

SUPPLEMENT

Atherosclerosis

New clinical probes for molecular imaging of atherosclerosis. SHU 555 C. While the USPIO agent ferumoxtran paved the way for clinical molecular MRI of plaque macrophages, newer USPIOs may offer faster pathways to FDA approval. Metz et al. tested the ability of SHU 555 C (Supravist; Bayer Schering Pharma AG, Berlin, Germany), a carboxydextran-coated ferucarbotran nanoparticle, to image carotid inflammation in 12 patients scheduled for CEA (1). At an injected dose of 40 mg Fe/kg body weight, SHU 555 C enhanced MR allowed both early first-pass T1 contrast angiography to define plaque vascularity followed by late (18 hours post-injection) mild T2*-weighted signal loss from SHU 555 C deposition into the plaque. Plaques with late T2* SHU 555 C signal loss (inflammation) corresponded with those exhibiting higher early T1 enhancement (vascularity). CEA histopathology specimens confirmed co-localization of SHU 555 C with resident macrophages. Therefore, SHU 555 C has the potential to localize two important vulnerable plaque markers, vascularity and inflammation, in a single agent, although further studies are needed.

¹¹C-acetate PET imaging of fatty acid synthesis. As fatty acids are commonly found in atheromas associated with foam cell maturation, Derlin et al. retrospectively examined the relationship between CT vascular calcification and carotid, aortic, and iliac artery uptake of ¹¹C-acetate, a PET agent that reports on fatty acid synthesis (2). Nearly 90% of the 36 patients analyzed demonstrated ¹¹C-acetate uptake with mean TBR 2.5, and >80% of patients also had calcified vessels (483 sites identified). However, only 29% (64 of 220 lesions) of the enhanced ¹¹C-

acetate regions demonstrated detectable calcification, and even fewer (13%) of the total calcified segments had significant ^{11}C -acetate uptake, suggesting that only select plaques have biological fatty acid synthesis. In combination with FDG PET/CT inflammation detection, ^{11}C -acetate may provide complementary information to more fully describe plaque atherogenesis and vulnerability. Furthermore, ^{11}C -acetate PET imaging may uncover additional important biological insights into new atherosclerosis therapies, such as the novel reverse cholesterol transport and anti-inflammatory liver X receptor agonist R211945, recently reported by Vucic et al., that exhibited more FDG PET regression than atorvastatin in atherosclerotic rabbits (3). Although further studies must be performed to better characterize the vascular selectivity of ^{11}C -acetate, these promising results imply that it may report on a novel plaque metabolic process.

Preclinical advances. *MMP inhibition and plaque stability.* Another promising potential target for plaque stabilization are matrix metalloproteinases (MMP), enzymes that can degrade and destabilize the thin collagen-rich fibrous cap. To assess the impact of MMP inhibition on atheroma collagen content, a selective oral MMP-13 inhibitor was administered by gavage to ApoE^{-/-} mice or controls (4). After 10 weeks of treatment, carotid plaque MMP activity was evaluated *in situ* with MMPsense 680 (an enzyme-activatable NIRF molecular agent that reports on MMP-13), which was co-injected with iron-oxide nanoparticles to label resident macrophages, and then imaged with multichannel confocal intravital fluorescence microscopy (IVFM). Despite no significant change in atheroma size, smooth muscle cell infiltration, and plaque macrophage content, the MMP-13 inhibitor diminished plaque MMP-13 activity. Histology revealed enhanced intimal and fibrous cap collagen content, and thicker collagen

fibrils as detected by picosirius red staining. Plaque MMPs can also be evaluated non-invasively using I I I-indium RP782 ($^{111}\text{In-RP782}$) and micro-SPECT imaging, as demonstrated in aortic atheroma of ApoE^{-/-} mice (5). Compared to mice that received 3 months of high-fat diet, those that were given 2 months high-cholesterol food followed by 1 month normal chow had significantly less aortic $^{111}\text{In-RP782}$ uptake (0.14 ± 0.05 vs. 0.36 ± 0.05 ; $p=0.02$) with histologic evidence of decreased macrophage content suggesting less local inflammation. As MMP are important targets for plaque inflammation and stability, they continue to be tested in pre-clinical studies with hope for eventual clinical application.

