Poster Abstracts* from the Multimodality Cardiovascular Molecular Imaging Symposium

SNM and the SNM Molecular Imaging Center of Excellence are sponsoring a symposium designed to help disseminate the latest cardiovascular molecular imaging research and the most promising related clinical applications. The Multimodality Cardiovascular Molecular Imaging Symposium will be held at the National Institutes of Health (NIH), April 30–May 1, and will include a series of lectures from invited experts in the field, panel discussions, and a moderated poster session. This multidisciplinary meeting is attracting individuals from both the basic science and the clinical communities. In this special section of *JNM,* we are publishing the 28 original abstracts selected for poster presentation at this symposium.

This meeting has been designed to address a shift in emphasis from treatment of cardiovascular disease to prevention of disease, with the application of molecular imaging primarily to advance health care and in part to control the escalating costs. The application of molecular imaging may provide unique molecular and pathophysiologic insights that will allow a more personalized approach to evaluation and management of cardiovascular disease. The conference will build on a similar and successful symposium held at NIH in 2004 (*J Nucl Med*. 2004;45[3]:28N) and will bring together individuals from multiple scientific disciplines with the goal of promoting the emerging field of cardiovascular molecular imaging. You may register at www.snm.org/cvmi2009.

The 2004 conference served as the basis for the first textbook dedicated to the field of cardiovascular molecular imaging. The 2009 meeting is designed to continue this momentum. A series of papers generated by the speakers/moderators for each of the sessions will be published by *JNM* as a special supplement on cardiovascular molecular imaging.

Speakers have been chosen from multiple scientific disciplines, including chemistry, engineering, physics, molecular biology, cardiovascular physiology, and imaging sciences. The agenda focuses on advances in multimodality targeted imaging of the cardiovascular system, including imaging of cardiovascular receptors, stem cell therapy, vascular biology, myocardial metabolism, atherosclerosis, angiogenesis, cardiomyopathies, ischemia, and infarction. This gathering of leading researchers in the field will help chart the direction of cardiovascular molecular imaging for the next decade.

The abstracts presented here represent some of the most interesting ideas in molecular imaging research as applied to cardiovascular disease. We have put particular emphasis on encouraging participation by junior scientists. The first section contains those abstracts selected to receive Young Investigator Travel Awards. Abstracts are also presented related to new molecular probes and imaging technologies, as well as preclinical and clinical applications of molecular imaging.

On behalf of the symposium co-chairs, I invite you to join us in Bethesda at the end of this month to hear these talented investigators present the details of their research and to attend our educational program focused on multimodality cardiovascular molecular imaging.

Albert J. Sinusas
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Abstract Reviewers

*Abstracts are listed alphabetically by first author within each section.

SECTION 1

Young Investigator Travel Award Winners

1

X-Ray- and MRI-Visible Microencapsulated Mesenchymal Stem Cell for Cell Delivery and Tracking on Clinical Scanners. Y. Fu[1], D. Kedzioreik[1], R. Ouwerkerk[1], S. Shea[2], T. Ehtiati[2], G. Huang[1], N. Azene[1], R. Krieg[3], J.W.M. Bulte[1], F. Wacker[1], D.L. Kraithman[1]; 1. Johns Hopkins University, School of Medicine, Baltimore, Maryland; 2. Siemens Research Corporate, Inc., Baltimore, Maryland; 3. Siemens AG Healthcare Sector, Enlangen, Germany

