The anti-core 1 O-glycans targeting monoclonal antibody NEO-201 recognizes and reduces the quantity of naïve regulatory T cells in PBMCs of cancer patients

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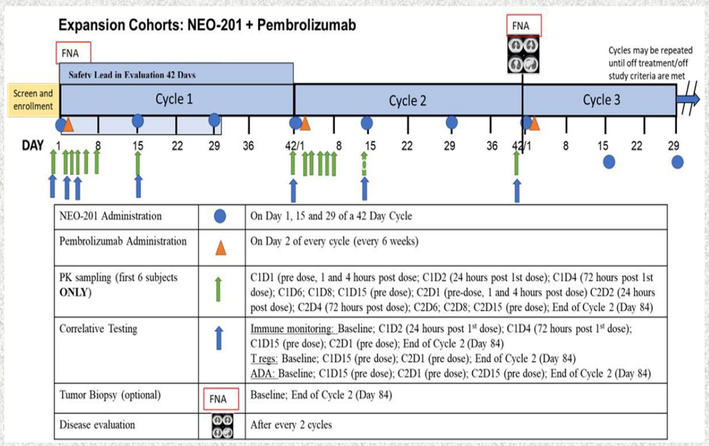
Introduction

NEO-201 is a humanized IgG1 monoclonal antibody which binds to Core 1 and/or extended Core 1 O-glycans expressed by several human solid and blood tumors, as well as neutrophils, but it does not bind to most normal tissues and human immune cell subsets (B cells, CD4+ T cells, CD8+ T cells, NK cells, monocytes). NEO-201 mediates killing of its target cells via ADCC and CDC. In Phase I clinical trial we observed that NEO-201 also binds to circulating regulatory T cells (cTregs) and causes a reduction of the quantity of cTregs in cancer patients with stable disease (SD). One of the reasons of the low response rates and resistance to PD-1/PD-L1 blockade in solid cancers may be due to the activity of Tregs in the tumor microenvironment (TME). The reduction in the percentage of cTregs following NEO-201 treatment supported the rationale of the ongoing phase II clinical trial (Clinical Trial NCT03476681) evaluating the efficiency of the combination of NEO-201 with pembrolizumab in adults with checkpoint inhibitors treatment-resistant solid tumors.

Experimental Design

Subjects with recurrent, locally advanced unresectable or metastatic Non-Small Cell Lung Cancer (NSCLC), Cervical Cancer, Head and Neck Squamous Cell Carcinoma (HNSCC), Uterine Carcinoma who have progressed during or after front-line standard of care treatment and have tumors resistant to checkpoint inhibitors are being enrolled. To evaluate which subset of Tregs is recognized by NEO-201, PBMCs from 5 cancer patients, enrolled in the ongoing phase II clinical trial, were profiled by flow cytometry for expression of specific Treg markers, including CD3, CD4, CD45RA, Foxp3 and NEO-201. The percentage of Tregs in PBMCs was analyzed before starting the treatment (C1D1 PRE) and at multiple time points after the first infusion with NEO-201, including C1D15 (14 days of the first infusion), C2D1 PRE (beginning of cycle 2, 42 days after first infusion), C3D1 PRE (beginning of cycle 3, 84 days after first infusion). The modulation of percentage of circulating Tregs after treatment with NEO-201+ pembrolizumab was correlated with clinical response.

1. Schema of Treatment: Safety Lead-In Course



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

2. Schema of Treatment: Expansion Cohorts

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)

Table 2: Collection of PBMCs for Tregs Analysis During Expansion Cohorts

Test/assay	Collection point
Tregs	Baseline (C1D1 PRE) C1D15 (14 days of the first infusion with NEO-201) C2D1 PRE (beginning of cycle 2, 42 days after first infusion with NEO-201) C3D1 PRE (beginning of cycle 3, 84 days after first infusion with NEO-201)