PET imaging of plaque hypoxia. PET agents alternative to FDG may offer new applications to detect high-risk vulnerable plaques in the coronary arteries. Silvola et al. evaluated the FDA-approved PET agent for hypoxia detection, 2-(2-nitro-1H-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl) acetamide ($^{18}\text{F-EF5}$) (6). Comparing atheroma-bearing mice to controls, $^{18}\text{F-EF5}$ signal was significantly higher in mice with large atheroma burdens and showed greater local plaque uptake when contrasted with non-atherosclerotic adjacent segments. Atheroma hypoxia detected by $^{18}\text{F-EF5}$ may thus represent a surrogate for lipid-rich plaque, and despite a long blood half-life that may hinder clinical translation, the expected low myocardial background of $^{18}\text{F-EF5}$ compared to FDG may enable improved coronary imaging.

Theranostic silencing of CCR2 expression on monocytes. Leuschner et al. reported on a new lipid nanoparticle that inhibits inflammatory monocytes by reducing their expression of the chemokine receptor CCR2, a molecule important in macrophage homing to inflamed tissues (7). By encapsulating a fluorescently-tagged short interfering RNA (siRNA) sequence within the

nanoparticle, monocyte CCR2 expression was silenced by degrading its cellular mRNA. To image the agent distribution in vivo in mouse cardiovascular disease models, the authors employed non-invasive fluorescence molecular tomography and ex vivo immunofluorescence to demonstrate reduced monocyte recruitment at inflamed sites including atheroma and infarcted myocardium. Importantly, by sparing non-inflammatory monocytes, CCR2-targeted monocyte inhibitory agents may limit toxicity compared to non-selective anti-inflammatory therapies. As the origin and biological characteristics of recruited inflammatory cells to growing atheroma becomes better understood, for example with new studies identifying that extramedullary sources of splenic monocytes (Ly-6C^{high} subtype) can infiltrate nascent plaques in atherosclerotic mice (8), ever more selective anti-inflammatory atherosclerosis therapeutics may be on the horizon.

Human ferritin cages as a macrophage-targeted nanoplatfom. Based on the natural affinity of macrophages for ferritin, targeted imaging agents comprised of bioengineered polypeptide apoferritin (iron-free ferritin) cages were developed as NIRF or MRI agents by coupling apoferritin to the NIR fluorochrome cyanine 5.5, or magnetite nanoparticles, respectively (9). In diabetic, high-fat fed mice, the imaging agents localized to the macrophage-rich lesions of ligated carotid arteries. Loading of other imaging agents or even therapeutic drugs into the apoferritin protein cage architecture is possible, providing versatility for this macrophage-avid agent.

Endothelial-targeted MRI molecular imaging agents. Inflamed, activated endothelial cells promote atherosclerotic lesion initiation, development, and rupture. Probes that identify markers of

endothelial dysfunction are being tested in animal models, with recent emphasis on MRI-detectable iron oxide particles labeled with specific endothelial biomarkers. McAteer et al. labeled microparticles of iron oxide (MPIO) with both P-selectin and vascular cell adhesion molecule-1 (VCAM-1), two important ligands expressed by activated endothelium that recruit circulating blood leukocytes, and injected them into ApoE^{-/-} mice (10). Labeled-MPIO were detectable at aortic atheroma by MRI 30 min after injection with the signal remaining stable for another 60 min, and correlated with macrophage density at histopathology ($r=0.53$, $p<0.01$). In another investigation, VCAM-1 functionalized USPIO could be visualized within the intima of aortic atheroma in ApoE^{-/-} mice 24 hours after injection using 17.6T high-field MRI (11). By electron microscopy, the VCAM-1 USPIO were observed within endothelial cells, which indicates that similar endothelial-targeted particles may be accessible to intravascular imaging approaches for clinical use.

Myocardial Infarction and Thrombosis

New insights into myocardial infarction diagnosis and treatment. *Molecular detection of recent myocardial ischemia.* While transient myocardial ischemia is often not detected by ECG or cardiac enzymes after a short-lived episode, new molecular imaging approaches may be able to identify these patients hours later by illuminating key cellular changes that persist following myocardial ischemia. Davidson et al. tested this theory in mice subjected to 10 minutes of peripheral (hindlimb tourniquet) or myocardial (LAD ligation) ischemia by evaluating the attachment of human P-selectin coated lipid microbubbles by ultrasound to injured vascular endothelium that express P-selectin on their surface (12). P-selectin microbubble deposition was increased at 45 minutes following peripheral ischemia, and remained significantly elevated

up to 6 hours. After myocardial ischemia, P-selectin microbubble attachment was greatest 1.5-3 hours after reperfusion, with continued significant increase at as much as 18 hours post-ischemia. Importantly, in both models P-selective deficient animals showed no targeted microbubble uptake. Given the prolonged detection window reported in this study, P-selectin ultrasound imaging holds promise as a sensitive technique to identify recent tissue ischemia.

