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Reduction of circulating naïve Tregs and gMDSCs and low levels of soluble MICA are prognostic for efficacy of combined NEO-201 and pembrolizumab.

Background: The humanized IgG1 monoclonal antibody NEO-201 binds to Core 1 and/or extended Core 1 O-glycans expressed by several human solid and blood tumors, as well as neutrophils, and mediates killing of cancer cells, neutrophils, regulatory T cells (Tregs) and granulocytic myeloid-derived suppressor cells (gMDSCs) via ADCC and CDC. Resistance to PD-1/PD-L1 blockade may be due to the accumulation of Tregs and gMDSCs in the tumor microenvironment (TME). NEO-201 was proven to bind and reduce the amount of circulating Tregs in cancer patients. This supports the rationale of the ongoing phase II clinical trial (NCT03476681) evaluating the activity of NEO-201 with pembrolizumab in adults with solid tumors resistant to prior checkpoint inhibitors. Furthermore, elevated serum levels of soluble MICA (sMICA) have been correlated with decreased NK cell activity.

Methods: Cancer patients were treated each cycle with 3 doses of NEO-201 1.5mg/kg every 2 weeks and pembrolizumab 400mg IV every 6 weeks and imaged for response every 2 cycles. PBMCs and serum from cancer patients pre- and at multiple time points post-treatment were used to evaluate the percentage of circulating gMDSCs and Tregs (flow cytometry) and sMICA levels (ELISA).

Results: NEO-201 recognizes naïve Tregs (nTregs: $CD3^+/CD4^+/CD45RA^+/Foxp3^{low}$ cells) but not effector Tregs (eTregs: $CD3^+/CD4^+/CD45RA^-/Foxp3^{high}$ cells). NEO-201 also binds to gMDSCs, defined as $HLA^-DR^{neg}/CD33^+/CD15^+/ CD14^{neg}/CD66b^+$ cells. In patients with durable SD, a patient with HNSCC (SD >11 months) showed >50% reduction of nTregs and >90% of gMDSCs at C3D1 (beginning of cycle 3) compared to baseline. One patient with cervical cancer (SD >8 months) showed 40% reduction of nTregs at C3D1 compared to baseline, while gMDSCs trended down to baseline levels at C3D1 after initial increase. Conversely, we observed a general uptrend of nTregs and gMDSCs in patients with PD after treatment. Median serum levels of sMICA pre-treatment were 33-fold higher in patients with PD compared to patients with SD. Levels of sMICA remained elevated in patients with PD and low in patients with SD at all time points post-treatment.

Conclusions: Depletion of circulating Tregs and gMDSCs may prevent their accumulation in the TME and enhance the efficacy of pembrolizumab in subjects with tumors resistant to checkpoint inhibitors. The decrease in circulating nTregs and gMDSCs after treatment with NEO-201 and

pembrolizumab was associated with durable SD. High levels of sMICA can impair ADCC mediated by NEO-201, resulting in poor clinical response. Low levels of sMICA in patients with SD, together with the reduction of both circulating nTregs and gMDSCs, could be favorable prognostic markers for clinical benefit following treatment with NEO-201 and pembrolizumab. Ongoing enrollment in this clinical trial will validate these findings in larger cohorts.