**Effect of charges of radiolabeled Nanobodies on kidney retention**

Maxine Crauwels¹,², Sam Massa²,³, Charlotte Martin⁴, Steven Ballet⁴, Mathias D’Huyvetter¹, Vicky Caveliers¹,⁵, Tony Lahoutte¹,⁵, Sophie Hernot¹, Nick Devoogdt¹, Catarina Xavier¹, Serge Muyldermans²

¹In Vivo Cellular and Molecular Imaging Laboratory, Vrije Universiteit Brussel (VUB) – Brussels, Belgium
²Laboratory of Cellular and Molecular Immunology, VUB
³Myeloid Cell Immunology, VIB
⁴Research Group of Organic Chemistry, VUB
⁵Nuclear Medicine Department, UZ Brussel, Belgium

Radiolabelled Nanobodies (Nbs) are used as diagnostics, therapeutics and theranostics. Kidneys are their main route of elimination from the body and previous studies revealed the impact of C-terminal charges of radiolabeled Nbs on kidney retention. The removal of polar positively charged amino acids decreased kidney retention. We hypothesized that negatively charged amino acids at the C-terminal end of Nbs or spread over the Nb surface might reduce their kidney retention.

An anti-HER2 Nb was site-specifically coupled, via the Sortase reaction, to different probes (H-GGGXnYK(DTPA-CHX-A’’)-NH₂, with n=0 or 4 and X= A, R, E or D). Alternatively, NbT1, NbT2, NbT3 and NbT4 (with decreasing pI from T1 to T4) were coupled to the DTPA-CHX-A’’ chelator. Sortase reaction yields were between 20% and 60%. All Nbs were labelled with ¹⁷⁷Lu. High radiochemical purity (99%), and radiochemical yields ranging from 65% to 87% were obtained.

When administered in mice, α-HER2 Nbs coupled to H-GGGXnYK(DTPA-CHX-A’’)-NH₂, with n = 0 resulted in 26.6±3.1% of the injected activity (%IA) in the kidneys at 1 hr post injection (p.i.). For n = 4 and X = A, R, E or D, accumulations of 30.2±2.5%, 48.8±2.2%, 44.5±7.7% and 42.5±2.0% IA were observed, respectively. For Nbs where charges are more evenly distributed over their surface, we observed an accumulation of 50.2±8.3% IA for NbT1 at 1 h p.i.. For NbT2, NbT3 and NbT4 accumulations of 39.8±5.7%, 33.3±3.6% and 14.2±1.3% IA were noted. The %IA measured 3 and 24 hrs p.i. showed the same tendency.

Introducing extra negative or positive charges at the C-terminal of Nbs resulted in unwanted higher kidney retention. However, Nbs of lower pI tend to have reduced kidney retention.