Molecular imaging of the immune response and post-MI inflammation. In a significant step forward, Li et al. developed a two-photon intravital microscopy molecular imaging system to successfully monitor leukocyte recruitment into inflamed myocardium *in vivo* on beating hearts (13). After transient LAD occlusion in mice, green fluorescent protein-expressing neutrophils were tracked binding and traversing the coronary veins at sites of myocardial injury, where they accumulated into clusters in the ischemic zone. Blood vessels were visualized in a separate fluorescence channel through injection of non-targeted Q-dots. Transplanted hearts from mutant animals with specific leukocyte binding receptor defects revealed impaired neutrophil recruitment, demonstrating the potential power of this technique to probe post-MI mechanisms of injury and investigate new therapeutics.

Further highlighting the need for better understanding of *in vivo* leukocyte dynamics after acute MI, a study by Lee et al. employing serial FDG PET/CT/MRI of post-MI inflammation in mice revealed significant monocyte infiltration in the remote (non-infarcted) myocardium (14). While FDG activity was strongly associated with the infarcted territory, flow cytometry detected significant remote myocardial monocyte recruitment (10^4 /mg myocardium) compared to non-infarct control mice that peaked later (approximately day 10) than the infarct zone.

Monocyte/macrophage-related protease activity measured by a NIRF protease sensor was also elevated in the remote myocardium of explanted hearts at day 6 post-MI. Detailed knowledge of the timecourse and mechanisms of monocyte recruitment to the infarcted and remote myocardium may help guide therapies to limit infarct size and adverse ventricular remodeling.

Molecular MRI of myocardial necrosis. DNA exposed during myocardial cell death is a potential specific target for determining acute tissue necrosis and infarct size during acute MI. Huang et al. tested this theory in post-MI mice by MRI with a DNA-binding gadolinium chelate (Gd-TO) imaged 2 hours after intravenous injection, a time point where non-targeted gadolinium contrast had washed out of the infarct zone (15). Peak Gd-TO signal was observed 9-18 hours after coronary ligation, and disappeared by 72 hours corresponding to cellular debris clearance. *In vitro* experiments revealed no significant Gd-TO uptake by inflammatory cells, making this agent useful in highly inflamed tissues. In addition to detection of myocyte death in acute MI, Gd-TO also may have a role for use in heart failure and transplant rejection.

SPECT imaging of post-MI MMP activity. Serial SPECT imaging with the broad spectrum MMP nuclear tracer ^{99m}Tc -RP805 in pigs following acute MI highlighted molecular changes during left ventricular remodeling and inflammatory patterns after injury (16). At 1 week post-MI, ^{99m}Tc -RP805 uptake increased >4-fold in the infarct zone where it remained elevated compared to non-infarcted regions for another 4 weeks (24.5 ± 2.7 vs. 7.2 ± 1.5 ; $p<0.05$). *Ex vivo* MMP tissue zymography of individual MMP isoforms demonstrated dominant early MMP-9 upregulation followed by late MMP-2 activation, and infarct-related left ventricular remodeling documented

by cardiac MRI associated with the degree of tissue MMP activity ($r=0.38$). MMP-targeted agents may have future clinical use in predicting left ventricular remodeling after MI.

Long-term PET stem cell tracking after MI. PET-based stem cell tracking approaches are clinically-viable as demonstrated by Perin et al. who utilized reporter gene PET/CT to successfully visualize stem cells serially over 5 months in a porcine MI model (17). The authors detection strategy relied on delivering mesenchymal stem cells retrovirally engineered to express a herpes simplex virus type I thymidine kinase mutant (HSV1-sr39tk) gene product into infarcted regions, followed by later intravenous administration of the PET nucleoside analogue 1-(29-deoxy-29-fluoro-b-D-arabinofuranosyl)-5-ethyl-uridine (^{18}F -FEAU), which becomes phosphorylated by HSV1-sr39tk intracellularly trapping the PET tracer to allow late detection of still-viable resident stem cells. Further refinement of robust non-invasive stem cell molecular tracking approaches will strengthen the ability to interrogate *in vivo* cell-based transplant biology.

