Development of novel bimodal PET/MRI probes targeting PD-L1

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Positron emission tomography (PET) is an imaging technique that is widely used in early detection and treatment follow up of many diseases, including cancer. It uses radiolabelled molecules to visualise molecular events such as tumour metabolism, receptor expression and drug metabolism pathways. With the increasing knowledge of immunological mechanisms in cancer cells, the need for monitoring these processes led to the development of the immuno-PET, which uses radiolabelled monoclonal antibodies (mAb) or their fragments. Accordingly, immuno-PET allows to accurately identify the presence and accessibility of target of interests and provide a rapid assessment of tumour response to a variety of treatments in a timely fashion. Furthermore, immuno-PET images can provide information about the heterogeneity of both target expression and therapeutic response.

In routine clinical practice PET is combined with computed tomography (CT), which adds the anatomical reference to the acquired images. **Recently, several studies have demonstrated that integrating PET into a magnetic resonance scanner is a promising new imaging modality, which may replace PET/CT in selected cancer scenarios**. The high spatial resolution of MRI in combination with the very high sensitivity and specificity of PET, may provide valuable insights into the functional and molecular characteristics of cancer. However, the number of hybrid agents applicable in PET/MR imaging is very limited so far. Therefore, in this project **we aim to investigate the potential of a novel bimodal PET/MRI nanoprobe targeting programme cell death ligand (PD-L1).** Recently, the substantially improved response rates to anti-PD-L1 therapeutics were reported in case of patients with PD-L1 overexpression. Therefore, such a bimodal probe could guide the selection of patients who most likely will benefit from immune checkpoint inhibitors.

The proposed **bimodal probe** will consist of: **i) superparamagnetic iron oxide nanoparticles** (SPIONs) proven to act as MRI contrast agents (CA); **ii) atezolizumab (anti-PD-L1 mAb)**; **iii) zirconium-89 (^{89}Zr)**, a PET radioisotope.

Initially we will evaluate the physicochemical properties of the probe (morphology, size, hydrodynamic size and zeta potential, as well as radiopharmaceutical stability), followed by binding specificity, internalisation, and cytotoxicity *in vitro*. Moreover, we will investigate the probe *in vivo* using mice bearing subcutaneous PD-L1+ve and PD-L1-ve xenografts. The biodistribution studies will confirm whether the probe specifically recognises PD-L1 and allow to establish the conditions for PET/MRI imaging studies. Finally, PET imaging will be co-registered with MR data to precisely describe nanoprobe disposition/retention *in vivo*. Images will be quantified, and probe uptake in the tumour and selected organs correlated with e.g. PD-L1 expression measured by immunohistochemistry.

These studies will confirm whether molecular imaging using our bimodal probe specifically targeting PD-L1 has potential to non-invasively monitor the expression level of PD-L1. Furthermore, it will help to decide on how to proceed with future studies utilizing these radio-nano-probe for magnetic hyperthermia and to target different proteins (e.g. EGFR). Of note, the reports published so far on the combination of immuno-PET and MRI are mostly focused on imaging of infectious diseases, not cancer visualisation. Therefore, **this early-stage project is highly innovative and has tremendous translational potential** that could lead to the development of novel imaging biomarkers for specific cancer detection and monitoring of therapeutic strategies.