+} cells) between 3 cancer patients with stable (SD) and 3 cancer patients with progressive disease (PD) at different time points by flow cytometry analysis. gMDSCs were gated from alive PBMCs.
Data are presented as median of percentage of viable cells expressing gMDSCs markers

Cancer patients with SD: Patient 7 (SD > 11 months) showed reduced gMDSCs (93.86%) at C3D1 compared to baseline (0.014% vs 0.228%). Patient 6 showed a marked reduction of gMDSCs (87.42%) at C1D15 compared to C1D1 PRE (0.074% vs 0.588%). Patient 1 (SD > 8 months) showed initial increase of gMDSCs at C1D15 and C2D1 compared to C1D1 but trended down at C3D1 (0.146% vs 0.114%).
Cancer patients with PD: general uptrend of circulating gMDSCs in patients with PD.

3. Cancer Patients with SD Showed a Decrease of Median Arginase-1 Levels Compared to Cancer Patients with PD



Comparison of serum levels of arginase-1 between 3 cancer patients with stable (SD) and 3 cancer patients with progressive disease (PD). Data are presented as median of arginase-1 serum levels.

4. Cancer Patients with PD had Higher Neutrophil-to-Lymphocyte ratio (NLR) Compared to Cancer Patients with SD

Patient#	Visit Date	Visit#	ALC	ANC	NLR
1	2021/12/06	Screen	2.88	2.02	0.70
Cervical (SD)	2022/01/18	C2D1	2.31	8.61	3.72
	2022/04/12	C3D1	1.20	4.58	3.81
4	2022/07/11	Screen	0.72	5.70	7.91
Endometrial (PD)	2022/07/26	C1D15	0.41	1.65	4.02
	2022/08/23	C2D1	0.77	19.37	**25.15**
5	2022/08/22	Screen	1.12	3.67	3.27
Endometrial (PD)	2022/09/13	C1D15	1.15	2.08	1.80
	2022/10/12	C2D1	1.13	4.39	3.88
6	2022/08/31	Screen	0.37	3.01	8.13
NSCLC (SD)	2022/09/21	C1D15	0.32	3.31	**10.34**
	2022/11/02	C2D15	0.33	2.99	**9.06**
7	2022/10/04	Screen	0.99	3.85	3.88
HNSCC (SD)	2022/10/19	C1D15	0.89	4.33	4.86
	2022/11/16	C2D1	0.72	3.32	4.61
8	2022/10/11	Screen	1.39	5.69	4.09
Endometrial (PD)	2022/10/25	C1D15	0.93	9.60	**10.32**
	2022/11/22	C2D1	1.02	7.78	7.62
	2023/01/03	EOT	1.01	10.56	**10.45**

Absolute Neutrophil Count (ANC)/Absolute Lymphocyte Count (ALC) [NNR].
Correlation between NLR and physiological stress levels:
1-3 – normal
6-8 – mild
9-18 – moderate,
and >18 – severe.

Progressive disease (PD)
Stable disease (SD)

5. Characteristics of Cancer Patients with 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.

Conclusion

We compared cancer patients with SD and PD at first radiological assessment (C3D1 PRE). Among patients with SD we observed a general downtrend of circulating gMDSCs after treatment. On the other hand, there was a general uptrend of circulating gMDSCs in patients with PD. Additionally, patients with PD had NLR >10 at C2D1, suggesting moderate to severe physiological stress compared to patients with SD who had NLR level <10.

Recent studies highlight the host's inflammatory response in tumor development and progression of various cancers. In our study, patients with SD had a downtrend of circulating gMDSCs, arginase-1 levels and normal to mild NLR compared to patients with PD, suggestive of good prognosis for treatment with NEO-201 and pembrolizumab. Ongoing enrollment in this clinical trial will validate these findings in larger cohorts.

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