Procedures for the production of ^{90}Nb-labeled monoclonal antibodies and stability studies.

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Introduction: The positron emitter ^{90}Nb is a promising radionuclide for applications in positron emission tomography (PET). ^{90}Nb has a favorable half-life (14.6 hours), which conveniently allows extended labeling procedures including quality control. Furthermore, the half-life enables the visualization of medium and slow biological processes such as pharmacokinetics of nanobodies (fragments of antibodies), polymers and antibodies. Another advantage of ^{90}Nb are the decay parameters: relatively high positron branching of 51% and a rather low β^+-energy of E_{mean}= 662 keV (E_{max}= 1,5 MeV), which provides good quality and high resolution images even with low amounts of ^{90}Nb.

Experimental: Labeling strategies were developed with retuximab (MabThera®). As chelator for ^{90}Nb deferoxamine (Df) was used. Deferoxamine coupled to antibodies via two difference methods.

In the first method N-SucDf [1] was used. In short, the chelator Df was converted into N-succinyl- deferoxamine B (N-sucDf) and hydroxamate groups of N-sucDf were temporarily blocked with Fe(III). N-sucDf-Fe was esterified with TFP, and subsequently TFP-N-sucDf-Fe was coupled to the mAb. Thereafter, Fe(III) was removed by transchelation to ethylene-diaminetetraacetic acid (EDTA) (formation of [Fe(III)EDTA]).

The novel bifunctional chelator p-isothiocyanatobenzyl-deferoxamine B (Df-Bz-NCS) [2] from Macrocyclics (Dallas, USA) was employed in the second approach. In short, while gently shaking, a threefold molar excess of Df-Bz-NCS (in 20 µl DMSO) was added to the mAb (2-10 mg in 1 ml 0.1M $NaHCO_3$ buffer solution, pH 9.0), and incubated for 30 min at 37 °C. Non-conjugated chelator was removed by size exclusion chromatography using a PD-10 column (GE Healthcare Life Sciences) and 0.9% sodium chloride / gentisic acid 5 mg/ml (pH 5.0) as eluent.

After modification of the antibody, labeling with ^{90}Nb was applied. After an anion-exchanger column, purified no-carrier added ~30 MBq of ^{90}Nb in 200 µl 6M HCl / 0.01M oxalic acid were neutralized with 180 µl of 6M NaOH and 230 µl 1M NaOH. Thereafter, 1.2 ml (N-SucDf) and 390 µl (Df-Bz-NCS) of 0.5M HEPES buffer was added to create favorable condition for the antibodies. Finally, retuximab (5 mg/ml) 250 µl (N-SucDf) and 1 ml 1.5 mg/ml (Df-Bz-NCS) was added. The final volume in both procedures was 2 ml. The mixtures were incubated for 60 min at room temperature under gently stirring. Labeling kinetics were checked by ITLC and after 60 min by HPLC analysis. Residues of free ^{90}Nb were separated by using a PD-10 column. Products were analyzed by ITLC and HPLC.

Stability studies were performed in buffer solution (pH 7) at room temperature for 18 days or with a 4-fold excess of fresh fetal calf serum (FCS) at 37 °C for 9 days.

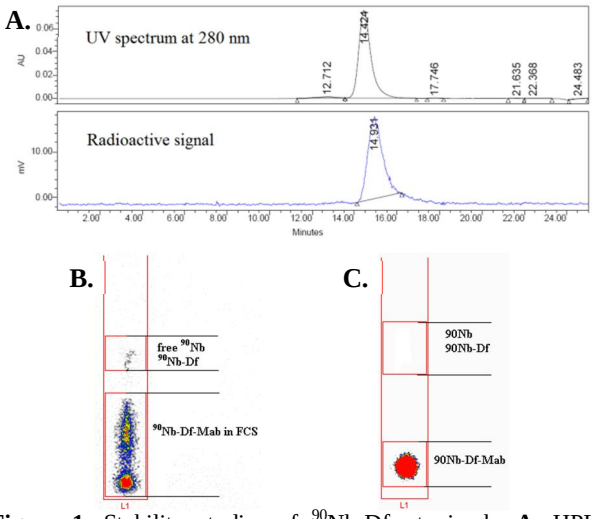

Figure 1. Stability studies of ^{90}Nb-Df-retuximab: **A.** HPLC profile of stability studies at room temperature at 4 days. **B.** ITLC image of stability studies in FCS at 3 days. **C.** ITLC image of stability studies at room temperature at 4 days.

Results: The analysis via HPLC (430 nm UV) showed a coupling yield of the first N-sucDF-method of around 1.5 Df-molecules per molecule of rituximab.

In the second approach, the coupling yield could not be determined.

For both approaches the labeling yield after 1 hour was more than 90% (91% ITLC, 94% HPLC). Labeling kinetics showed already a labeling yield of 77% after 15 min and 90% after 50 min. The PD-10 column purification gave a product purity of ≥ 97%.

The stabilities studies revealed only minimal difference between both methodologies. At room temperature in buffer solution at pH 7, in both cases, less than 1% of degradation was observed after 18 days. In fetal calf serum less than 5% degradation were observed up to 5 days. After 5 days, degradation slightly increased to reach 10% after 9 days.

References

[1] Verel I. *et al., J. Nucl. Med.* 2003, 44, 1663–1670.

[2] Perk L.R. *et al., Eur. J. Nucl. Med. Mol. Imag.* 2010, 37, 250-259.

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