

**IDENTIFICATION OF THE O-GLYCAN EPI TOPE TARGETED BY AN ANTI-HUMAN CARCINOMA MONOCLONAL ANTIBODY (MAB) NEO-201**

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**Background** NEO-201 is a humanized IgG1 mAb reactive against multiple human carcinomas, but not normal epithelial tissues. NEO-201 can mediate antitumor activity through multiple mechanisms such as antibody-dependent cellular cytotoxicity, complement dependent cytotoxicity, and blockade of the CEACAM5/CEACAM1 immune checkpoint inhibitory pathway. In addition to solid tumors, flow cytometry analysis has demonstrated that NEO-201 binds to 98.9% of CD15<sup>+</sup> granulocytes, human regulatory T cells as well as various human hematological neoplastic cell lines. However, NEO-201 does not bind to other immune subsets and to the majority of CD4<sup>+</sup> T cells. Furthermore, we have demonstrated that NEO-201 binds to mammalian expressed rhCEACAM6 but not bacterial expressed rhCEACAM6. These findings suggest that NEO-201 binds to glycans linked to specific proteins. Glycosylation is an important post-translation modification of protein and is affected by oncogenesis. Aberrant O-glycans may serve as potential targets to improve the monitoring and treatment of cancers. Based on this information, this study was designed to focus on the identification of the O-glycan binding epitope of NEO-201.

**Methods** An O-glycan array consisting of 94 O-glycans was used to identify the O-glycans targeted by NEO-201. O-glycan profiles were elucidated from human pancreatic cancer cell line (CFPAC-1), human hematological neoplastic cell lines (HL60, U937, K562) and human neutrophils. Different truncated C-terminus of CEACAM6 and CEACAM5 gene constructs were designed and the truncated CEACAM6 and CEACAM5 proteins were expressed in mammalian expression system to identify the NEO-201 binding region in CEACAM6 and CEACEAM5.

**Results** The O-glycan array analysis shows that NEO-201 interacts with O-glycans 01, 02 (Tn antigens), 06 (Core 1), 023 (Core 2), 026 (Core 3) and 039 (Core 4). The Core-1 binding interaction was the strongest of any observed. The O-glycan profiling studies demonstrated that CFPAC-1 and human neutrophils express mostly the Core 1 profile. HL-60 expresses mainly the extended Core 1 profile, U937 expresses mainly the extended Core 1 and Core 2 profiles and K562 expresses only the Core 2 profile. Flow cytometry analysis demonstrated that NEO-201 binds to CFPAC-1, human neutrophils, HL60 and U937 cells but not to K562. We also proved that in both CEACAM5 and CEACAM6 NEO-201 binds to regions containing threonine. GalNAc residue can be added onto threonine to form O-glycans.

**Conclusions** This study demonstrated that NEO-201 binds strongly to Core 1 and/or extended Core 1 O-glycans and confirms our finding that NEO-201 binds only mammalian expressed rhCEACAM6 express O-glycans.

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