

Theranostic $^{89}\text{Zr}/^{225}\text{Ac}$ anti-CEACAM5 radioimmunotherapy for metastatic castration-resistant prostate cancer

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Background: Radiotheranostics targeting prostate-specific membrane antigen (PSMA) and androgen receptor (AR) pathway-directed therapies have improved outcomes in metastatic castration-resistant prostate cancer (mCRPC), yet patients with AR-independent or PSMA-negative/low-expressing tumors lack effective treatment options. Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) has emerged as a promising alternative cell-surface target in this subset, providing a rationale for developing CEACAM5-directed imaging and therapeutic strategies. This study evaluates a CEACAM5-targeted radiotheranostic pair using the high-affinity anti-CEACAM5 IgG1 monoclonal antibody tusamitamab, formulated as [^{89}Zr]Zr-DFO-tusamitamab for PET imaging and [^{225}Ac]Ac-macropa-tusamitamab for alpha-emitter therapy. In parallel, we explore pretargeted radioimmunotherapy (PRIT) using anti-CEACAM5/anti-DOTA bispecific antibodies engineered within the self-assembling dis-assembling antibody (SADA) platform to enable high-avidity tumor targeting followed by rapid systemic clearance prior to radioligand delivery.

Methods: Tusamitamab was conjugated with desferrioxamine B (DFO) or macropa and radiolabeled with zirconium-89 or actinium-225. Studies were performed in nude mice bearing NCI-H660 xenografts or neuroendocrine prostate cancer PDOX models. Radioimmunoconjugates were evaluated for stability, immunoreactivity, and in vitro binding. Tumor targeting was assessed by PET/CT and biodistribution. Therapeutic efficacy was tested across administered activities of 1.85–18.5 kBq/mouse. SADA-PRIT constructs were evaluated for assembly/disassembly behavior, CEACAM5 binding, and in vivo tumor localization.

Results: Tusamitamab-chelator conjugates (ratios 1–4) retained low-nM affinity comparable to parental antibody. [^{89}Zr]Zr-DFO-tusamitamab and [^{225}Ac]Ac-macropa-tusamitamab were radiolabeled in high yields (30 MBq/nmol and 2.8 MBq/nmol) and remained stable for 240 hours. Both demonstrated ~70% immunoreactivity. Biodistribution showed high CEACAM5-specific tumor uptake at 120 hours (29.2 ± 3.2 %IA/g for Zr-89 and 35.9 ± 7.8 %IA/g for Ac-225), with robust targeting also observed in PDOX xenografts. Therapy studies demonstrated complete responses by week 3 in all Ac-225-treated mice. Dosimetry revealed high tumor-absorbed dose (3404.16 Gy/MBq) relative to blood (256.37 Gy/MBq), bone marrow (92.29 Gy/MBq), and kidneys (63.91 Gy/MBq), yielding therapeutic indices of 13.3, 36.9, and 53.3, respectively. Histopathology at 20 weeks confirmed complete tumor regression at the inoculation site. Renal evaluation showed dose-dependent cortical and corticomedullary tubular injury consistent with radiotoxicity, while intestine, testes, urinary bladder, and most livers were unremarkable. Splenic reactive hyperplasia occurred across groups, most prominently at 500 nCi. Hematologic analysis showed dose-dependent anemia without thrombocytopenia, and serum biochemistry demonstrated mild BUN elevations consistent with early renal azotemia. Preliminary CEACAM5-SADA bispecific antibody studies confirmed strong tumor localization and rapid systemic clearance.

Conclusions: CEACAM5-targeted [^{89}Zr]Zr-DFO-tusamitamab and [^{225}Ac]Ac-macropa-tusamitamab demonstrate potent antitumor activity, high tumor-to-normal tissue absorbed dose ratios, and a favorable therapeutic window in mCRPC models. Development of CEACAM5-SADA-PRIT further expands the

translational potential of CEACAM5-directed radiotheranostics by enabling modular, high-precision radioligand delivery.

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