18F-Anti-MMR-nanobodies for PET imaging of tumor-associated macrophage subtypes as surrogate markers for tumor hypoxia.

Anneleen Blykera1, Catarina Xavier2, Ilse Vaneyckens2, Damya Laoui3, Nick Devooagdt3, Matthias D’huyvetter3, Tony Lahoutte3,4, Jo A. Van Ginderachter3 and Vicky Caveliers1,2

1In vivo Cellular and Molecular Imaging (ICMI), Vrije Universiteit Brussel, Belgium; 2 Nuclear Medicine, UZBrussels, Belgium, 3Laboratory of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Brussels, Belgium.

Summary
Tumor-associated macrophages (TAMs) are an important component of the tumor stroma. Macrophages can mainly be divided in two subtypes: the M1 and M2 macrophages. This latest subtype of macrophages, having a more malignant phenotype, express the Macrophage Mannose Receptor (MMR). Previously in our University it was proven that anti-MMR nanobodies (single domain antibodies) targeting MMR+ TAMs accumulated in hypoxic regions1. As reduced oxygen levels in a tumor influences the clinical outcome, the development of anti-MMR nanobody probes for PET imaging of tumor hypoxia is very interesting. The pharmacokinetic profile of nanobodies is optimal for imaging with short-lived radioisotopes such as 18F. Here we present the development of 18F-labeled anti-MMR nanobodies using the 18F-SFB method.

Methods
Synthesis of 18F-SFB
The automated synthesis and purification of 18F-SFB was optimized on the Synthera platform comprising two-coupled synthesis modules (v1 and v2). The 18F-SFB prosthetic group is produced using the original disposable 18F-FDG cassette (IFP18F-nucleophilic) and editing the existing 18F-FDG-script.

Figure 1: The synthesis of 18F-SFB

Conjugation between 18F-SFB and nanobody
After incubation of the nanobody with 18F-SFB for 10 min at room temperature and pH 8.5, the radiolabeled nanobody was purified with a Sephadex G25 disposable column (PD-10).

Assessment of radiochemical purity was performed by RP-HPLC. Stability of 18F-Nanobody was evaluated by incubation in PBS or human serum for up to 2 h and analyzed by size exclusion chromatography (SEC).

Figure 2: Conjugation of 18F-SFB with Nb

Ex-vivo biodistribution and tumor targeting
C57Bl/6-mice have been subcutaneously inoculated with 3·10^6 3LL-R cells on the left hindlimb. After tumor growth, the tracer was injected and 1 and 3 hours post injection the animals were dissected and the activity distribution within the different organs and tissues measured ex vivo. Tumors grown in MMR-deficient (Knock-out) mice were used to prove specificity.

Results
Synthesis and conjugation
18F-SFB can be successfully synthesized using the Synthera platform with a yield of about 40%.

The radiochemical purity was >99% after AFFINIMIP purification. The formed 18F-SFB is coupled to the Nb with a yield of 10% using 1mg/ml Nb at pH 8,5 reacting 30’ at RT. 18F-anti-MMR nanobody was obtained with >97% radiochemical purity. The labelled Nanobody was stable in PBS and human serum and keeps his functionality after labeling with fluorine.

Figure 3: purified 18F-SFB

Ex-vivo biodistribution and tumor targeting
The biodistribution profile (1h and 3h p.i.) showed specific uptake in liver, spleen, tumor and clearance via the kidneys. Specificity has been confirmed by comparison with MMR-deficient mice. The degree of kidney uptake of fluorinated Nanobody was remarkably 88% lower compared to 225mTc analogs.

Figure 5: Increased tumor targeting and specificity was observed for 18F-SFB anti-MMR-Nb in wild-type (WT) compared to MMR/KO tumor bearing mice (3h p.i.).

Conclusions
We showed for the first time the successful labeling of anti-MMR targeting Nanobodies with 18F, using the Synthera module. This PET-probe targets the MMR-receptor in-vivo, enabling his use in the clinic.