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PRESENTATIONS: TUESDAY, APRIL 18

CLINICAL RESEARCH EXCLUDING TRIALS

Therapeutic Antibodies, Including Engineered Antibodies

#5654

A therapeutic humanized anti-carcinoma monoclonal antibody (mAb) NEO-201 can also target human granulocytic myeloid-derived suppressor cells (gMDSCs) and regulatory T (Tregs) cells

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Background**:** NEO-201 is a humanized IgG1 mAb reactive against multiple human cancers but not against most normal epithelial tissues. NEO-201 binds to core 1 or extended core 1 O-glycans expressed by its target cells, including neutrophils, various carcinomas, and some human hematological malignancies. NEO-201 can mediate antitumor activity through antibodydependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), and blockade of the CEACAM5/CEACAM1 ICI pathway. A previous study using flow cytometry demonstrated that NEO-201+/CD4+ T cells were also CD25+/CD127-/Foxp3+/CD15s+ using PBMCs from healthy donors (HD). NEO-201 can kill these Treg cells through CDC *in vitro*. NEO-201 does not bind to the majority of CD4+ T cells and to other immune subsets. Human gMDSCs are increased in cancer patients and are a population of immature MDSCs deriving from immature neutrophils and alternative activation of mature neutrophils. gMDSC are characterized by HLA-DR-, CD11b+, CD33+, CD15+phenotype.We have

shown that NEO-201 recognizes and kill human neutrophils through ADCC. This current investigation was designed to evaluate whether NEO-201 can target and mediate ADCC activity against human gMDSCs.

Methods: gMDSCs were generated from human neutrophils from 5 HD isolated using EasySepTM direct human neutrophil isolation kit. Isolated neutrophils were cultured in complete RPMI1640 medium supplemented with human GM-CSF and human IL-6 for 7 days. Phenotypic analysis by flow cytometry was performed on the generated gMDSCs using NEO-201 and mAbs against human CD33, HLA-DR, CD15, CD14, CD66b. Flow cytometry based ADCC assay was performed using gMDSCs stained with both CD33 and HLA-DR as target. PBMCs from a separate HD were used as effectors at different E:T ratios. The ADCC activity of NEO-201 was evaluated comparing the percentage of CD33+/HLA-DR- viable cells in gMDSCs incubated with medium alone to the percentage of CD33+/HLADR-viable cells incubated with PBMCs alone and with PBMCs plus NEO-201.

Results: Flow cytometry analysis revealed that gMDSCs can be generated from human neutrophils after 7 days of culture with GM-CSF and IL-6 and that they express the following phenotype: HLA-DR-/CD33+/CD15+/CD14-/CD66b+. NEO-201 bound to the majority of these gMDSCs. NEO-201 was functional in mediating ADCC to kill these gMDSCs.

Conclusion: This study demonstrated that NEO-201 can be used to identify and kill suppressive gMDSCs in addition to Treg cells. Depletion of suppressive Tregs and gMDSCs in the TME could be an effective strategy to prevent hyperprogressive disease when anti-PD-1 is used in cancer immunotherapy. These data support the rationale for the ongoing phase II clinical trial using NEO-201 in combination with pembrolizumab in checkpoint refractory patients with metastatic solid tumors.