Promising non-invasive thrombosis molecular imaging strategies. *Molecular MRI of fibrin deposition in atheroma.* Fibrin is an important marker of atherosclerotic plaque vulnerability to rupture. The clinical MRI-detectable fibrin-targeted gadolinium contrast agent EP-2104R was tested in atherosclerotic ApoE^{-/-} mice and compared with *ex vivo* tissue fibrin immunostaining and gadolinium accumulation by inductively coupled mass spectroscopy (18). 3 months after initiation of a high-fat diet, brachiocephalic artery atheroma imaged 90 min following administration of 10 $\mu\text{mol/kg}$ EP-2104R exhibited enhanced EP-2104R plaque signal (contrast-to-noise ratio ~ 25 , $p < 0.05$ compared to control) that was diminished in a subset of mice co-

administered daily pravastatin (contrast-to-noise ratio ~ 15 , $p < 0.05$ compared to 3 months high-fat diet without statin). Sites of plaque EP-2104R enhancement correlated with *ex vivo* gadolinium concentration and immunohistochemical fibrin staining ($r^2 = 0.70$ and 0.82 , respectively), validating the *in vivo* molecular MRI findings. Thus, EP-2104R may allow noninvasive MRI detection of fibrin deposition on plaques.

P-Selectin SPECT reporter. Fucoidin, a high-affinity P-selectin ligand that binds to activated platelets and endothelium, was labeled with ^{99m}Tc for SPECT imaging (19). In rat models of aortic aneurysm with mural arterial thrombosis and aortic valve infective endocarditis, platelet-rich thrombi were detected with high-sensitivity by ^{99m}Tc -fucoidin at target-to-background ratios (TBR) of 5.2 and 3.6, respectively. ^{99m}Tc -fucoidin SPECT also revealed endothelial activation up to 2 hours after myocardial ischemia-reperfusion injury induced by coronary occlusion in the rat (TBR 4.1). All *in vivo* studies were validated by *ex vivo* autoradiography and tissue P-selectin staining to demonstrate signal co-localization. Since P-selectin detection is a prominent endovascular marker of inflammation and cell activation, molecular imaging of P-selectin holds considerable promise for future cardiovascular applications in thrombosis and endothelial dysfunction.

Platelet-targeted ultrasound microbubbles. Activated platelets incorporating into arterial thrombi were successfully imaged in living mice using lipid-shell ultrasound microbubbles coated with a specific antibody that detects activated glycoprotein IIb/IIIa (GPIIb/IIIa) platelet receptors (20). Compared to non-specific control microbubbles, GPIIb/IIIa-targeted microbubbles (1.5×10^7 microbubbles injected) localized significantly to non-occlusive murine carotid arterial thrombi

induced by chemical injury (topical ferric chloride) 20 minutes after injection. Urokinase mediated thrombolysis (500 IU/g) diminished GPIIb/IIIa-targeted microbubble binding as assessed 30 minutes after thrombolysis following re-injection of the GPIIb/IIIa microbubble imaging agent, suggesting that GPIIb/IIIa-targeted microbubbles may furthermore allow non-invasive tracking of the efficacy of thrombolysis.

Vasculitis, Dissection, and Aneurysm

Molecular imaging of aneurysm inflammation: PET, MRI, and optical techniques. Nahrendorf et al. labeled previously described monocyte/macrophage targeted near-infrared fluorescent dextran-coated cross-linked iron oxide particles (CLIO) with the PET tracer ^{18}F to identify aortic aneurysm inflammatory foci in ApoE^{-/-} mice infused with angiotensin II to induce aneurysm formation (21). PET/CT imaging performed 10-12 hours after ^{18}F -CLIO injection demonstrated significantly greater radiotracer uptake in aneurysms than in non-aneurysmal control mouse aortas (2.46 ± 0.48 vs. 0.82 ± 0.05 ; $p < 0.05$), and was confirmed to be >90% monocyte/macrophage specific by a combination of immunohistochemistry, fluorescence microscopy, and flow cytometry analysis. A subset of mice underwent splenectomy to deplete monocyte reservoirs, leading to both decreased aneurysm formation and diminished ^{18}F -CLIO aortic signal. ^{18}F -CLIO signal was also potentially predictive of aneurysm rupture, as those mice that died during the study protocol and those with enlarging aneurysms had the greatest ^{18}F -CLIO aneurysm uptake.

A separate study employed superparamagnetic particles of iron oxide (SPIO) alone to evaluate early inflammation during aneurysm formation in angiotensin II infused ApoE^{-/-} mice (22).