Objectives: To enable the use of allogeneic mesenchymal stem cells (MSCs) to treat ischemic arterial diseases, we hypothesize that a perfluorooctylbromide (PFOB) containing cell microencapsulation method would improve MSCs viability and enable the monitoring of MSC delivery with noninvasive tracking of the engraftment using clinical X-ray and MR imaging systems. **Methods:** Microencapsulation of bone marrow-derived rabbit MSCs ($1.5x10^6$cells/ml) was performed by extruding rabbit MSCs mixed with PFOB-loaded alginate through a syringe pump into 100 mM $CaCl_2$ solution, followed by cross linking with poly-L-lysine. MSC viability post-encapsulation was determined *in vitro*. *In vitro* PFOB microcapsule visibility was studied in agarose phantoms using clinical X-ray (Siemens Axiom Artis dFA) and ^{19}F MRI (Tim-Trio; 3D-TrueFISP, 3.9/2.0 ms, 1.5x1.5x2.0 mm^3, and 15 min 43 s acquisition). *In vivo* imaging studies were performed in New Zealand White (NZW) rabbits, which received 5-7 injections of PFOB microcapsules and unlabeled microcapsules in opposing thigh muscles. X-ray angiograms, c-arm CT (8s DR), and ^{19}F MR images were acquired 1-7 days after injection and repeated up to 5 weeks post-injection. Postmortem histology was performed to confirm the presence and mechanical stability of PFOB microcapsules three days after transplantation. **Results:** MSC viability decreased <10±3% immediately after encapsulation and was maintained up to 4 weeks *in vitro* (88±5%). C-arm CT and ^{19}F MR imaging of PFOB microcapsule phantoms demonstrated the ability to detect as few as 2 and 5 microcapsules, respectively. *In vivo* visibility of PFOB microcapsules by X-ray was demonstrated relative to unlabeled microcapsules with persistence of intact microcapsules up to 5 weeks post delivery. Using ^{19}F MRI, all PFOB microcapsule injections were clearly identified in rabbit medial thigh. Fusion of c-arm CT images and postmortem histology showed high concordance of injection sites. TUNEL staining of the recovered PFOB microcapsules from the rabbit thigh demonstrated high MSC viability at three days post-injection. **Conclusions:** A novel, perfluorinated microcapsule made with clinically approved perfluorocarbon provides an ideal microenvironment for maintaining MSC viability *in vitro* and *in vivo*. PFOB offers the unique feature of multimodality imaging to monitor MSC delivery and track engraftment using clinical X-ray angiographic and MRI systems.

2

Imaging of Angiotensin II Receptor Expression After MI. T. Higuchi, K. Fukushima, J. Xia, M. Javadi, J. Fox, W. Mathews, Z. Szabo, F. Bengel; Johns Hopkins University, Baltimore, Maryland.

Objectives: The renin-angiotensin system is thought to play an essential role in left ventricular remodeling after myocardial infarction. Therefore, it represents an attractive molecular imaging target. We aimed to determine the feasibility of PET imaging of angiotensin II receptor (ATR) expression in a rodent model. **Methods:** Myocardial infarction was created by ligation of the left coronary artery in male Wistar rats (220-250g). Multi-tracer autoradiography was conducted at different times after experimental myocardial infarction (n=29). In order to determine the time course of ATR expression (after tissue incubation with I-125-SI AT II, 50pM), its regional relation to the area at risk (determined by 5mCi Tc-99m-TF injection during coronary re-occlusion prior to sacrifice), and infarct area (determined by 2uCi Tl-201 injection prior to reocclusion and sacrifice). Next, C-11 KR31173 (KR) an ATR-specific PET tracer previously validated in renal studies was tested in a single model (n=16). **Results:** ATR upregulation was found in the infarct area at 3days, 1week and 3weeks after MI, but not in the acute phase at 1hour and 1day with multi-tracer autoradiography. Significant C-11 KR PET tracer focal uptake was observed in the MI area by autoradiography. Infarct/remote uptake ratio = 0.9±1.03, 2.9±0.7, 3.2±0.6, 1.5±0.2 at control, 1day, 3days, 1week, 3weeks and 3months, respectively. Blocking with the antagonist SK-1080 (2mg/kg) confirmed C-11 KR uptake specificity, and small animal PET imaging visualized the increased C-11 KR uptake non-invasively. **Conclusions:** Following myocardial infarction, ATR expression is upregulated and peaks at 1-3 weeks. This upregulation can be detected noninvasively using C-11 KR PET. The usefulness to predict postinfarct ventricular remodeling needs to be demonstrated in subsequent studies.

3

Precise Targeting of X-Ray-Visible Microencapsulated Mesenchymal Stem Cells Using C-Arm CT for Interrogation of a Bioluminescent Reporter Gene Probe. D.A. Kedziorek[1], P. Walczak[1], Y. Fu[1], T. Ehtiati[2], A.B. Brost[3], N. Azene[1], G. Huang[1], J.W.M. Bulte[1], F. Wacker[1], D.L. Kraitchman[1]; 1. Johns Hopkins University, Baltimore, Maryland; 2. Siemens Corporate Research, Baltimore Maryland; 3. University of Erlangen-Nurembert, Erlangen, Germany.

