A HER2 targeted polylysine dendrimer nanoparticle radiotheranostic demonstrates excellent tumor accumulation, rapid clearance from circulation, and promising performance in PET-CT imaging

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Background

- Development of targeted radiotheranostics for human epidermal growth factor receptor type 2 positive (HER2⁺) tumors has lagged advances made in other receptor systems such as prostate specific membrane antigen (PSMA) in prostate cancer.
- Myelosuppression is a dose limiting toxicity in clinical studies of Trastuzumab monoclonal antibody-based radioconjugates due to slow clearance of this high molecular weight radiotherapeutic from the circulation.[1]
- Conjugation of cancer therapeutics such as taxanes, irinotecan/SN38, or Bcl2-family inhibitors to highly optimized dendrimer (DEP®) nanoparticles improves pharmacokinetics (PK), tumor uptake and antitumor efficacy *in vivo*.[2]
- DEP® dendrimer nanoparticles can be used as targeted radiotheranostics with potential to achieve superior PK, enhanced tumor killing, and reduced toxicity (improved therapeutic index) compared to traditional antibody-based radiotheranostics.[3]
- A radio-imaging and biodistribution study of the HER2-targeted dendrimer-based radiodiagnostic, DEP® HER2-zirconium, was conducted in mice implanted with HER2⁺ tumors.
- The study compared performance of DEP® HER2-zirconium in positron emission tomography-computed tomography (PET-CT) imaging, and in the kinetics of tumor and normal tissue uptake, to radiolabeled Trastuzumab control.

Materials and Methods

- A HER2 targeted VHH (single domain antibody [sdAb], or antigen binding fragment of heavy chain only camelid antibody) was covalently linked to a generation 4 dendrimer with 16 deferoxamine (DFO) chelation groups on its surface (DEP® HER2_DFO(16)).
- Trastuzumab was covalently linked to DFO (Trastuzumab_DFO(2)).
- Human and dog HER2 receptor extracellular domains (ECDs) were immobilised (3 µg/mL) onto a CM5 chip and surface plasmon resonance (SPR) analysis of binding conducted using a Biacore 8K+ instrument. Biacore Insight Evaluation software was used to evaluate the binding kinetics, using the in-built single cycle kinetics analysis. Data were fit to a 1:1 binding model.
- DEP® HER2_DFO(16) and Trastuzumab_DFO(2) were successfully radiolabeled with ^{89}Zr to high specific activity.
- Female BALB/c nude mice were inoculated subcutaneously with BT474 HER2⁺ breast cancer cells. When tumors reached 150mm , a single dose (3MBq) of DEP® HER2_^{89}Zr (DEP® HER2-zirconium) or Trastuzumab_^{89}Zr was administered intravenously. Uptake was evaluated in tissues by *ex vivo* gamma scintillation counting (n=3/group) from 4h-12d. *In vivo* biodistribution (n=3/group) was determined using quantified PET-CT data (4h-5d). PET-CT images were obtained on day 2 and 4.

Figure 1A: Schematic representation of the monoclonal antibody control bioconjugate. The anti-HER2 humanized monoclonal antibody, Trastuzumab, was covalently linked (through a DBCO linker) to approximately two chelators (one on each of the heavy chains). DFO was used for the PET isotope, ^{89}Zr, and DOTA for therapeutic isotope, ^{177}Lu.

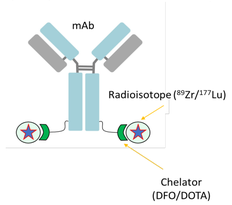
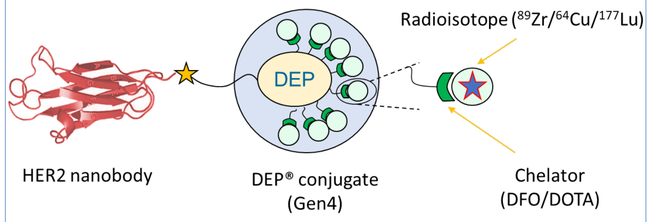


Figure 1B: Schematic representation of the DEP® HER2 radiotheranostics platform. The lead bioconjugate consists of HER2 targeting nanobody clone 2D3 linked via an engineered C terminal cysteine residue to a generation 4 polylysine dendrimer. The generation 4 dendrimer is covalently linked, through the epsilon amino group of lysine, to 16 chelator molecules (16x DFO for PET isotope ^{89}Zr, 16x DOTA for PET isotope ^{64}Cu, or therapeutic isotope ^{177}Lu).