Detailed histopathology compared to *in vivo* MRI SPIO detection at day 14 after angiotensin II

administration determined that SPIO were retained in the shoulder region and external layer (adventitia) of AAA, with a direct relationship between iron-rich macrophage populations and T2-weighted MRI SPIO signal loss ($r=0.96$, $p<0.05$). As SPIO have larger physical diameter than USPIO, they may be size-restricted from deeper tissues and are well suited to interrogate more superficial structures as observed in this study.

MRI of aneurysm collagen deposition. Extracellular matrix remodeling, predominately type I collagen, may be related to the mechanical stability of growing aneurysms. To test this hypothesis, Klink et al. employed a collagen-specific fluorescent paramagnetic micelle nanoparticle agent, CNA-35, for serial MRI imaging of aneurysmal mice (23). In collagen rich stable aneurysms, MRI of CNA-35 micelles injected 32 hours prior to allow sufficient blood clearance caused significantly greater aneurysm enhancement compared to non-specific control micelles (80% vs. 30%; $p<0.01$), and non-aneurysmal mice had no appreciable aortic wall CNA-35 micelle signal. In contrast, more advanced aneurysms complicated by dissection and rupture demonstrated less CNA-35 enhancement. As extracellular matrix destruction during aortic aneurysm growth may have implications for later events, future investigations may prove molecular MRI of collagen as a useful adjunctive predictive tool.

SPECT integrin-receptor macrophage imaging. Adding to the armamentarium of macrophage detection agents, Razavian et al. demonstrate that macrophages can be detected in injured vascular tissue by targeted SPECT imaging of $\alpha_v\beta_3$ integrin macrophage cell surface receptors with ^{99m}Tc -NC100692, an agent previously described to identify angiogenesis (24). Two weeks after unilateral carotid artery chemical injury in hypercholesterolemic ApoE^{-/-} mice, ^{99m}Tc -

NCI00692 uptake in the injured carotid 2 hours after administration was significantly greater than the contralateral sham-operated control (0.52 ± 0.09 vs. 0.08 ± 0.02 ; $p < 0.001$). Up to 4 weeks post-injury, this relationship remained statistically significantly elevated, although to a lesser extent. Immunostaining and real-time polymerase chain reaction localized enhanced tissue $\alpha_v\beta_3$ integrin receptor levels in this large artery vascular injury model to macrophages, rather than endothelial or smooth muscle cells, extending a new use for this agent in vascular inflammation and remodeling.

MRI tracking of vascular repair by endothelial progenitor cells. Vascular trauma frequently results in endothelial cell loss or dysfunction, which can lead to thrombosis, inflammation, and restenosis. As endothelial progenitor cells (EPC) have demonstrated potential benefit for early endovascular repair, Chen et al. utilized an *ex vivo* labeling approach to tag murine EPC harvested from the spleen with Fe_2O_3 -poly-L-lysine SPIO for *in vivo* MRI tracking (25). Upon intravenous reinjection, SPIO-labeled EPC homed to the guidewire-injured left common carotid arteries of mice on serial MRI imaging performed over 15 days post-injury, validated histopathologically by iron particle layering in the subendothelium. EPC may provide a novel means for endovascular therapy to re-endothelialize stents or other injured vasculature, and despite the potential for reporting on retained non-viable cells, SPIO may provide ample early understanding of EPC fate.

Ultrasound microbubble imaging of endothelial P-selectin in diabetes. Ischemic tissue injury results in a robust inflammatory response prompting vascular remodeling that recruits new blood vessels, a process that is blunted in diabetes. To assess the role of endothelial activation in the diabetic

angiogenic cascade, endothelial P-selectin expression was examined using targeted ultrasound microbubbles in wild-type and obese insulin-resistant (db/db, leptin-receptor deletion) mice subjected to complete hindlimb arterial ligation (26). Partial restoration of flow occurred only in control mice on contrast-enhanced ultrasound perfusion imaging, and while baseline *in vivo* ultrasound microbubble P-selectin expression was chronically elevated 4-fold in db/db mice compared to controls, the controls exhibited 10-fold augmentation of P-selectin microbubble binding day 1 after limb ischemia that was absent in the db/db animals due to deficient monocyte recruitment. P-selectin is an important endothelial inflammatory marker that binds circulating mononuclear cells, and this study highlights the chronically increased diabetic inflammatory state, which lack the important spatiotemporal inflammatory boost required to initiate angiogenesis and promote tissue healing.