Introduction: Poor cell survival and difficulties with visualization of cell delivery are major problems with current cell transplantation methods. To protect cells from early destruction, microencapsulation methods have been developed. The addition of a contrast agent to the microcapsule also could enable tracking by MR, ultrasound, and X-ray imaging. In the presented study, mesenchymal stem cells (MSCs) were transfected with reporter gene and microencapsulated in either unlabeled or perfluorooctylbromide (PFOB) impregnated capsules and transplanted intramuscularly in a rabbit model of peripheral arterial disease. Rather than systemic injections of the reporter gene probe, c-arm CT was used to target the reporter probe to the X-ray-visible microcapsules. **Methods:** MSCs were transfected with triple fusion reporter gene containing red fluorescent protein, truncated thymidine kinase (SPECT/PET reporter) and firefly luciferase (bioluminescence (BLI) reporter). For microencapsulation, the classical alginate method (Lim F, Sun AM, *Science.* 1980;210:908–910) was used with the addition of 12% PFOB to create X-ray-visible capsules. The microcapsules were incubated with D-luciferin and injected intramuscularly in the rabbit thigh. BLI was performed immediately after capsules transplantation as well as 1 and 2 days post injection. C-arm CT was performed using the 8sDR preset on days 1 and 2 to target luciferin to the PFOB capsules with a custom needle targeting software (X-Loc). Unlabeled capsules were injected blindly on days 1 and 2. **Results:** Encapsulation did not block the MSC bioluminescence. The BLI signal from PFOB encapsulated MSCs was reduced by 5% compared to a similar number of nonencapsulated MSCs. All injection sites of PFOB encapsulated MSCs were targeted successfully using c-arm CT with BLI revealing viable cells 1 and 2 days post transplantation. Blind luciferin injections to the thigh muscles with unlabeled microcapsules resulted in successful BLI signal detection in ~15% of the injection locations. **Conclusions:** Viability of encapsulated MSCs in X-ray-visible microcapsules can be monitored with non-invasive BLI in conjunction with reporter gene targeting using clinical X-ray imaging modalities. The reporter gene probe could be precisely targeted using c-arm CT, thereby avoiding large systemic injections of a reporter probe.

4

Molecular Imaging of Atherosclerotic Plaques Targeted on Oxidized LDL Receptor LOX-1 Using SPECT/CT and Magnetic Resonance. D. Li, A. Patel, A. Klibanov, C.K. Kramer, G.A. Beller, D.K. Glover, C.H. Meyer; University of Virginia, Charlottesville, Virginia.

Objectives: Oxidized LDL receptor LOX-1 plays a crucial role in atherosclerotic lesions. This study was designed to detect and assess atherosclerotic plaque *in vivo* using SPECT and CMR based molecular imaging targeted to LOX-1. **Methods:** LDLR-/- and double LDLR/LOX-1-/- mice on an atherogenic diet for > 16 weeks were used for CMR based imaging. Apo E-/- mice on western diet >20 weeks were used for SPECT imaging. The imaging probe consisted of liposomes decorated with LOX-1 antibody (LOX-1 Ab) [or nonspecific IgG (nIgG)], gadolinium (or ^{111}In) and DiI fluorescence markers. MRI at 7.0T and microSPECT/CT were

performed from aortic root to arch at baseline and 24 hrs after intravenous injection of 150 µl of probe containing LOX-1 Ab (or nIgG) with 0.075 mmol Gd/kg (or 600 µCi of ^{111}In), followed by excision of the aorta for phosphor ex-vivo imaging and frozen cross-sections. The ex-vivo fluorescence images, H&E staining, apoptosis and MMP9 were examined using aortic frozen sections. **Results:** Fluorescence imaging revealed that the apo E-/- or LDLR-/- mice injected with the LOX-1 Ab probe showed significant uptake in atherosclerotic plaque. There was little fluorescence signal in plaques in the mice injected with the nIgG probe or double LDLR/LOX-1-/- mice injected with the LOX-1 Ab probe. The MR images consistently showed a strong post-contrast signal on atherosclerotic plaques at 24 hours in LDLR-/- mice (n=7) injected with LOX-1 Ab probe but not in those (n=5) injected with nIgG probe. The increased signal was not observed in double LDLR/LOX-1-/- mice (n=3) injected with the LOX-1 Ab probe. The % contrast to noise ratio (CNR) and % normalized enhancement ratio (NER) were significantly higher at 24 hours in LDLR-/- mice compared to other two groups (both $P<0.05$, fig 1). SPECT/CT imaging showed hotspots in the aortic arch in all apo E-/- mice (n=8) injected with the LOX-1 Ab probe corresponding with focal uptake areas observed by phosphor imaging of the excised aortas. There was no hotspot in the aortic arch in all apo E-/- mice (n=7) injected with nIgG probe. Further studies showed that the imaging signal of LOX-1 Ab probe colocalized with the increased apoptotic cells and elevated MMP9 expression in atherosclerotic plaque. **Conclusions:** LOX-1 can be used as a target for multimodality molecular imaging of atherosclerotic plaque in vivo. Further studies of in vivo imaging of LOX-1 may help to determine whether LOX-1 is associated with plaque vulnerability.

