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Poster Presentations - Preclinical and Experimental Imaging

Abstract 3912: Pre-clinical developments of the G3 Designed ankyrin repeat protein (DARPin) for *invivo* assessment of HER2 expression .

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Background:

Breast cancer HER2 molecular imaging can potentially identify disease relapse, inform treatment decisions and assess treatment responses. Molecular imaging relies upon achieving high tumour:blood and tumour:normal tissue ratios. The G3 DARPin is a small protein with picomolar affinity for HER2, based on the ankyrin repeat scaffold that is expressed in humans. The hexahistidine (His_6) tagged G3 DARPin labelled with $^{99m}Tc(CO)_3$ can image HER2+ SK-OV-3 tumours [Zahnd et al. Cancer Res 2010;70:1595-605].

Alteration of the His_6 tag to a negatively charged and hydrophilic histidine-glutamate $(HE)_3$ tag can reduce background liver uptake, while enabling tag mediated purification by immobilised metal affinity chromatography [Hofstrom et al. J Med Chem 2011;54;3817-26]. We hypothesized that the biodistribution of ^{111}In and ^{125}I G3 DARPin could be optimised by altering the N-terminal domain.

Methods:

His_6, HE_3 and untagged G3 were produced in E. coli and or P. pastoris and labelled directly with ^{125}I or with DOTA via a C-terminal cysteine for ^{111}In. BALB/c mice were injected with 0.3 MBq of ^{111}In or ^{125}I G3. The optimal G3 construct was assessed with ^{111}In and ^{125}I in HER2+ human breast tumour (BT474)-bearing mice.

Results:

Biodistribution of the DARPins was evaluated in BALB/c mice at 4 and 24 h. Results showed that ^{111}In-HE_3-G3 had lower or similar uptake to ^{111}In-His_6-G3 and ^{111}In-untagged-G3 in 11 different normal tissues tested. Superiority of HE_3-G3 for normal tissue uptake was also observed when the DARPins were labelled with ^{125}I.

HE_3-G3 was assessed in HER2+ tumour-bearing mice. The tumour uptake for ^{125}I-HE_3-G3 was approximately 2 fold higher than ^{111}In-HE_3-G3 at 4 h. However, ^{111}In-HE_3-G3 tumour uptake was better maintained, so that by 24 h ^{111}In-HE_3-G3 tumour uptake was approximately 1.5 fold higher than ^{125}I-HE_3-G3. Normal tissue uptake was generally lower

for ^{111}In-HE_3-G3 than ^{125}I-HE_3-G3 at 4 h, except in the kidneys which were higher for ^{111}In-HE_3-G3 throughout. At 24 h, the differences in normal tissue uptake between ^{111}In-HE_3-G3 and ^{125}I-HE_3-G3 were smaller. ^{111}In-HE_3-G3 had faster serum clearance than ^{125}I-HE_3-G3, resulting in higher normal tissue:blood ratios for all assessed tissues except stomach. As a consequence, the tumour:blood ratios for ^{111}In-HE_3-G3 were the most impressive, > 150:1 at 4 h and > 300:1 at 24 h . ^{111}In-HE_3-G3 microSPECT/CT imaging demonstrated tumour uptake at 2 and 4 h.

Conclusions:

N-terminal tags effect tissue biodistribution of G3. HE_3-G3 radiolabelled with ^{111}In and ^{125}I had lower uptake in normal tissues compared to untagged or His_6 tagged G3. ^{111}In-HE_3-G3 achieved and maintained the highest tumour:blood ratios over 24 h. Based on its superiority, development will focus on the radiolabelled C-terminal cysteine DOTA conjugated HE_3-G3 for SPECT and PET HER2 imaging.

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