

Cryo-EM Structure of uPAR Bound to an Antibody Developed for Targeted Cancer Therapy. Implications for Domain Flexibility, uPA-binding and Molecular Imaging

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The urokinase-type plasminogen activator receptor (uPAR), a GPI-anchored membrane-tethered glycoprotein belonging to the LU protein domain superfamily, is currently gaining momentum as a promising molecular target for treatment of various solid cancers. For patient stratification, a high-affinity uPAR-targeting peptide (AE105) shows promise in the detection of primary cancer lesions as well as occult metastasis by positron emission tomography (PET) imaging. uPAR-targeting by AE105 is also used for optical imaging in fluorescence-guided surgery of *e.g.* head-and-neck cancers. Recently, an antibody-drug conjugate (ADC, FL1-PNU) consisting of a monoclonal anti-uPAR antibody (FL1) conjugated to a potent anthracycline derivative PNU, proved to be highly efficient in the eradication of pancreatic ductal adenocarcinoma (PDAC) in surrogate mouse models, leading to long-term remission. In the current study, we determined the cryo-EM structures of FL1 in complex with two different uPAR conformations. Combined with SPR kinetic data, our structures define the FL1•uPAR binding interface and provide essential insights into how FL1 binding impacts uPAR domain flexibility, subsequent ligand (uPA, vitronectin, and AE105) binding, and how these properties will affect uPAR-targeting with FL1-ADCs and the accompanying patient stratification with PET imaging. Our findings, combined with the promising preclinical evidence of FL1-PNU efficacy in PDAC models lay the foundation for potential clinical studies of FL1-PNU in treatment of PDAC and other uPAR-expressing cancers.