A site-specific radiolabelling strategy of Nanobodies for PET imaging

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Introduction: Nanobodies, or single-domain antibody fragments (sdAbs), are antigen-binding fragments derived from Camelid heavy-chain-only antibodies (VHH). Their high affinity and specificity, as well as their fast clearance kinetics make them excellent probes for PET imaging.

Methods: The sdAb was engineered to have the recognition motif of the Sortase A enzyme, allowing site-specific coupling of the BFCs at its C-terminal. Characterisation was performed through Mass Spectrometry (ESI-Q-TOF), SDS-PAGE and Western Blot.

NOTA-sdAb was radiolabelled with 68Ga and RESCA-sdAb with Al-18F. Radiochemical purity (RCP) and stability were assayed using SEC and iTLC.

Results: Site-specific functionalised sdAb with NOTA or RESCA was obtained with high purity (≥99%) in 52% and 59% yields respectively.

Radiolabelling of NOTA-sdAb with 68Ga was performed in a 76% decay-corrected radiochemical yield (DC-RCY), ≥99% RCP with apparent molar specific activity of 63 GBq/µmol. The radiolabelled probe was stable in vitro.

Radiolabelling of RESCA-sdAb with Al-18F was performed in a 29% DC-RCY and with a RCP ≥99%.

Conclusion: The Sortase A enzyme coupling allowed to obtain a site-specifically labelled probe for 68Ga or Al-18F radiolabelling using NOTA or RESCA chelators. The next step is comparison of in vivo stability and in vivo tumor targeting studies of both radiolabeled probes, and to select the most suitable probe for clinical translation.

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement N° 675417. Crauwels M. is funded by FWO (G086615N). Broos K. is funded by the Agency of Innovation by Science and Technology. Lecocq Q. is funded by the FWO-SB grant (1S24218N).Keyaerts M is a senior clinical investigator of FWO.