Cardiomyopathy, Transplant, and Allograft Rejection

SPECT imaging of right ventricular myocardial apoptosis. Arrhythmogenic right ventricular cardiomyopathy (ARVC), characterized by fibrofatty replacement of the right ventricular myocardium, is a cause of malignant arrhythmias and sudden cardiac death, but has variable expression and therefore strategies are needed to better identify the risk of ARVC.

Recognizing that ARVC is associated with enhanced myocyte apoptosis, Campian et al. employed the apoptosis reporter ^{99m}Tc -annexin V in 6 patients fulfilling ARVC criteria (27). Compared to the left ventricle and interventricular septum, ^{99m}Tc -annexin V SPECT signal 4 hours after agent administration was greater in the right ventricular free wall ($p \leq 0.05$). Three ARVC patients in particular demonstrated marked increase in right ventricular ^{99m}Tc -annexin V signal compared to the others (1.79 vs. 0.98, $p = 0.001$), possibly indicating a more active

phenotype, although no clear clinical difference was appreciated between these two groups. Future studies evaluating myocardial apoptosis and clinical outcomes in ARVC should prove fruitful.

USPIO MRI detection of myocarditis. More specific tools for diagnosing myocarditis and monitoring treatment response would be clinically useful. Moon et al. tested the ability of MRI macrophage molecular imaging with USPIO as a means to classify inflammation in an experimental rat model of autoimmune myocarditis (28). 24 hours after USPIO injection (10 mg Fe/kg), MRI demonstrated a significant increase in myocardial contrast-to-noise ratio over baseline MRI in myocarditis animals compared to controls (1.08 vs. 0.48, $p < 0.001$). Overall, USPIO-enhanced MRI was able to successfully characterize the degree of myocardial inflammation compared to standard histopathological grading, except in cases where the inflammation was only mild. Repeated USPIO MRI studies in the same animals 5 days apart revealed the ability of USPIO to longitudinally track inflammation in myocarditis over time, which could be explored in subsequent studies evaluating the response to anti-inflammatory therapies.

SPECT imaging of neurohumoral activation in heart failure. Agents that suppress beta-adrenergic tone and the renin-angiotensin-aldosterone axis are mainstays of heart failure management, but means to objectively assess the amount of effective blockade in a particular individual are lacking. Dilsizian et al. investigated the SPECT agent technetium-99m-labeled lisinopril (Tc-Lis) to identify tissue angiotensin converting enzyme (ACE) activity *in vivo* (29). ACE overexpressing transgenic rats administered Tc-Lis demonstrated myocardial enhancement by micro-

SPECT/CT 120 min after injection with a 5-fold increase compared to normal control rats. Pretreatment with non-labeled lisinopril decreased Tc-Lis signal uptake demonstrating low non-specific binding of the Tc-Lis agent. If clinically translated, monitoring ACE inhibition with SPECT Tc-Lis may therefore allow tailored pharmacologic therapy for cardiomyopathy patients.

Monitoring stem cell transplants with SPECT and PET. In order to understand the fate of stem cells and optimize techniques for transplantation, reliable long-term tracking of injected pluripotent cells must be achieved. Reporter genes that can be interrogated by nuclear imaging platforms are most promising, as they require host cell machinery for expression and therefore report on viable, engrafted stem cells. Liu et al. studied the human simplex virus thymidine kinase (TK) PET reporter gene transduced into human cardiac progenitor cells (hCPC) and transplanted (1×10^6 cells) into the peri-infarct border zone of mice following MI (30). Two weeks after hCPC transplant, left ventricular function improved by MRI and echocardiography. PET imaging demonstrated declining hCPC viability over the study, with the amount of residual PET signal at 4 weeks proportional to the initial PET uptake immediately post-transplant suggesting that successful early engraftment is key to later stem cell maintenance. In a pig MI model, Templin et al. tested a different reporter gene, the sodium iodide symporter (NIS), which was transgenically incorporated into human-induced pluripotent stem cells (hiPSC) (31). Ten days after MI, hiPSC were intramyocardially delivered to the infarcted tissue and then followed by ^{123}I SPECT imaging to assess transplanted cell survival. NIS-reporter SPECT imaging in this model was able to successfully monitor engrafted hiPSC for as long as 15 weeks after injection, and furthermore histopathology revealed hiPSC-derived endothelial cell vascularization. These

and other reports demonstrate that nuclear reporter gene approaches can successfully image stem cells over the long term non-invasively and *in vivo*.

