

Supplementary Materials: Tracking Radiolabeled Endothelial Microvesicles Predicts Their Therapeutic Efficacy: A Proof-of-Concept Study in Peripheral Ischemia Mouse Model Using SPECT/CT Imaging

Romain Giraud, Anaïs Moyon, Stéphanie Simoncini, Anne-Claire Duchez, Vincent Nail, Corinne Chareyre, Ahlem Bouhlel, Laure Balasse, Samantha Fernandez, Loris Vallier, Guillaume Hache, Florence Sabatier, Françoise Dignat-George, Romaric Lacroix, Benjamin Guillet and Philippe Garrigue

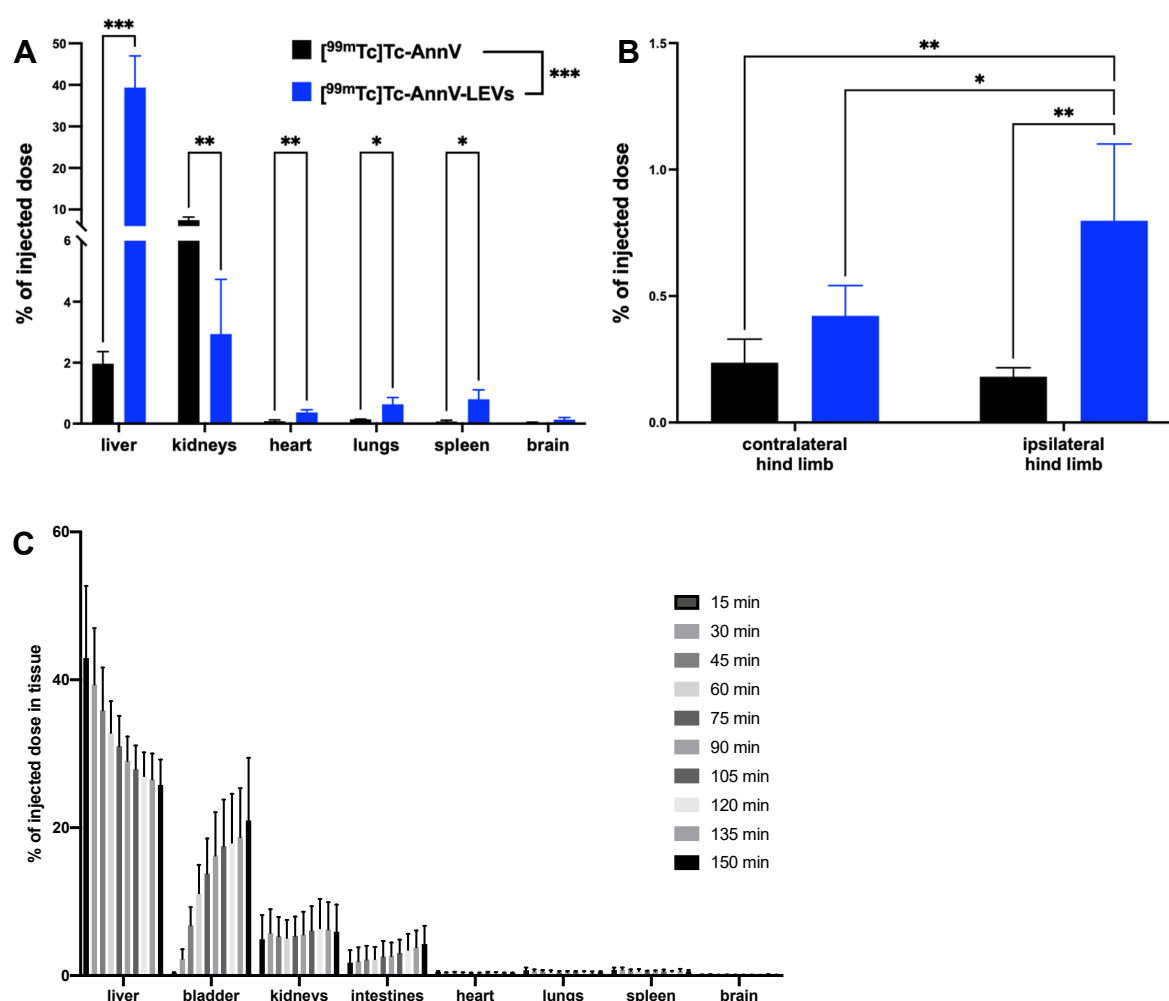


Figure S1. SPECT signal quantification in organs and hind limbs expressed as percentage of the injected dose (%ID). **A and B:** MicroSPECT/CT signal quantifications of free $[^{99m}\text{Tc}]\text{Tc-AnnV}$ biodistribution (black bars, $n = 3$) and $[^{99m}\text{Tc}]\text{Tc-AnnV-LEV}$ biodistribution (blue bars, $n=10$) 30 min after injection (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$). **C:** MicroSPECT/CT