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Developing Designed Ankyrin Repeat Proteins (DARPin) for HER2 imaging

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Background

HER2 imaging can potentially assist in clinical management. The G3 DARPin, a small protein based on human ankyrin repeats¹, was developed as a novel imaging agent. The DARPin has picomolar affinity for HER2 and is generated as a recombinant protein with N-terminal tags for purification. The bio-distribution of G3 was tested to evaluate the influence of tags and choice of radiolabel.

Method

The DARPin was generated in *P. pastoris*, and purified with either a hexahistidine (His₆ tag) or a more negatively charged hydrophilic histidine-glutamate (HE₃ tag). Untagged G3 was used as a control. The purified proteins were labelled with ¹²⁵I (using iodogen) or ¹¹¹In (via site-specific attachment of DOTA). Bio-distribution was assessed in BALB/c mice or nude mice bearing HER2+ human (BT474) breast tumours.

Results

¹¹¹In-His₆-G3 and untagged ¹¹¹In-G3 had significantly higher liver uptake than ¹¹¹In-HE₃-G3 at 4 h (p=0.001) and 24 h (p=0.001 and p=0.002 respectively) post administration. Superiority of the HE₃ tag was also observed with ¹²⁵I-radiolabelled-G3. HE₃-G3 was therefore taken forward for testing in HER2+ tumour-bearing mice.

Tumour uptake of ¹²⁵I-HE₃-G3 and ¹¹¹In-HE₃-G3 were similar at 4 h but the ¹¹¹In-HE₃-G3 tumours retained more radioactivity over time, resulting in ~3-fold higher than value for ¹¹¹In-HE₃-G3 (*residualizing radionuclide*) than ¹²⁵I-HE₃-G3 (*non-residualizing*) at 24 h. ¹¹¹In-HE₃-G3 also had faster serum clearance than ¹²⁵I-HE₃-G3, resulting in higher normal tissue: blood ratios for all assessed tissues (except stomach). Tumour: blood ratios of >300:1 were achieved at 24 h whilst maintaining ~8% of the injected radioactivity/g of tumour. Pre-administration of trastuzumab did not alter HER2 tumour ¹¹¹In-HE₃-G3 uptake. HER2+ tumour imaging was demonstrated by micro SPECT/CT.

Conclusion

The HE₃-G3 was superior to His-G3 or untagged G3 and ¹¹¹In was superior to ¹²⁵I. Clinical development will focus on chelate radiolabelled HE₃-G3 for SPECT and PET HER2 imaging.

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References

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