^{89}Zr-immuno-PET in translational development of biopharmaceuticals

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Position of immuno-PET in the development of biopharmaceuticals

Biologicals gained attention over the past decades thanks to their therapeutic success especially in oncology. In the EU vision of personalised treatment, the right treatment should be provided to the right patient and at the right dose and at the right time to increase chances of successful therapy, reduce toxicity and ultimately increase cost-effectiveness. The molecular imaging technique Positron Emission Tomography (PET) with zirconium-89 is highly attractive thanks to ^{89}Zr half-life (78.41h) matching the biological half-life of monoclonal antibodies (mAbs). This thesis provides an overview of ^{89}Zr-immuno-PET imaging using current and emerging radiolabeling tools and preclinical imaging to facilitate translation to the clinic of new antibody constructs. By improving the radiochemistry toolbox and better understanding quantification using preclinical PET cameras, new opportunities can be translated in the clinic. On top of providing insights on the position of ^{89}Zr-immuno-PET imaging in the context of biopharmaceuticals development, this thesis gives an overview of current radiolabeling methods with ^{89}Zr, ^{64}Cu and ^{68}Ga, but also less common radiometals: 52Mn, ^{86}Y, ^{66}Ga, ^{44}Sc, and ^{18}F as in [^{18}F]AlF. Chelator-radionuclide pairs and radiolabeling conditions are discussed along with recent preclinical and clinical trends. Even though multiple novel chelators have been developed and seem promising *in vitro*, thusfar many failed to outperform well-known conventional chelators such as DFO, DOTA and NOTA which are still the most widely used chelators in the clinic.

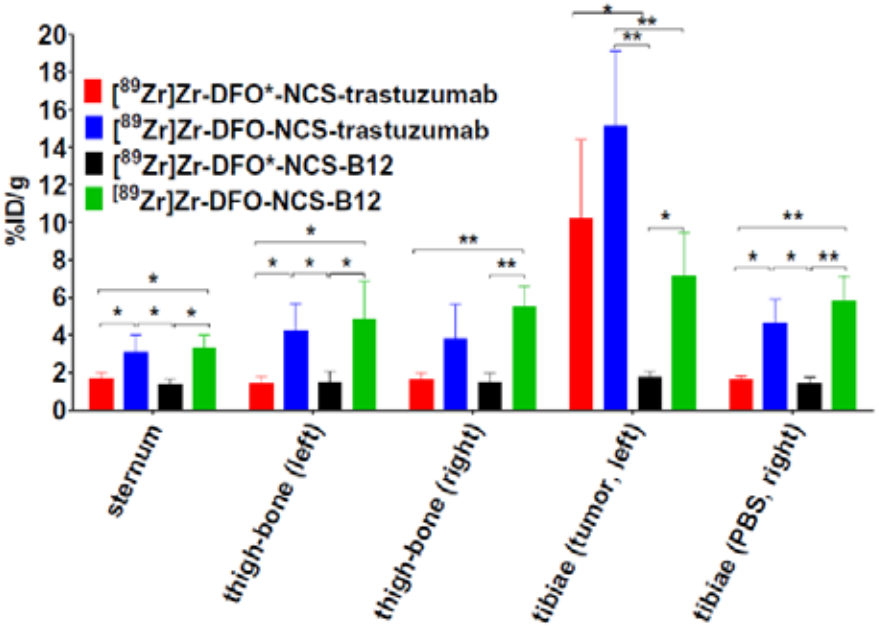
Evaluation of new types of biopharmaceuticals with ^{89}Zr-immuno-PET

Probody® therapeutics are new types of constructs which possess antigen binding domains masked by a peptide cap only converted to active antigen binding antibodies in the tumor environment by removal of the caps by tumor-associated proteases, locally overexpressed. Probodies aim at widening the therapeutic window while Probody drug conjugates (PDCs) aim at delivering selectively their payload to tumors via widely expressed antigens, such as CD166. CX-2009, a PDC with a toxic DM4 payload attached was evaluated by performing ^{89}Zr-immuno-PET and biodistribution studies in CD166-positive lung cancer mouse model in comparison with its Probody (CX-191), unmasked antibody drug conjugate (CX-1031), and parental mAb derivatives (CX-090). Tumor uptake was similar for all constructs 72h p.i. with a highest uptake of 21.8 ± 2.3 ([^{89}Zr]Zr-CX-2009), 21.8 ± 5.0 ([^{89}Zr]Zr-CX-191), 18.7 ± 2.5 ([^{89}Zr]Zr-CX-1031) and 20.8 ± 0.9 %ID/g ([^{89}Zr]Zr-CX-090) at 110 µg injected, demonstrating that enzymatic activation inside the tumor was not a limiting factor for tumor uptake and justifying the clinical evaluation of Probody® therapeutics.

