Anti-CD38 nanobodies as theranostic agents for multiple myeloma

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Theranostic agents include molecules with a combined diagnostic and therapeutic capability and nanobodies (Nbs) are increasingly used in this field. Nbs are single-domain antigen-binding fragments derived from Camelidae heavy-chain antibodies. They have several advantages such as their small size leading to better tissue penetration, favourable pharmacological properties and ability to recognise small, buried epitopes. The current project aims to use nanobodies directed against CD38 as diagnostic and therapeutic tools in the management of multiple myeloma (MM) disease, an incurable malignancy characterized by the accumulation of monoclonal plasma cells in the bone marrow. The differentiation marker CD38 is highly expressed on myeloma cells isolated from patients with a newly diagnosed or a relapsing disease. Our Nb, retained for its affinity, stability, favorable biodistribution and capacity to recognize myeloma cells in a xenograft model, was coupled to Indium-111 and Lutetium-177 and intravenously injected in tumor bearing mice to assess its diagnostic and therapeutic value.

The functionality of ^{111}In-DTPA-Nb2F8 was verified by saturation binding experiments (serial dilutions of the conjugated Nb) on CD38^{+} RPMI-8226 cells and internalisation experiments were performed to assess their therapeutic potential. A minor internalisation (about 20% of the initial bound activity) was observed in the first hours and remained stable during 24H. Micro-SPECT/CT images of mice bearing CD38^{+} RPMI-8226 tumors and injected with ^{111}In-DPTA-Nb2F8 showed specific tumor targeting 1 hour and at least until 48 hours after injection with a low background signal already 1 hour post-injection (p.i.), except kidneys. The in/ex vivo biodistribution data revealed uptake values in tumor of 3.1% IA/g and 1.4% IA/g, 1 hour and 48 hours p.i., respectively, for ^{111}In-DTPA-Nb2F8 while ^{111}In-DTPA-NbCTRL noted a tumor uptake of 0.54% IA/g and 0.1% IA/g organ 1 hour and 48 hours p.i., which is significantly lower than anti-CD38 Nbs confirming the specific targeting of these Nbs. Similar biodistributions were found with ^{177}Lu-DTPA-Nb2F8. The tumor uptake values were at early time point around 4.5% IA/g organ without significant modification up to 48 H post-injection. Kidneys uptake values peaked at 18% IA/g 1 hour post-injection and then decreased to 2% IA/g at 24 H p.i. and 1% IA/g at 48 H p.i. Radioactivity concentration in the other major organs and tissues was low, with values below 2% IA/g at early time points and decreasing over time. In order to assess the therapeutic potential of this nanobody, mice with subcutaneously injected RPMI-8226 cells were randomly categorised into 3 groups (n=10). Mice in each group received 3 intravenous (i.v.) injections (D20, D24 and D27) of a phosphate-buffered saline (PBS) containing either 37MBq ^{177}Lu-DTPA-Nb2F8, 37MBq ^{177}Lu-DTPA-NbCTRL, or PBS. While both vehicle and ^{177}Lu-DTPA-NbCTRL showed progression of tumor masses, the tumors that received Nb2F8 all regressed and tumor development was significantly delayed

In conclusion, this is the first report on CD38-binding Nbs used for the identification and treatment of MM cells by conjugating them to diagnostic and therapeutic isotopes.