Molecular PET imaging of transplant rejection. Determining transplant rejection presently requires endomyocardial biopsy, although the application of new molecular imaging agents may replace invasive screening with non-invasive detection strategies. In rat heart and kidney transplant rejection models, Hitchens et al. investigated VS-580H (V-sense; Celsense, Pittsburgh PA), a commercially available fluorine-19 perfluorocarbon emulsion agent, to perform *in vivo* labeling of macrophages for cellular MRI (32). Fluorine-19 MRI imaging has the advantage of negligible tissue background signal given the absence of endogenous cellular fluorine as well as being highly quantitative, but requires an additional ^1H scan sequence for anatomical co-registration. 24-hours after injection, VS-580H localized extensively to the rejecting organ bed, where dual-channel fluorescence microscopy confirmed a predominately tissue macrophage origin of the MRI signal. *In vivo* macrophage labeling approaches such as with fluorine-19 agents or previously reported USPIO could eventually lead to new non-invasive organ transplant monitoring protocols.

Supplemental References

1. Metz S, Beer AJ, Settles M, et al. Characterization of carotid artery plaques with USPIO-enhanced MRI: assessment of inflammation and vascularity as *in vivo* imaging biomarkers for plaque vulnerability. *Int J Cardiovasc Imaging* 2011;27:901-12.
2. Derlin T, Habermann CR, Lengyel Z, et al. Feasibility of ^{11}C -acetate PET/CT for imaging of fatty acid synthesis in the atherosclerotic vessel wall. *J Nucl Med* 2011;52:1848-54.
3. Vucic E, Calcagno C, Dickson SD, et al. Regression of inflammation in atherosclerosis by the LXR agonist R211945: a noninvasive assessment and comparison with atorvastatin. *JACC Cardiovasc Imaging* 2012;5:819-28.

4. Quillard T, Tesmenitsky Y, Croce K, et al. Selective inhibition of matrix metalloproteinase-13 increases collagen content of established mouse atherosclerosis. *Arterioscler Thromb Vasc Biol* 2011;31:2464-72.
5. Razavian M, Tavakoli S, Zhang J, et al. Atherosclerosis plaque heterogeneity and response to therapy detected by in vivo molecular imaging of matrix metalloproteinase activation. *J Nucl Med* 2011;52:1795-802.
6. Silvola JM, Saraste A, Forsback S, et al. Detection of hypoxia by [18F]EF5 in atherosclerotic plaques in mice. *Arterioscler Thromb Vasc Biol* 2011;31:1011-5.
7. Leuschner F, Dutta P, Gorbатов R, et al. Therapeutic siRNA silencing in inflammatory monocytes in mice. *Nat Biotechnol* 2011;29:1005-10.
8. Robbins CS, Chudnovskiy A, Rauch PJ, et al. Extramedullary hematopoiesis generates Ly-6C(high) monocytes that infiltrate atherosclerotic lesions. *Circulation* 2012;125:364-74.
9. Terashima M, Uchida M, Kosuge H, et al. Human ferritin cages for imaging vascular macrophages. *Biomaterials* 2011;32:1430-7.
10. McAteer MA, Mankia K, Ruparelina N, et al. A leukocyte-mimetic magnetic resonance imaging contrast agent homes rapidly to activated endothelium and tracks with atherosclerotic lesion macrophage content. *Arterioscler Thromb Vasc Biol* 2012;32:1427-35.
11. Michalska M, Machtoub L, Manthey HD, et al. Visualization of vascular inflammation in the atherosclerotic mouse by ultrasmall superparamagnetic iron oxide vascular cell adhesion molecule-1-specific nanoparticles. *Arterioscler Thromb Vasc Biol* 2012;32:2350-7.
12. Davidson BP, Kaufmann BA, Belcik JT, Xie A, Qi Y, Lindner JR. Detection of antecedent myocardial ischemia with multiselectin molecular imaging. *J Am Coll Cardiol* 2012;60:1690-7.
13. Li W, Nava RG, Bribriescio AC, et al. Intravital 2-photon imaging of leukocyte trafficking in beating heart. *J Clin Invest* 2012;122:2499-508.
14. Lee WW, Marinelli B, van der Laan AM, et al. PET/MRI of inflammation in myocardial infarction. *J Am Coll Cardiol* 2012;59:153-63.
15. Huang S, Chen HH, Yuan H, et al. Molecular MRI of acute necrosis with a novel DNA-binding gadolinium chelate: kinetics of cell death and clearance in infarcted myocardium. *Circ Cardiovasc Imaging* 2011;4:729-37.
16. Sahul ZH, Mukherjee R, Song J, et al. Targeted imaging of the spatial and temporal variation of matrix metalloproteinase activity in a porcine model of postinfarct