Improvement of the radiochemistry tool box for Zirconium-89

The novel octadentate chelator DFO* was studied in depth *in vitro* and *in vivo* in comparison with desferrioxamine (DFO), current standard for ^{89}Zr-immuno-PET, DFOSq, also reported as potential successor of DFO and DFO*Sq included to evaluate the extra hydroxamate or squaramide group contribution to ^{89}Zr complexation. DFO* is an octadentate chelator and showed in an earlier study superior stability over DFO, resulting in a significantly reduced bone uptake. [^{89}Zr]Zr-DFO*-NCS-trastuzumab and [^{89}Zr]Zr-DFO*Sq-trastuzumab showed excellent stability *in vitro*,

at 37 °C in serum for seven days and under chelator challenging conditions for 24h, superior to their [^{89}Zr]Zr-DFO counterparts. In breast cancer xenograft mice, DFO* derivatives were more stable than DFO derivatives especially in bones. DFOSq did not outperform the DFO derivative, suggesting that the Squaramide is not improving in vivo stability. Cetuximab, directed against the Epidermal-Growth-Factor-Receptor was used in xenograft mice and again DFO* was superior over DFO regarding bone uptake. In an intratibial bone metastasis model, [^{89}Zr]Zr-DFO*-trastuzumab, [^{89}Zr]Zr-DFO-trastuzumab, [^{89}Zr]Zr-DFO*-B12 and [^{89}Zr]Zr-DFO-B12 (a non-targeting control mAb) were evaluated and the DFO*-conjugate appeared superior over the DFO-conjugate with a tumour-specific signal in bone tumors. At 144 h p.i., [^{89}Zr]Zr-DFO*-NCS-trastuzumab and the non-binding control [^{89}Zr]Zr-DFO*-NCS-B12 demonstrated low and comparable uptake in tibiae without tumor involvement (1.6±0.2 and 1.5±0.3 %ID/g, respectively) while [^{89}Zr]Zr-DFO-NCS-trastuzumab and [^{89}Zr]Zr-DFO-NCS-B12, showed an elevated uptake (4.7±1.3 and 5.7±1.4 %ID/g, respectively) (figure 1 and 2). Overall, the studies confirmed DFO* to be the candidate for the future of ^{89}Zr-immuno-PET applications.

