

## Targeting Urokinase Plasminogen Activator for Radioimmunotherapy Using an Internalizing Human Antibody

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**Background:** The plasminogen activation system (PAS) - consisting of the secreted serine protease urokinase plasminogen activator (uPA), the receptor of uPA (uPAR) and the endogenous inhibitor of uPA (PAI-1) - is overexpressed in a number of adenocarcinomas with poor clinical outcome. Enzymatically active uPA is known to cleave substrates that promote tumor growth and metastasis. Active uPA, therefore, represents a promising target for therapeutic intervention. Detailed here is the development of a novel uPA-targeted human antibody for radioimmunotherapy (RIT).

**Methods:** A human antibody specific for secreted and uPAR bound forms of active uPA was discovered using a human Fab phage display library. This antibody, U33 IgG, was labeled with  $^{111}\text{In}$  for *in vitro* characterization studies in prostate cancer cell lines and for *in vivo* SPECT/CT imaging and biodistribution studies in PC3 xenograft mice. The therapeutic efficacy of  $^{177}\text{Lu}$  labeled U33 IgG ( $^{177}\text{Lu}$ -U33 IgG) was evaluated *in vitro* and *in vivo* in an RIT study using a single dose of 100 $\mu\text{Ci}$  in PC3, DU145, and LNCaP xenograft mice.

**Results:** The *in vitro* characterization studies in prostate cancer cell lines found that the U33 IgG mimicked the function of PAI-1 resulting in internalization of the uPA-U33 complex when bound to uPAR. This mechanism of internalization resulted in a sensitive SPECT/CT imaging probe with a high tumor uptake of 43.2% injected dose per gram (%ID/g) in PC3 xenografts. Subsequently,  $^{177}\text{Lu}$ -U33 IgG was found to be effective *in vitro* at selectively killing the uPA expressing cell lines PC3 and DU145, but not the uPA null LNCaP. As an RIT agent,  $^{177}\text{Lu}$ -U33 IgG had no effect on the LNCaP tumors, but dramatically reduced the tumor burden in the PC3 and DU145 xenografts.

**Conclusions:** U33 IgG is a unique antibody that activates internalization of the U33/uPA/uPAR complex through a complex mechanism. Because of this novel internalization mechanism, U33 IgG could be sequestered inside cancer cells resulting in a potent RIT agent when coupled to  $^{177}\text{Lu}$ . These data lay the foundation for further studies to evaluate the utility of U33 IgG as an RIT agent and potential translation into humans.

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