- remodeling: relationship to myocardial dysfunction. *Circ Cardiovasc Imaging* 2011;4:381-91.
17. Perin EC, Tian M, Marini FC, 3rd, et al. Imaging long-term fate of intramyocardially implanted mesenchymal stem cells in a porcine myocardial infarction model. *PLoS One* 2011;6:e22949.
 18. Makowski MR, Forbes SC, Blume U, et al. In vivo assessment of intraplaque and endothelial fibrin in ApoE(-/-) mice by molecular MRI. *Atherosclerosis* 2012;222:43-9.
 19. Rouzet F, Bachelet-Violette L, Alsac JM, et al. Radiolabeled fucoidan as a p-selectin targeting agent for in vivo imaging of platelet-rich thrombus and endothelial activation. *J Nucl Med* 2011;52:1433-40.
 20. Wang X, Hagemeyer CE, Hohmann JD, et al. Novel single-chain antibody-targeted microbubbles for molecular ultrasound imaging of thrombosis: validation of a unique noninvasive method for rapid and sensitive detection of thrombi and monitoring of success or failure of thrombolysis in mice. *Circulation* 2012;125:3117-26.
 21. Nahrendorf M, Keliher E, Marinelli B, et al. Detection of macrophages in aortic aneurysms by nanoparticle positron emission tomography-computed tomography. *Arterioscler Thromb Vasc Biol* 2011;31:750-7.
 22. Yao Y, Wang Y, Zhang Y, et al. In vivo imaging of macrophages during the early-stages of abdominal aortic aneurysm using high resolution MRI in ApoE mice. *PLoS One* 2012;7:e33523.
 23. Klink A, Heynens J, Herranz B, et al. In vivo characterization of a new abdominal aortic aneurysm mouse model with conventional and molecular magnetic resonance imaging. *J Am Coll Cardiol* 2011;58:2522-30.
 24. Razavian M, Marfatia R, Mongue-Din H, et al. Integrin-targeted imaging of inflammation in vascular remodeling. *Arterioscler Thromb Vasc Biol* 2011;31:2820-6.
 25. Chen J, Jia ZY, Ma ZL, Wang YY, Teng GJ. In vivo serial MR imaging of magnetically labeled endothelial progenitor cells homing to the endothelium injured artery in mice. *PLoS One* 2011;6:e20790.
 26. Carr CL, Qi Y, Davidson B, et al. Dysregulated selectin expression and monocyte recruitment during ischemia-related vascular remodeling in diabetes mellitus. *Arterioscler Thromb Vasc Biol* 2011;31:2526-33.
 27. Campian ME, Tan HL, van Moerkerken AF, Tukkie R, van Eck-Smit BL, Verberne HJ. Imaging of programmed cell death in arrhythmogenic right ventricle cardiomyopathy/dysplasia. *Eur J Nucl Med Mol Imaging* 2011;38:1500-6.

28. Moon H, Park HE, Kang J, et al. Noninvasive assessment of myocardial inflammation by cardiovascular magnetic resonance in a rat model of experimental autoimmune myocarditis. *Circulation* 2012;125:2603-12.
29. Dilsizian V, Zynda TK, Petrov A, et al. Molecular imaging of human ACE-I expression in transgenic rats. *JACC Cardiovasc Imaging* 2012;5:409-18.
30. Liu J, Narsinh KH, Lan F, et al. Early stem cell engraftment predicts late cardiac functional recovery: preclinical insights from molecular imaging. *Circ Cardiovasc Imaging* 2012;5:481-90.
31. Templin C, Zweigerdt R, Schwanke K, et al. Transplantation and tracking of human-induced pluripotent stem cells in a pig model of myocardial infarction: assessment of cell survival, engraftment, and distribution by hybrid single photon emission computed tomography/computed tomography of sodium iodide symporter transgene expression. *Circulation* 2012;126:430-9.
32. Hitchens TK, Ye Q, Eytan DF, Janjic JM, Ahrens ET, Ho C. ¹⁹F MRI detection of acute allograft rejection with in vivo perfluorocarbon labeling of immune cells. *Magn Reson Med* 2011;65:1144-53.