

Doctoral thesis „**Development of PET imaging agents with potential applications in immunotherapy**” mgr inż. **Anny Kastelik-Hryniewieckiej**.

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Abstract

The aim of this doctoral thesis was to design and evaluate bimodal PET/MRI probes to enhance precision imaging and support therapeutic decision-making, particularly in the context of immunotherapy. Two principal objectives were defined: firstly, to improve the stability of ^{89}Zr -based PET agents for immunoPET applications, and secondly, to develop bimodal probes suitable for combined PET/MRI imaging, reflecting the growing clinical interest in this hybrid modality.

The first objective involved the design of a novel chelator for ^{89}Zr , based on deferoxamine (DFO) but modified with a bisphosphonate moiety to enhance complex stability. Two synthetic pathways were proposed and experimentally explored; however, the final target structure could not be obtained. The second objective focused on the functionalisation of commercially available superparamagnetic iron oxide nanoparticles (SPIONs), coated with dextran, to create bimodal probes. These were conjugated with the Atezolizumab (anti-PD-L1 monoclonal antibody), DFO, and ^{89}Zr .

The resulting probes were characterised in terms of their physicochemical properties and biological behaviour. Two radiolabelling methods were optimised and compared, alongside two-step bioconjugation protocol. Amount of antibody and chelator introduced into the SPION structure was confirmed with appropriately selected analytical methods. Structural confirmation was achieved via X-ray photoelectron spectroscopy (XPS), while size and surface charge were assessed using dynamic light scattering (DLS). Quantitative assays were developed to determine the degree of functionalisation. *In vitro* studies demonstrated target specificity, internalisation, and cytotoxicity, while relaxivity measurements at two magnetic field strengths revealed the influence of surface modifications on MRI contrast properties. *In vivo* PET/CT and MRI imaging of mice bearing PD-L1-positive tumour xenografts, followed by *ex vivo* biodistribution, showed rapid accumulation of the probes in the hepatobiliary system.

To enable the integration of nanoparticle-based probes into the portfolio of the Radiopharmacy and Preclinical PET Imaging Unit (ZRO), it was necessary to develop and implement synthetic and analytical protocols previously unused in the Unit. The methodologies established in this project lay the groundwork for future adaptation of similar compounds, varying in isotope or targeting vector and contribute to the expansion of radiopharmaceutical tools for personalised and theranostic applications.

Keywords: PET/MRI, radiopharmaceutical, contrast agent, bimodal probes, immunotherapy, oncological diagnostics, relaxivity