

## ***In vivo* SPECT imaging of liver inflammation in a murine model of nonalcoholic steatohepatitis.**

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**Introduction.** We recently developed <sup>99m</sup>Tc-cAbVCAM1-5, a single domain antibody (sdAb) derived radiotracer directed against the inflammatory marker Vascular Cell Adhesion Molecule 1 (VCAM-1). This imaging agent has been fully validated for the non-invasive imaging of murine atherosclerotic lesions (1-2), and is currently under clinical translation for this application. However, VCAM-1 is considered as a potent inflammatory marker not only in the field of atherosclerosis, but in several other clinically relevant pathophysiological settings such as chronic liver inflammation. Thereby, <sup>99m</sup>Tc-cAbVCAM1-5 imaging was here evaluated in a nutritional mouse model of nonalcoholic steatohepatitis (NASH).

**Methods.** Thirty 12-wks old male C57Bl/6J mice were used (n=10/group). Mice were either fed a standard diet (STD) or methionine and choline deficient diet (MCD) to induce NASH. Longitudinal SPECT imaging was performed at baseline and at 4 wks and 8 wks following diet onset using either the anti VCAM-1 (V) or an irrelevant control sdab (C). Results were expressed as standardized uptake values (SUV). Following the 8 wks time point mice were euthanized and post mortem analysis were performed.

**Results.** MCD diet induced a 10-fold increase in serum alanine transaminase (ALT) levels both at 4 and 8 wks in comparison to STD diet (p<0.001). Moreover Oil Red O staining showed large lipid vacuoles in the liver of MCD fed mice. Finally, as demonstrated by ELISA, a significant 5-fold increase in the level of hepatic VCAM-1 expression was observed in MCD in comparison to STD fed group (MCD: 5.6±1.7 vs STD: 1.1±0.5 ng/mg, P<0.001).

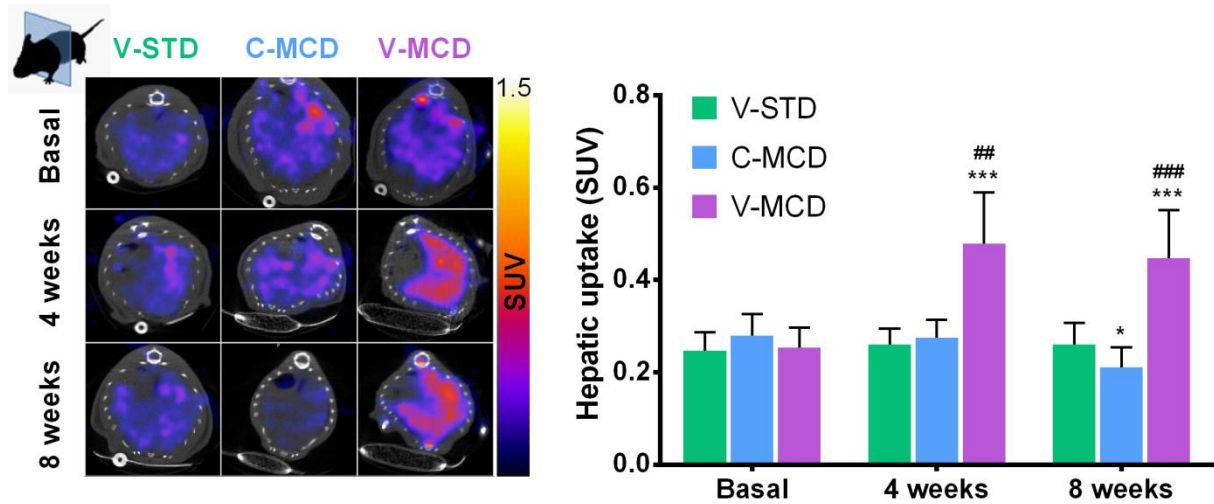
Robust <sup>99m</sup>Tc-cAbVCAM1-5 uptake in the liver of MCD fed mice (V-MCD) was readily observable by SPECT at 4 wks and remained unchanged at 8 wks. In comparison, modest liver uptake was found in control group fed a standard diet (V-STD) and in control group injected with the irrelevant control sdab (C-MCD) at all investigated time points. Image quantification further confirmed these results. Indeed <sup>99m</sup>Tc-cAbVCAM1-5 hepatic uptake significantly increased in MCD fed mice between baseline and 4 wks, and then remained unchanged at 8 wks (SUV = 0.25±0.04; 0.48±0.11 and 0.45±0.10 respectively, p<0.001 vs baseline at 4 and 8 wks), whereas no significant change was found in control groups. Moreover, SPECT imaging quantification performed at 8 wks significantly correlated with ex vivo biodistribution and with the level of VCAM-1 expression (p<0.001 for both).

**Conclusions.** The present data demonstrate that MCD diet increases VCAM-1 hepatic levels in mice. Robust and specific uptake of <sup>99m</sup>Tc-cAbVCAM1-5 was visible as early as 4 wks following MCD diet onset. <sup>99m</sup>Tc-cAbVCAM1-5 can therefore be employed for the non-invasive longitudinal imaging of

NASH in mice, and further studies are ongoing in order to evaluate the sensitivity of this technique for the monitoring of NASH therapy.

[1] Circ Res. 110(7):927-37. 2012

[2] J Nucl Med. 55(10):1678-84. 2014



Representative transversal sections obtained at the level of the liver at baseline, 4 wks and 8 wks together with image quantifications. No change in hepatic uptake was found in control diet nor in control sdab groups (V-STD and C-MCD) whereas robust  $^{99m}\text{Tc-cAbVCAM1-5}$  uptake was found in MCD fed mice at 4 and 8 wks (V-MCD). \*  $p<0.05$  and \*\*\*  $p<0.001$  vs V-STD; ##  $p<0.01$  vs basal. ###  $p<0.001$  vs basal.