The O-glycan Epitope Targeting Anti-Human Carcinoma Monoclonal Antibody (mAb) NEO-201 can also target human regulatory T cells (Tregs)

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Abstract:
Introduction:NEO-201 is a humanized IgG1 mAb that targets 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). Functional analysis revealed that NEO-201 mediates the killing of its target cells through ADCC and CDC. Previous study has shown that approximately 4.6% of $CD4^+$ T cells express NEO-201 target antigen and that those $CD4^+$ T cells have Tregs phenotype. In addition, in a recent published phase 1 study we observed that NEO-201 binds to $CD4^+$/ $CD25^+$/, $CD127^-$/, $Foxp3^+$/, $CD15s^+$ cells from peripheral blood mononuclear cells (PBMCs) from cancer patients with solid tumors. The purpose of this study was to further characterize the phenotype of the specific subset of $CD4^+$ T cells expressing the NEO-201 target antigen.

Experimental Design: Human PBMCs were obtained from 7 healthy donors (HD) collected at Osaka University and 6 cancer patients from our ongoing phase II clinical trial (Clinical Trial NCT03476681) evaluating the efficiency of the combination of NEO-201 with pembrolizumab in adults with solid tumors resistant to checkpoint inhibitors. Phenotypic analysis was performed by flow cytometry using NEO-201 and antibodies CD3, CD4, CD25, CD45RA, FoxP3, and CD15s. The same gating strategy was applied for both normal donors and patients to evaluate which fraction of $CD4^+$ T cells is recognized by NEO-201. Fraction (Fr) 1 is naïve Treg, Fr ll is effector Treg, Fr lll is non-Treg, Fr lV is effector CD4 T cells and Fr V is naïve CD4 T cells.

Results: Flow cytometry analysis of PBMCs revealed that NEO-201 recognizes naïve Tregs (nTregs: $CD3^+/CD4^+/CD45RA^+/Foxp3^{low}$ cells) while it does not bind to effector Tregs (eTregs: $CD3^+/CD4^+/CD45RA^-/Foxp3^{high}$ cells) in both HD and cancer patients. The % of nTregs in cancer patients was higher than HD. Preliminary results from the ongoing Phase II clinical trial showed that subjects with durable SD had a reduction of circulating nTregs after treatment with NEO-201 compared to baseline levels.

Conclusions: NEO-201 binds to human Tregs with significantly higher % of binding to Fr l and a greater % of Tregs expressing NEO-201 target antigen in cancer patients compared to HD. It is conceivable that naïve Tregs in cancer patients express high levels of Core 1 O-glycans. Furthermore, when subjected to TCR stimulation, naïve Tregs undergo proliferation and differentiate into eTregs. eTregs can then infiltrate into tumor microenvironment (TME). These data suggest that depletion of circulating nTregs in cancer patients after NEO-201 treatment may prevent the differentiation of nTregs into eTregs and their accumulation in the TME. This study suggests the potential use of NEO-201 to reduce the Treg-mediated suppression of anticancer immunity.

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