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Publication date:
2019

Citation for published version (APA):
**Effect of charges of radiolabeled Nanobodies on kidney retention**

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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-GGG\textsubscript{x}YK(DTPA-CHX-A’’)-NH\textsubscript{2}, with n=0 or 4 and X= A, R, E or D). Alternatively, NbT1, NbT2, NbT3 and NbT4 (with decreasing pl 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 \textsuperscript{177}Lu. High radiochemical purity (99%), and radiochemical yields ranging from 65% to 87% were obtained.

When administered in mice, α-HER2 Nbs coupled to H-GGG\textsubscript{x}YK(DTPA-CHX-A’’)-NH\textsubscript{2}, 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 pl tend to have reduced kidney retention.