Results

3. NEO-201 recognizes naïve Tregs

Flow Cytometry

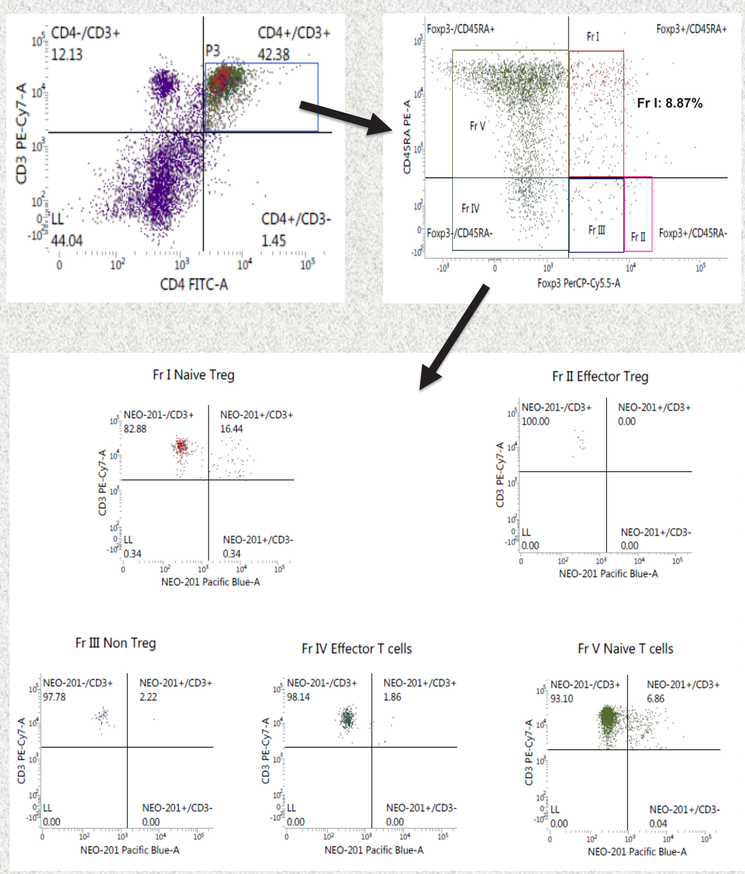


Figure depicts the binding of NEO-201 to naïve Tregs (Pat 1 C1D1 PRE)

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 plot : 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.

4. Reduction of quantity of naïve Tregs post treatment is associated with stabilization of the disease

Flow cytometry analysis of PBMCs revealed that NEO-201 recognizes naïve Tregs (Fraction I: $CD3^+/CD4^+/CD45RA^+/Foxp3^{low}$ cells).

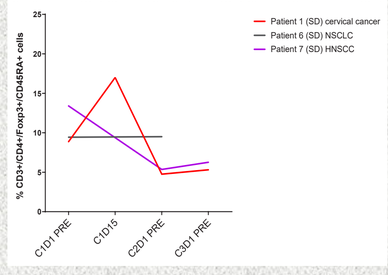
Based on this observation, we compared the percentage of naïve Tregs population (fraction I) from PBMCs in three cancer patients with stable disease (SD) and two cancer patients with progressive disease (PD) pre and post treatment, and we correlated the modulation of levels of circulating naïve Tregs with the clinical response.

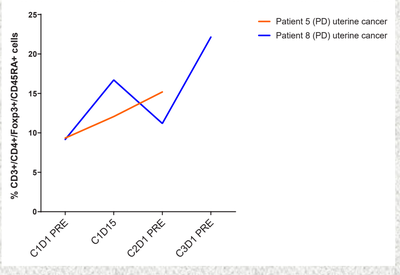
PATIENTS WITH SD

general downtrend of naïve Tregs after treatment

PATIENTS WITH PD

general uptrend of naïve Tregs after treatment





Patient 1, cervical cancer: reduction of 46.34% (4.76% vs 8.87%) and 40.14% (5.31% vs 8.87%) of naïve Tregs at C2D1 and C3D1 respectively, compared to baseline levels.

Patient 7, HNSCC: reduction of 60.08% (5.35% vs 13.40%) and 52.22% (6.26% vs 13.40%) of naïve Tregs at C2D1 and C3D1 respectively, compared to baseline levels.

Patient 6, NSCLC: No change in percentage of naïve Tregs has been detected at C2D1 compared to baseline levels (9.50% vs 9.44%).

In two patients with PD (both with uterine cancer), the percentage of naïve Tregs increased after the treatment compared to baseline levels. This phenomenon correlates with PD reported at the first re-staging (prior C3D1).

Patients with disease stabilization (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 10 months after first infusion with both antibodies
- **Patient is still receiving study treatment**

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

Following TCR stimulation, naïve Tregs proliferate and differentiate into highly suppressive effector Tregs. Infiltration of TME by effector Tregs has been correlated with poor prognosis and survival in various types of cancer. Partial decrease of circulating naïve Tregs after treatment with NEO-201 was associated with stabilization of disease in cancer patients analyzed in this study. These data suggest that depletion of circulating naïve Tregs may prevent the differentiation of naïve Tregs into effector Tregs and their accumulation in the TME. In this regard, the combination of NEO-201 with pembrolizumab can result in an enhancement of the efficacy of pembrolizumab in cancer patients with checkpoint inhibitors treatment-resistant solid tumors.