Results

Table 1: SPR analysis of interaction between free anti-HER2 nanobody (clone 2D3), unlabelled DEP® HER2 radiotheranostic bioconjugates, or unlabelled Trastuzumab bioconjugate and the ECDs of either human (left) or dog (right) HER2 receptor. The equilibrium dissociation constants (K_D) are expressed in nM.

		Human HER2 ECD			Dog HER2 ECD		
Construct	**MW (kDa)**	**K_a ($M^{-1}s^{-1}$)**	**k_d (s^{-1})**	**k_D (nM)**	**K_a ($M^{-1}s^{-1}$)**	**k_d (s^{-1})**	**k_D (nM)**
Free HER2 VHH	13.7	7.12E+05	1.13E-03	**1.59**	6.99E+05	1.95E-03	**2.79**
DEP® HER2_DFO(16)	50.8	2.13E+05	5.35E-04	**2.51**	1.15E+05	7.59E-04	**6.62**
DEP® HER2_DOTA(16)	47.6	4.11E+05	6.42E-04	**1.56**	3.88E+05	1.16E-03	**2.98**
Trastuzumab_DFO(2)	150	2.48E+05	2.84E-04	**1.14**	nd	nd	nd

Table 2: Radiochemistry parameters. Assessment of radiochemical purity (RCP), radiolysis, and serum stability of DEP® HER2 radioconjugates and Trastuzumab radioconjugate. RCP of Trastuzumab_^{89}Zr determined by radio-size exclusion chromatography (SEC)-HPLC (radio-SEC-HPLC) while RCP of the smaller DEP® HER2_^{89}Zr and DEP® HER2_^{64}Cu bioconjugates was determined by radio-reverse phase (RP)-HPLC (radio-RP-HPLC). All three bioconjugates showed excellent (> 95%) RCP at t=0. (Auto)radiolysis was measured by incubation in PBS for 5 days (for ^{89}Zr radioconjugates) and 1 day (for the ^{64}Cu radioconjugate). Serum stability was measured by incubation in 50% serum for 5 days (for ^{89}Zr radioconjugates) and 1 day (for the ^{64}Cu radioconjugate). Both DEP® HER2 radioconjugates were resistant to radiolysis and demonstrated excellent stability in serum (RCP of both > 90%).

Bioconjugate	**Specific Activity MBq per µg**	**Molar Activity MBq per mol**	**Percentage RCP (t=0)**	**Percentage RCP (t=5d) (PBS)**	**Percentage RCP (t=5d) (50% serum)**
DEP® HER2_^{89}Zr	0.1	2.03 x 10^{10}	100.0	94.2	93.6
Trastuzumab_^{89}Zr	0.034	2.03 x 10^{10}	95.0	75.2	48.5
Bioconjugate	**Specific Activity MBq per µg**	**Molar Activity MBq per mol**	**Percentage RCP (t=0)**	**Percentage RCP(t=1d) (PBS)**	**Percentage RCP (t=1d) (50% serum)**
DEP® HER2_^{64}Cu	3.85	5.48 x 10^{12}	98.2	92.8	93.1

Figure 2A: *Ex vivo* tissue biodistribution of DEP® HER2_^{89}Zr in mice bearing subcutaneous human BT474 HER2⁺ breast tumors. Radioconjugate was injected at t=0. Biodistribution was evaluated between 4h and 12d. Mean ± SEM (n=3). **Sustained high level tumor uptake (1d-5d) was observed, along with fast clearance from blood.**

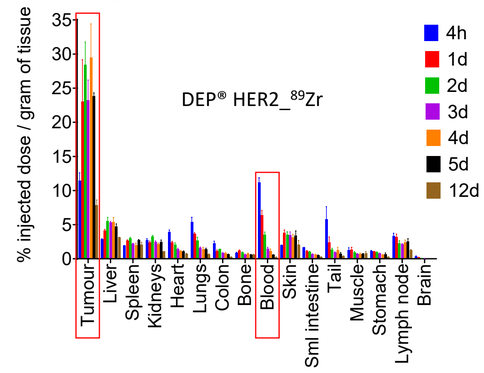


Figure 2B: *Ex vivo* tissue biodistribution of Trastuzumab_^{89}Zr in mice bearing subcutaneous human BT474 HER2⁺ breast tumors. Radioconjugate was injected at t=0. Biodistribution was evaluated between 4h and 12d. Mean ± SEM (n=3). **Sustained high level tumor uptake (1d-12d) was observed with slower clearance from blood**.

