**Pre-clinical developments of the G3 Designed ankyrin repeat protein (DARPin) for *in* *vivo* assessment of HER2 expression**

**Short Title:**HER2 G3 DARPin imaging

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*Abstract:*  
**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 (His6) tagged G3 DARPin labelled with 99mTc(CO)3 can image HER2+ SK-OV-3 tumours[Zahnd *et al*. Cancer Res 2010;70:1595-605].  
Alteration of the His6 tag to a negatively charged and hydrophilic histidine-glutamate (HE)3 tag can reduce background liver uptake, while enablingtag mediated purification by immobilised metal affinity chromatography [Hofstrom *et al.* J Med Chem 2011;54;3817-26]. We hypothesized that the biodistribution of 111In and 125I G3 DARPin could be optimised by altering the N-terminal domain.  
**Methods:**  
His6, HE3 and untagged G3 were produced in *E. coli and* or *P. pastoris* and labelled directly with 125I or with DOTA via a C-terminal cysteine for 111In. BALB/c mice were injected with 0.3 MBq of 111In or 125I G3. The optimal G3 construct was assessed with 111In and 125I 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 111In-HE3-G3 had lower or similar uptake to 111In-His6-G3 and 111In-untagged-G3 in 11 different normal tissues tested. Superiority of HE3-G3 for normal tissue uptake was also observed when the DARPins were labelled with 125I.  
HE3-G3 was assessed in HER2+ tumour-bearing mice. The tumour uptake for 125I-HE3-G3 was approximately 2 fold higher than 111In-HE3-G3 at 4 h. However, 111In-HE3-G3 tumour uptake was better maintained,  
so that by 24 h 111In-HE3-G3 tumour uptake was approximately 1.5 fold higher than 125I-HE3-G3. Normal tissue uptake was generally lower for 111In-HE3-G3 than 125I-HE3-G3 at 4 h, except in the kidneys which were higher for 111In-HE3-G3 throughout. At 24 h, the differences in normal tissue uptake between 111In-HE3-G3 and125I-HE3-G3 were smaller. 111In-HE3-G3 had faster serum clearance than 125I-HE3-G3, resulting in higher normal tissue:blood ratios for all assessed tissues except stomach. As a consequence, the tumour:blood ratios for 111In-HE3-G3 were the most impressive, > 150:1 at 4 h and > 300:1 at 24 h . 111In-HE3-G3 microSPECT/CT imaging demonstrated tumour uptake at 2 and 4 h.  
**Conclusions:**  
N-terminal tags effect tissue biodistribution of G3. HE3-G3 radiolabelled with 111In and 125I had lower uptake in normal tissues compared to untagged or His6 taggedG3. 111In-HE3-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 HE3-G3 for SPECT and PET HER2 imaging.  
  
  
  
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