Affinity Maturation and characterization of a Novel O-glycan Epitope Targeting Anti-Human Carcinoma Monoclonal Antibody (mAb) PB-223

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Introduction:

Tumor-targeting mAbs can be leveraged to stimulate innate anti-cancer immunity. NEO-102 (Ensituximab) is a chimeric human IgG1 mAb targets a glycosylated variant of MUC5AC with specificity to colorectal and pancreatic cancer. In a phase 2 study, NEO-102 pretreated patients with advanced, refractory colorectal cancer showed promising results (Kim R. et al. Clin Cancer Res 2020). Enhancing binding affinity stands as a prominent avenue within the realm of antibody engineering, offering the potential to significantly augment the therapeutic efficacy of antibodies. Thus, the objective of this investigation is to enhance the binding affinity of NEO-102.

Experimental Design:

This research entails the engineering to the VH and VL sequences of NEO-102 through Fast Screening for Expression Biophysical Properties and Affinity, with the aim to maintain the binding to target antigen while achieving a lower KD. To this end, we constructed a library for saturation mutagenesis into all residues in the VH and VL region of the antibody. Flow cytometry analysis was used to compare the binding affinity between the NEO-102 and the affinity maturation generated clone (PB-223) to various tumor cell lines. O-glycan microarray was utilized to identify the O-glycan binding epitope of the PB-223. IHC tumor tissue microarrays, and antibody internalization were performed on PB-223.

Results:

Clone PB-223 was selected through the affinity maturation process, The KD of NEO-102 was measured at 4.24-E09, while PB-223 exhibited a significantly improved KD of 1,23E-09. Flow cytometry analysis revealed that the PB-223 displayed markedly enhanced binding to multiple tumor cell lines and IHC analysis indicate that PB-223 demonstrated the ability to bind to a broader range of tumor types compare to NEO-102 but not to normal tissues. PB-223 binds strongly to truncated Core-2 O-glycan. PB-223 can be internalized in the PB-223 antigen positive cell line.

Conclusion:

We have successfully generated a high affinity clone PB-223 which comprises a KD at least 4-fold less than NEO-102. Flow cytometry and IHC analysis suggest that PB-223 can bind to a wider spectrum of tumor types than NEO-102 but not to normal tissues. Furthermore, we have identified the Core-2 O-glycan as the binding epitope of PB223 and PB-223 can be internalized in the PB-223 antigen positive cell line. This finding position PB223 as a potential candidate for the development of antibody-drug conjugates (ADC) for treatment of various human carcinomas.