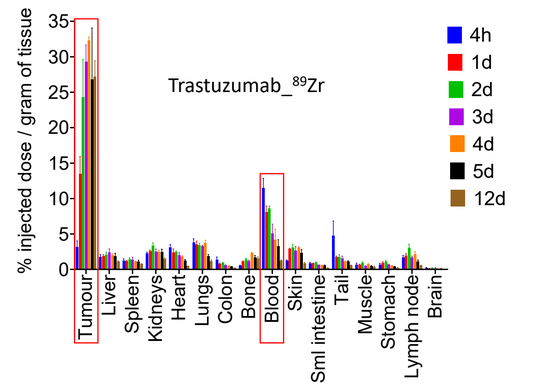


Figure 3: Comparison of blood levels and tumor:blood ratios between DEP® HER2_^{89}Zr and Trastuzumab_^{89}Zr radioconjugates. DEP® HER2_^{89}Zr demonstrated faster clearance from blood (left y axis) and sustained elevated tumor:blood ratios (right y axis) compared to Trastuzumab_^{89}Zr.

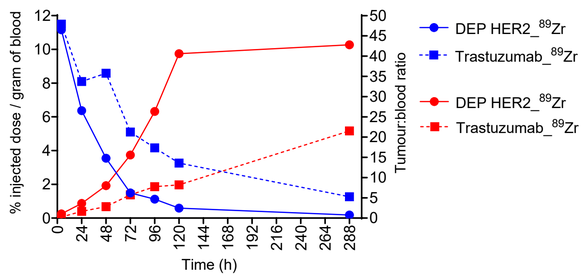


Figure 4: Maximum intensity projection (MIP) PET-CT images of BT474 HER2⁺ tumor-bearing mice dosed with either DEP® HER2_^{89}Zr or Trastuzumab_^{89}Zr. Radioconjugates administered at t=0. Representative mice are shown at day 2 (left side) or day 4 (right side) after injection. The scale bar (% ID/g) is shown to the right. **In addition to high level uptake in tumor, signals were observed in heart for Trastuzumab_^{89}Zr (which is likely to reflect higher levels in the circulation), and liver for DEP® HER2_^{89}Zr, as indicated. Deposition of ^{89}Zr in shoulder and hip joints was prominent for Trastuzumab_^{89}Zr but not for DEP® HER2_^{89}Zr (arrows).**

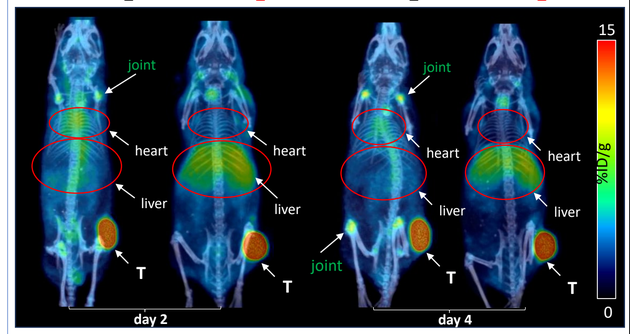
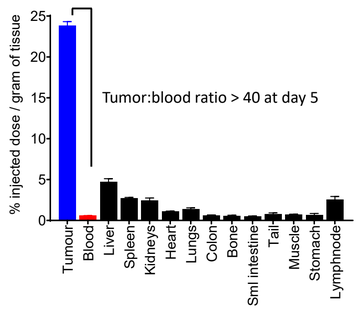


Figure 5: DEP® HER2_^{89}Zr resulted in high tumor:blood ratios.



Conclusions

DEP® HER2_^{89}Zr (DEP® HER2-zirconium) demonstrated imaging benefits in a HER2+ breast cancer model:

- More rapid tumor accumulation and superior PK vs. HER2 monoclonal antibody, labelled with zirconium, Trastuzumab_^{89}Zr ;
- A favourable biodistribution profile, with excellent imaging contrast between tumor and normal tissues
- Highly desirable “fast-in”/”fast-out” kinetics, with rapid accumulation in tumor and rapid clearance from the bloodstream;
- High tumor:organ ratios, delivering excellent specificity in imaging the tumor in HER2+ breast cancer
- High tumor:blood ratios, suggesting potential advantages of DEP® technology for radiotherapeutic applications.

References:

1. Bhusari P. *et al.,* (2017) Int. J. Cancer. 140(4):938-947
2. Patterson C.M., *et al.* (2021) Commun. Biol. 4(1):112
3. Akhtar, N., *et al.* (2023) J. Pharm. Sci. 112(3):844-858

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