

## IMMUNOTHERAPEUTIC POTENTIAL OF THE HUMANIZED ANTI-MUC16 ANTIBODY AR9.6 AGAINST PDAC

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**Background** Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease with poor overall survival, owing partly to its late-stage presentation and resistance to standard of care.<sup>1</sup> Mucins are largely glycosylated proteins that have been found to be aberrantly expressed in multiple adenocarcinomas, including PDAC.<sup>2</sup> Mucin-16 (MUC16/CA125) is one such heavily glycosylated member that is overexpressed in >65% of PDACs and has been shown to correlate to poor prognosis.<sup>3</sup> This study investigates the tumor mitigating mechanisms and thereby, therapeutic potential of the humanized anti-MUC16 monoclonal antibody (huAR9.6) in models of PDAC. **Methods** The enzyme-linked immunosorbent assay (ELISA) was performed using recombinantly produced MUC16 epitopes to assess the binding affinity of huAR9.6, as described previously.<sup>4</sup> The wound healing assay using isogenic PDAC cell lines treated with huAR9.6, isotype control huIgG and vehicle was performed to assess the effect on cell migration. To determine the *in vivo* therapeutic potency, PDAC cells were orthotopically implanted into the pancreas of athymic nude mice which were then randomized to huAR9.6, isotype control huIgG, and vehicle treatment groups. Furthermore, *in vitro* functional assays to assess the antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) capacity of huAR9.6 were performed using PDAC cell lines.

Results huAR9.6 showed a strong binding affinity to the tandem repeat region of the SEA domain 5 of MUC16. Treatment of PDAC cells with huAR9.6 reduced their migratory potential, indicating its anti-tumorigenic role. Mice treated with huAR9.6 showed a significant decrease in tumor weight and volume as compared to the huIgG and vehicle treated groups. The proliferative marker Ki67 was also significantly lowered in the pancreatic tumors of huAR9.6 treated mice, as compared to controls. Further, *in vitro* ADCC assays using freshly isolated human peripheral blood mononuclear cells (PBMCs) as effector cells showed an increase in cell death of PDAC cells treated with huAR9.6, as compared to cells treated with huIgG control. CDC assays using human serum also showed increased cytotoxicity of PDAC cells in the presence of the huAR9.6 antibody as compared to controls.

**Conclusions** The results strongly suggest that huAR9.6 binds to MUC16 and limits PDAC growth via cytotoxicity mechanisms including ADCC and CDC. This validates the therapeutic potential of huAR9.6 in models of PDAC and adds value to the previously established diagnostic ability of this antibody [5]. These studies are conducted with the overarching goal of facilitating the translation of huAR9.6 to treat PDAC in the clinic.

### REFERENCES

1. A. N. Hosein, S. K. Dougan, A. J. Aguirre, and A. Maitra, Translational advances in pancreatic ductal adenocarcinoma therapy, *Nature Cancer* 2022; 3:3, no. 3, pp. 272–286, Mar. 2022, doi: 10.1038/s43018-022-00349-2.
2. M. A. Hollingsworth and B. J. Swanson, Mucins in cancer: protection and control of the cell surface, *Nature Reviews Cancer* 2004; 4:1, vol. 4, no. 1, pp. 45–60, 2004, doi: 10.1038/nrc1251.
3. D. Thomas *et al.* Isoforms of MUC16 activate oncogenic signaling through EGF receptors to enhance the progression of pancreatic cancer, *Molecular Therapy*, vol. 29, no. 4, pp. 1557–1571, Apr. 2021, doi: 10.1016/j.ymt.2020.12.029/ATTACHMENT/632DD631-FCE4-4079-AC9E-940B42278BB0/MMC1.PDF.

4. B. White, M. Patterson, S. Karnwal, and C. L. Brooks, 'Crystal structure of a human MUC16 SEA domain reveals insight into the nature of the CA125 tumor marker,' *Proteins: Structure, Function, and Bioinformatics*, vol. 90, no. 5, pp. 1210–1218, May 2022, doi: 10.1002/PROT.26303.
5. S. K. Sharma *et al.*, ImmunoPET of Ovarian and Pancreatic Cancer with AR9.6, a Novel MUC16-Targeted Therapeutic Antibody, *Clinical Cancer Research*, vol. 28, no. 5, pp. 948–959, Mar. 2022, doi: 10.1158/1078-0432.CCR-21-1798/3019395/CCR-21-1798.PDF.

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