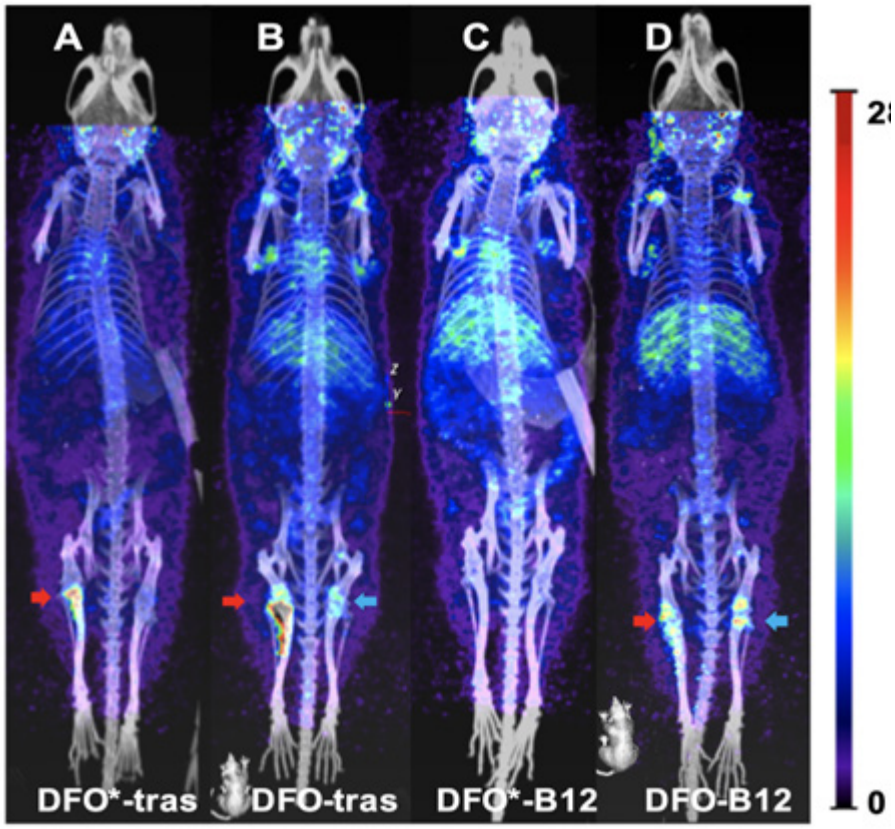


Figuur 1. Biodistribution of [^{89}Zr]Zr-DFO*-NCS-trastuzumab, [^{89}Zr]Zr-DFO-NCS-trastuzumab, [^{89}Zr]Zr-DFO*-NCS-B12, and [89Zr]Zr-DFO-NCS-B12 in collected bones 144 h p.i. of 100 µg per construct. Uptake expressed as %ID/g (Mean ±SD, n=5-6 animals per group). Significant differences between the four constructs are marked with asterisks (* p<0.05, ** p<0.01)

Reliable preclinical quantification

Finally, this thesis provides in-depth comparison between PET imaging and *ex vivo* biodistribution quantification to evaluate the potential of preclinical PET imaging as a reliable quantification method that could make *ex vivo* biodistribution superfluous. Phantom studies with a NanoScan PET/CT and PET/MR were performed with the most used PET radionuclides (^{11}C, ^{68}Ga, ^{18}F and ^{89}Zr). The cameras performed similarly: the highest recovery coefficient in the 5 mm rod was obtained with ^{18}F (80%), followed by 76-77% for ^{11}C and ^{89}Zr and finally ^{68}Ga (54%). Both scanners were evaluated after injection of [^{18}F]FDG and [^{89}Zr]Zr-DFO-NCS-trastuzumab in breast cancer tumor bearing mice and performed equally well regarding tumor quantification with average PET/*ex vivo* ratios of 0.8-0.9 with PET-assessed uptake consistently lower than *ex vivo* values with biases comparable for both cameras and ^{18}F-and ^{89}Zr-labelled tracers. In the brain, [^{18}F]FDG-PET/*ex vivo* ratios were excellent (1.07±0.03 and 1.03±0.10 for the PET/CT and PET/MR, respectively) suggesting that brain is suitable for quantitative imaging of ^{18}F-tracers but not for ^{89}Zr-radiolabeled-mAbs, probably due to poor brain penetration of ^{89}Zr-labeled mAbs and lack of specific targeting. In kidney and liver more disparities were observed. Preclinical cameras and *ex vivo* biodistribution quantification, requires fully described standardised protocols for reliability, reproducibility and inter-study comparisons.

Altogether, this research offers a state-of-the-art overview on promising developments regarding ^{89}Zr-immuno-PET imaging. It provides new insights on preclinical studies including quantification with preclinical scanners and new tools for radiolabeling biologicals confirming ^{89}Zr-immuno-PET imaging potential to evaluate new constructs. *In vitro* and *in vivo* superiority of the new chelator DFO* over the current gold standard for clinical ^{89}Zr-immuno-PET, DFO, was confirmed in various models and has resolved past issues regarding bone uptake. DFO* is thus considered as the successor of DFO for clinical applications. ♦



Figuur 2. PET images of mice injected with 110 µg of either [^{89}Zr]Zr-DFO*-NCS-trastuzumab (a), [^{89}Zr]Zr-DFO-NCS-trastuzumab (b), [^{89}Zr]Zr-DFO*-NCS-B12 (c), or [^{89}Zr]Zr-DFO-NCS-B12 (d) and scanned 144h p.i. All mice had received an intratibial injection of HER2 expressing BT-474 cells in the left leg and PBS in the right leg. Images are presented as Maximum Intensity Projections (MIP). Uptake in affected tibiae is indicated with red arrows and uptake in contralateral tibiae with blue arrows