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 DARPinss (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<sub>6</sub>, HE<sub>3</sub> and untagged G3 with a C-terminal cysteine (C) were produced in P. pastoris and labelled with 125<sup>I</sup> or 111<sup>In</sup> (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<sub>6</sub>-G3: HHHHHHGP-[G3 DARPin]-C
3) HE<sub>3</sub>-G3: HEHEHEGP-[G3 DARPin]-C

Results:
• 111<sup>In</sup>-HE<sub>3</sub>-G3 and 125<sup>I</sup>-HE<sub>3</sub>-G3 had lower or similar uptake in ten different normal tissues compared to His<sub>6</sub> and untagged G3.
• 111<sup>In</sup>-HE<sub>3</sub>-G3 had lower normal liver uptake [Fig. 3].
• 111<sup>In</sup>-HE<sub>3</sub>-G3 has faster serum clearance and its tumour uptake is maintained, resulting in higher tumour-to-blood ratios than 125<sup>I</sup>-HE<sub>3</sub>-G3 [Table 1].
• microSPECT/CT imaging demonstrated tumour uptake at 4 h [Fig. 4].

Conclusions:
• 111<sup>In</sup> and 125<sup>I</sup>-HE<sub>3</sub>-G3 had lower normal tissue uptake compared to untagged or His<sub>6</sub> G3.
• 111<sup>In</sup>-HE<sub>3</sub>-G3 achieved highest tumour-to-blood ratios and had low normal tissue uptake (except in the kidneys).
• HE<sub>3</sub>-G3 radiolabelled via a bifunctional chelator will be tested in a first in man study.

Table 1: 111<sup>In</sup> and 125<sup>I</sup>-HE<sub>3</sub>-G3 DARPin in female mice bearing HER2+ human breast tumours (mean ± SD)

<table>
<thead>
<tr>
<th>DARPin</th>
<th>Liver 4h</th>
<th>Blood 4h</th>
<th>Lung 4h</th>
<th>Kidneys 4h</th>
<th>Liver 24h</th>
<th>Blood 24h</th>
<th>Lung 24h</th>
<th>Kidneys 24h</th>
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<tbody>
<tr>
<td>111&lt;sup&gt;In&lt;/sup&gt;-HE&lt;sub&gt;3&lt;/sub&gt;-G3</td>
<td>8.8 ± 1.3</td>
<td>8.1 ± 0.9</td>
<td>11.3 ± 3.2</td>
<td>2.4 ± 0.6</td>
<td>8.1 ± 0.9</td>
<td>8.1 ± 0.9</td>
<td>11.3 ± 3.2</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>125&lt;sup&gt;I&lt;/sup&gt;-HE&lt;sub&gt;3&lt;/sub&gt;-G3</td>
<td>0.05 ± 0.01</td>
<td>0.03 ± 0.02</td>
<td>3.6 ± 0.2</td>
<td>0.1 ± 0.04</td>
<td>0.05 ± 0.01</td>
<td>0.03 ± 0.02</td>
<td>3.6 ± 0.2</td>
<td>0.1 ± 0.04</td>
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Fig 2: BT474 tumour assessed by HercepTest (3+).

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

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