signal quantifications of $[^{99m}\text{Tc}]\text{Tc-AnnV-LEV}$ biodistribution ($n = 10$) each 15 min, from 15 min post-injection to 150 min post-injection. The rapidly increasing signal quantified in the bladder from 45 min after injection presumes in vivo degradation of LEVs.

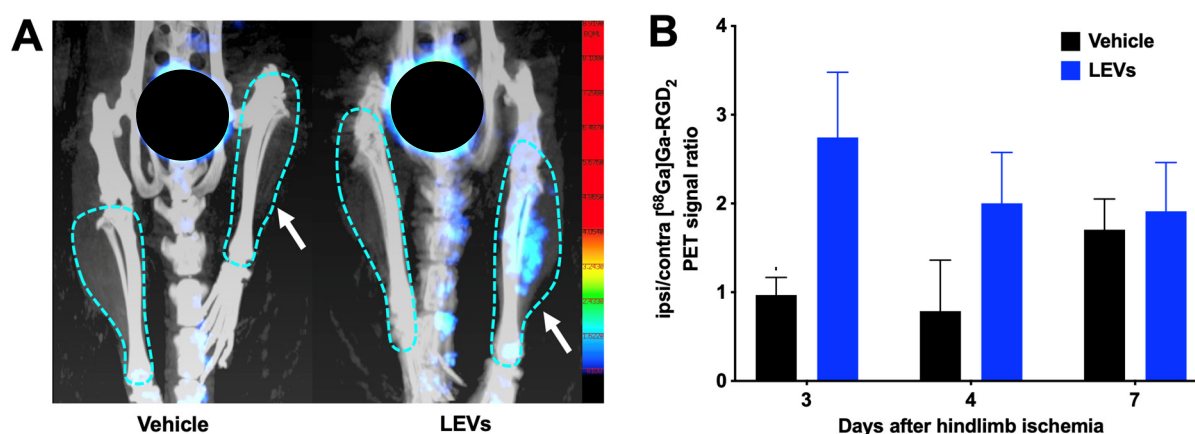


Figure S2. Angiogenic activation in a mouse model of hindlimb ischemia treated by LEVs or vehicle. (A) Representative images of ^{68}Ga [Ga-RGD₂] microPET/CT on day 3 after ischemia in vehicle- ($n = 3$, black bars) or LEV-treated mice ($n = 3$, blue bars). The blue dots delimit regions of interest, ipsilateral hind limb on the right and contralateral hind limb on the left side of each animal. (B) ^{68}Ga [Ga-RGD₂] microPET/CT quantifications on days 3, 4 and 7 after ischemia.

Follow-up and quantification of angiogenic activation by ^{68}Ga [Ga-RGD₂] microPET/CT imaging

