

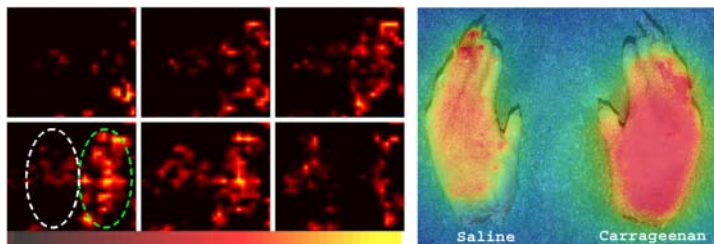
## <sup>99m</sup>Tc-labeled Bispecific Cytokine Ligand and SPECT Imaging of Inflammation

Interleukin-1 (IL-1) and Interleukin-18 (IL-18) are potent pro-inflammatory cytokines in various inflammation-related diseases. Their actions are regulated by the IL-1 receptor antagonist (IL-1ra) and IL-18 binding protein (IL-18bp). IL-1ra and IL-18bp can be linked using the Fc portion of human IgG1 to form a two-domain recombinant fusion protein that binds to inflammatory sites via IL-18 and IL-1 pathways. We were recently able to label newly developed IL-18bp-Fc-IL-1ra with <sup>99m</sup>Tc and preliminarily demonstrated the feasibility of the bispecific radioligand, <sup>99m</sup>Tc-IL-18bp-Fc-IL-1ra, for targeting inflammatory sites using high-resolution SPECT imaging in multiple-animal models.

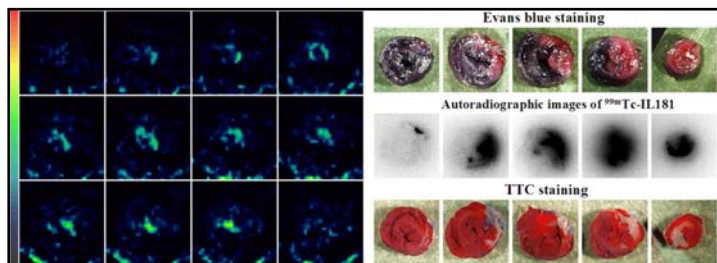
### IL-18bp-Fc-

IL-1ra labeling with <sup>99m</sup>Tc yielded greater than 95% RCP. <sup>99m</sup>Tc-IL-18bp-Fc-IL-1ra was stable in saline, rat serum, and whole blood. SPECT images of <sup>99m</sup>Tc-IL-18bp-Fc-IL-1ra were obtained after intravenous injection in a rat carrageenan-induced paw edema model, mouse skin with inflammation induced by 12-O-tetradecanoyl-phorbol-13-acetate, and an ischemic-reperfused rat heart model created by ligating the left coronary artery for 45 minutes followed by 2-hour reperfusion. Myocardial viability was determined by TTC staining. SPECT imaging showed higher <sup>99m</sup>Tc-IL-18bp-Fc-IL-1ra uptake in the paws with carrageenan-induced edema compared to the controls with saline. The regional inflammatory reaction in mouse skin was detectable by <sup>99m</sup>Tc-IL-18bp-Fc-IL-1ra imaging. Rat cardiac imaging showed radioactive accumulation in areas of the left ventricle that corresponded to ischemic areas at risk as determined by staining with Evans blue dye. Uptake of <sup>99m</sup>Tc-IL-18bp-Fc-IL-1ra in the target tissues was confirmed by autoradiography. Our imaging results indicate that targeting pro-inflammatory cytokines with the novel bispecific radioligand <sup>99m</sup>Tc-IL-18bp-Fc-IL-1ra may provide a suitable approach for specific detection of inflammatory sites.

This is an ongoing project to develop a non-invasive tool for specific imaging of inflammation. We currently focus on detecting inflammatory response in myocardial ischemia-reperfusion injury and atherosclerosis. The data with <sup>99m</sup>Tc-IL-18bp-Fc-IL-1ra have been used as preliminary studies for an NIH R01 grant submission entitled "Technetium-99m-labeled bispecific cytokine ligands and SPECT imaging of inflammation induced by myocardial ischemia and reperfusion." An abstract has been submitted to the 54<sup>th</sup> Annual Meeting of the Society of Nuclear Medicine (1).



**Figure 1.** *Left:* FastSPECT tomographic image (coronal slices) in rat model with carrageenan-induced paw edema using <sup>99m</sup>Tc-IL-18bp-Fc-IL-1ra 2 hours post-injection. The radioactivity in the right hind paw (green dashed mark) treated with inflammation was higher than that in the left hind paw with saline (white mark). *Right:* Co-registered autoradiographic images with the rat paws treated with carrageenan and saline.



**Figure 2.** *Left:* SPECT images (transaxial slices) of <sup>99m</sup>Tc-IL-18bp-Fc-IL-1ra 2-hour post-injection in a rat heart with ischemia-reperfusion. Hot spots were clearly visible on the left ventricular wall. *Right:* Transversal slices from the heart in left panel. Myocardial ischemic area (IAR) was determined by Evans blue dye staining. The distribution of <sup>99m</sup>Tc (dark area) identified by autoradiographic imaging matched with IAR, but was larger than the infarct on TTC staining.

1. Z. Liu, M. M. Hui, L. R. Furenlid, S. Lewis, J. M. Woolfenden, and H. H. Barrett, A novel <sup>99m</sup>Tc-labeled recombinant bispecific ligand targeting Interleukin-1 and Interleukin-18 for inflammation imaging, Submitted to the 54<sup>th</sup> Annual Meeting of the Society of Nuclear Medicine, Washington, DC, 2007.