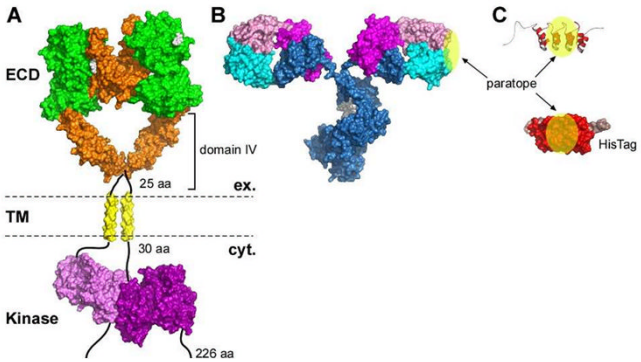
G3 designed ankyrin repeat protein for HER2 molecular imaging

R.Goldstein[1], B.Tolner[1], J.Leyton[2], M.Livanos[1], G.Bhavsar[1], K.Vigor[1], G.Nagy[3], S.Mather[2], A.Plückthun[3], J.Sosabowski[2], T.Meyer[1], K.Chester[1].
[1]UCL Cancer Institute, London, UK; [2]Barts Cancer Institute, London, UK; [3]University of Zurich, Zurich, CH.

Background:
HER2 breast cancer imaging could predict and assess treatment responses. Molecular imaging requires high tumour-to-blood and high tumour-to-normal tissue ratios for which radiolabelled DARPins (designed ankyrin repeat proteins) may be ideally suited. The G3 DARPin has picomolar affinity for HER2 and we have evaluated its potential for imaging HER2 in a preclinical model.

Fig 1. Size comparison A) HER2 B) IgG C) G3 DARPin



Aim: To evaluate the optimal conjugate and isotope for imaging with the G3 DARPin

Methods:
His_6, HE_3 and untagged G3 with a C-terminal cysteine (**C**) were produced in *P. pastoris* and labelled with ^{125}I or ^{111}In (via DOTA). Female BALB/c mice were injected with radiolabelled G3. The optimal construct was assessed in female HER2+ breast tumour (BT474) bearing mice [Fig 2].

1) Untagged-G3: GP-[G3 DARPin]-**C**
2) His_6-G3: HHHHHHGP-[G3 DARPin]-**C**
3) HE_3-G3: HEHEHEGP-[G3 DARPin]-**C**

Fig 2: BT474 tumour assessed by HercepTest (3+).

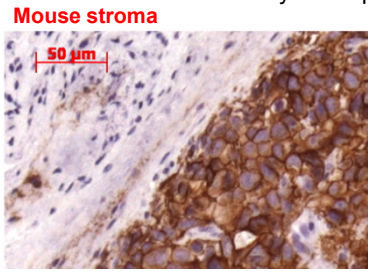


Fig 3: ^{111}In-G3 DARPins in BALB/c mice at 24 h, *p< 0.05.

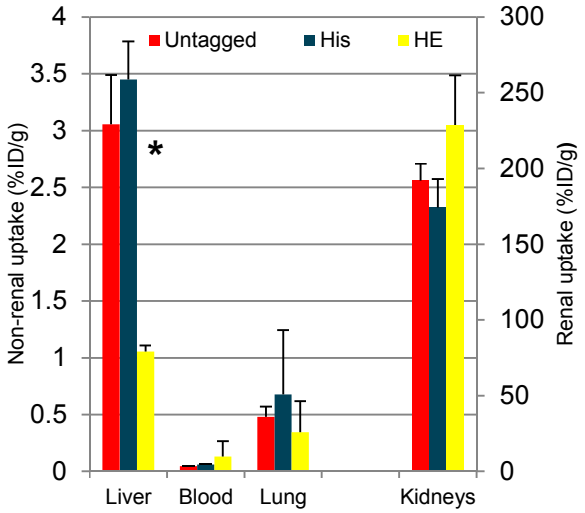
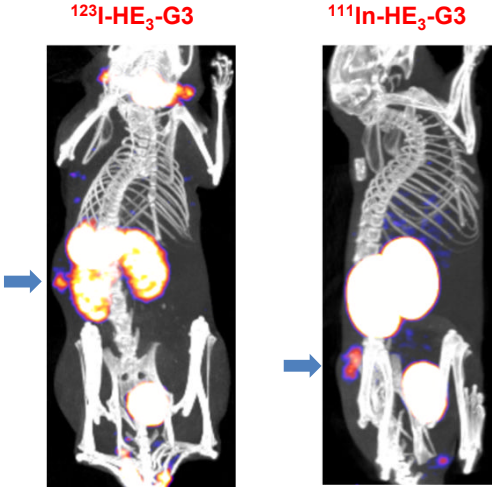


Table 1: ^{111}In and ^{125}I-HE_3-G3 DARPin in female mice bearing HER2+ human breast tumours (mean ± SD)

	^{111}In-HE_3-G3		^{125}I-HE_3-G3	
	4h	24h	4h	24h
Mean tumour uptake (%ID/g)	8.8 ±1.3	8.1 ±0.9	11.3 ±3.2	2.4 ±0.6
Mean blood Uptake (%ID/g)	0.05 ±0.01	0.03 ±0.02	3.6 ±2.2	0.1 ±0.04
Tumour-to-blood ratio	174.7 ±26.1	343.7 ±161.3	4.4 ±3.4	22 ±9.6

Results:
- ^{111}In-HE_3-G3 and ^{125}I-HE_3-G3 had lower or similar uptake in ten different normal tissues compared to His_6 and untagged G3.
- ^{111}In-HE_3-G3 had lower normal liver uptake [Fig. 3].
- ^{111}In-HE_3-G3 has faster serum clearance and its tumour uptake is maintained, resulting in higher tumour-to-blood ratios than ^{125}I-HE_3-G3 [Table 1].
- microSPECT/CT imaging demonstrated tumour uptake at 4 h [Fig. 4] .

Fig 4: microSPECT/CT of HER2+ tumour bearing mice at 4 h, (arrow identifies tumour)



Conclusions:
- ^{111}In and ^{125}I-HE_3-G3 had lower normal tissue uptake compared to untagged or His_6 G3.
- ^{111}In-HE_3-G3 achieved highest tumour-to-blood ratios and had low normal tissue uptake (except in the kidneys).
- HE_3-G3 radiolabelled via a bifunctional chelator will be tested in a first in man study.