^{68}Ga [Ga-RGD₂] was prepared by mixing 20 μg of NODAGA-RGD dimer peptide (RGD₂, ABX Biochemicals) with 150 μL of 4.0 $\text{mol}\cdot\text{L}^{-1}$ ammonium acetate buffer, then with 500 μL of a fresh ^{68}Ga [GaCl₃] solution eluted with pharmaceutical-grade 0.1 $\text{mol}\cdot\text{L}^{-1}$ hydrochloric acid (Eckert&Ziegler, Berlin, Germany) from a pharmaceutical-grade ^{68}Ge [Ge]/ ^{68}Ga [Ga] generator (Galliapharm®, Eckert&Ziegler, Berlin, Germany), at room temperature for 5 min. Radiochemical purity was assessed by instant thin layer radiochromatography using a radiochromatograph (miniGITA, Elysia-Raytest, Straubenhardt, Germany), iTLC-SG paper (Agilent, Santa Clara, USA) as stationary phase, a freshly prepared 0.1 $\text{mol}\cdot\text{L}^{-1}$ sodium citrate pH 5 solution as mobile phase A (Rf₀: ^{68}Ga [Ga-RGD₂] + ^{68}Ga [Ga-colloids]; Rf₁: free ^{68}Ga [GaCl₃]) and a 50:50 mixture of freshly prepared 1 $\text{mol}\cdot\text{L}^{-1}$ ammonium acetate solution with methanol as mobile phase B (Rf₀: free ^{68}Ga [GaCl₃] + ^{68}Ga [Ga-colloids]; Rf₁: ^{68}Ga [Ga-RGD₂]). A radiochemical purity above 95% enabled to validate the radiolabeling. Mice ($n = 3$ per condition) were injected with 6.0 ± 1.1 MBq/50 μL of ^{68}Ga [Ga-RGD₂] in the caudal vein on days 3, 4, and 7 post-ischemia. Twenty-minute-long micro-Positron Emitting Tomography coupled with Computed Tomography (microPET/CT) images were acquired 60 min after ^{68}Ga [Ga-RGD₂] injection on a NanoscanPET/CT camera (Mediso, Budapest, Hungary) under 1.5% isoflurane anesthesia. Quantitative ROI analysis of PET signal was performed on attenuation- and decay-corrected PET images using InterviewFusion software (Mediso, Budapest, Hungary) and tissue uptake values were expressed as a mean $\pm$ sd ischemic-to-contralateral (i/c) hindlimb signal ratio.

Endothelial LEV administration was associated with earlier angiogenic activation.

As soon as day 3 post-ischemia, ^{68}Ga [Ga-RGD₂] microPET/CT quantifications showed a higher i/c radiotracer uptake ratio in LEVs-treated animals compared to vehicle group (respectively 2.7 ± 0.7 and 0.9 ± 0.2 ; $n = 3$) maintained up on day 4 (respectively 2.0 ± 0.6 and 0.8 ± 0.6 , $n = 3$). Both groups presented an elevated i/c radiotracer uptake ratio on day 7 (vehicle: 1.7 ± 0.3 , LEVs: 1.9 ± 0.6 , $n = 3$).

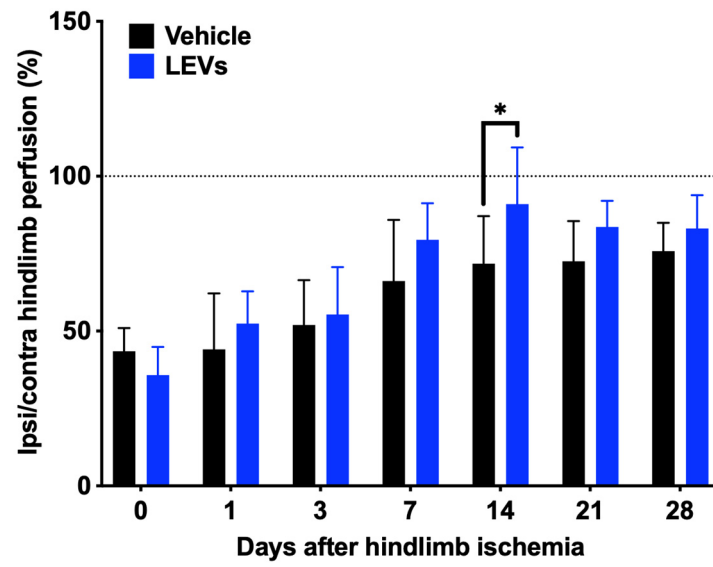


Figure S3. Quantitative analysis of LASER Doppler signal expressed as ischemic-to-contralateral muscle ratio (%; mean $\pm$ sd) from day 0 to day 28 in vehicle- ($n = 10$, black bars) or LEV-treated mice ($n = 10$, blue bars) (* $P < 0.05$).