Conjugation, labeling and initial *in vivo* assessment of an anti-VEGF monoclonal antibody labeled with niobium isotopes

V. Radchenko[1], P. Bouziotis[2], G. Loudos[3], S. Xanthopoulos[2], H. Hauser[4], M. Esienhut[4], B. Ponsard[5], F. Roesch[1]

[1]Institute of Nuclear Chemistry, Johannes Gutenberg University, Mainz, Germany; [2]Institute of Radioisotopes and Radiodiagnostic Products, N.C.S.R. "Demokritos", Athens, Greece; [3]Department of Medical Instruments Technology, Technological Educational Institute of Athens, Athens, Greece; [4]Radiopharmaceutical Chemistry, German Cancer Research Center, Heidelberg, Germany; [5]Department of Nuclear Material Science, Belgian Nuclear Research Centre, Mol, Belgium

^{90}Nb is a potential PET nuclide ($T_{1/2}$ = 14.6 h and high positron branching 53%). Promising results in labeling and *in vitro* evaluation of ^{90}Nb-labeled monoclonal antibodies confirm expectation about ^{90}Nb as an appropriate isotope for *immuno*-PET [1]. Our latest efforts are focused on the labeling of bevacizumab with Niobium-90, a new potential isotope for *immuno*-PET imaging

Bevacizumab (Avastin®) was pre-modified with Df-Bz-NCS [2]. In short, while gently shaking, a threefold molar excess of Df-Bz-NCS (in 20 µL DMSO) was added to the mAb (3 mg/mL in 1 ml 0.1 M $NaHCO_3$ buffer, pH 9.0), and incubated for 30 min at 37 °C. Non-conjugated chelator was removed by size exclusion chromatography (SEC) using a PD-10 column and 0.9% sodium chloride solution as eluent.

Cyclotron-produced ^{90}Nb was applied for PET imaging and reactor-produced ^{95}Nb ($T_{1/2}$ 35 days) was involved in biodistribution studies. Df-Bz-NCS-bevacizumab was labeled with $^{90/95}$Nb at room temperature in a volume of 2 ml under gently stirring for 60 min. Finally, $^{90/95}$Nb-Df-Bz-NCS-bevacizumab was purified by PD-10 column.

Metabolic stability of ^{95}Nb-Bevacizumab was studied in fresh human plasma. The plasma was collected and incubated with ^{95}Nb-Bevacizumab at 37°C. Aliquots of the sample were withdrawn at 60 min, 3 h, 3 d, 5 d and 7 d, and analyzed by TLC.

Female Athymic SCID mice (average weight 20 g, 5 weeks) were inoculated subcutaneously into the right front leg with M165 cells (1 x 10^7 cells/animal) in 100 µL fetal bovine serum-free medium. When tumors reached a size of 0.2 to 1 g (i.e. 10 to 15 days for all cell lines involved), biodistribution studies were performed. Mice were injected via the tail vein with (270 kBq/100 µg). Groups of three animals were sacrificed at 4, 24, 48 and 72 hours after injection of radiolabeled antibody. Tumors and organs (heart, liver, stomach, intestines, spleen, muscle, lungs, pancreas) were excised, blotted dry and weighed.

PET imaging was performed on an experimental, small field-of-view PET camera of the Medical Instruments Department of the Technological Educational Institute of Athens.

Tumor-bearing SCID mice were injected with 200 KBq/100 µg of ^{90}Nb-bevacizumab (volume 100 µL) and were anaesthetized by i.p. injection of 100 µl/10 g mouse body weight of a cocktail solution of ketamine/xylazine. Dynamic imaging was not performed, due to the low specific activity of the radiolabeled species. On the contrary, twenty-four hour static images were acquired. At 24 hours p.i. the tumor was clearly visualized.

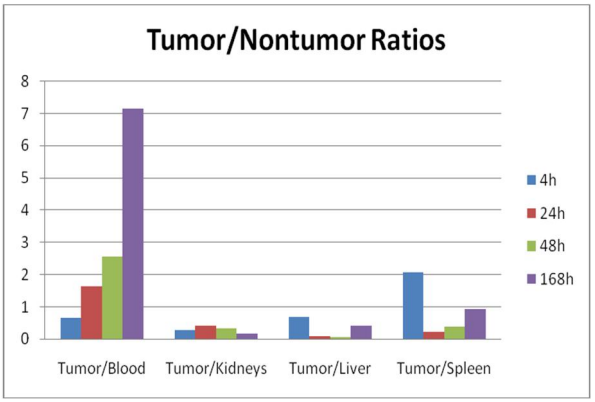

Figure 1: Tumor/Non-tumor ratios of ^{95}Nb-bevacizumab at 4, 24, 48 and 168 h p.i.

Yield of the total purified product ($^{95/90}$Nb) was 50-60%, while radiochemical purity was more than 97%. In vitro stability of ^{95}Nb-Bevacizumab was monitored up to 7 days post-labeling, showing less than 10% product degradation. *In vivo* biodistribution studies show increased tumour uptake and also a satisfactory tumour/blood ratio (Figures 1). Co-injection of an excess unlabeled antibody resulted in a significant decrease in radioactivity concentration in the tumor, indicating VEGF-mediated antibody uptake. Static PET imaging of ^{90}Nb-bevacizumab at 24 h p.i. showed a clear visualisation of the tumour.

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References

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