5

Molecular Imaging of Atherosclerotic Plaque with 64-Cu-Labeled Natriuretic Peptide and Positron Emission Tomography. Y. Liu[1], R. Rossin[1], D. Abendschein[2], J. Zheng[1], K. McCommis[1], G.E. Woodard[3], P. Woodard[1], M.J. Welch[1]; 1. Mallinckrodt Institute of Radiology; 2. Department of Medicine, Washington University School of Medicine, St Louis, Missouri; 3. National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, Maryland.

Objectives: Molecular imaging of vulnerable atherosclerotic lesions is of paramount importance for the identification of patients at risk for thrombo-ischemic event. Recently, the role of natriuretic peptides (NPs) in the pathogenesis of coronary atherosclerotic plaque was suggested, as these cardiac hormones have potent anti-proliferative and anti-migratory effects on vascular smooth muscle cells. In this study, the use of a 64-Cu-labeled NP fragment as imaging probe for non-invasive atherosclerotic plaque imaging with PET was investigated. **Methods:** The animal model was developed from male New Zealand white rabbits fed with cholesterol-rich diet before the right femoral artery was double-injured by air dessication and balloon overstretching, respectively, overtime. A plaque-targeted contrast agent (Gadodlurine M, Schering AG) was used to check the rabbit atherosclerotic lesion with 3T MRI.CANF (atrial natriuretic factor), which is a C-type NP analog, functionalized with DOTA and labeled with 64-Cu was used as radiotracer for the plaque imaging. After MRI scan, the rabbit was injected with 64-Cu-DOTA-CANF (104 MBq, ca. 7.5 Ìg CANF peptide) and a 1h dynamic scan was acquired on the microPET Focus-220. Fiducial markers attached to the animal bed and filled with a 64-Cu aqueous solution were used to correlate the MRI and microPET images. Histopathologic imaging and immunohistochemistry (IHC) of the ex-vivo atherosclerotic artery were performed to confirm the presence of atherosclerotic plaque and natriuretic peptide clearance receptor on the plaque. Both PET and IHC blocking studies with the CANF peptide were performed to confirm the receptor specific tracer uptake. **Results:** MRI clearly showed the accumulation of contrast agent surrounding the lesion. The 64-Cu-DOTA-CANF uptake at injury site was evidently visualized on microPET images, with the highest signal/background ratio (3.39±0.78) observed after the first injury. The IHC demonstrated the presence of NP clearance receptor on the plaque. The blocking studies proved the receptor mediated tracer uptake at the injury artery. With the blocking, the tracer uptake SUV was decreased from 1.42±0.02 to 1.06±0.06 ($P < 0.001$). **Conclusions:** This imaging studies show that 64-Cu-DOTA-CANF is a promising candidate tracer for PET imaging of atherosclerotic plaques.

6

Imaging Matrix Metalloproteinase Activation to Predict Aneurysm Expansion in Vivo. L. Nie[1], M. Razavian[1], J. Zhang[1], S. Tavakoli[1], L.W. Dobrucki[2], A.J. Sinusas[2], D.S. Edwards[3], M. Azure[3], M.M. Sadeghi[1]; 1. Cardiovascular Molecular Imaging Laboratory, Section of Cardiovascular Medicine, Yale University School of Medicine, and VA Connecticut Healthcare System, New Haven, Connecticut and West Haven, Connecticut; 2. Cardiovascular Molecular Imaging Laboratory, Section of Cardiovascular Medicine, Yale University School of Medicine, New Haven, Connecticut; 3. Lantheus Medical Imaging, North Billerica, Massachusetts

Objectives: Arterial aneurysm expansion and rupture is a major cause of morbidity and mortality. Matrix metalloproteinase (MMP) activation plays a key role in aneurysm expansion. RP782 is a novel ^{111}In-labeled tracer targeted at activated MMPs. We hypothesized that RP782 imaging can predict aneurysm expansion in vivo. **Methods and Results:** Arterial aneurysm was induced by exposing the left common carotid artery of apolipoprotein $E^{-/-}$ mice to $CaCl_2$ (0.9 M). The contralateral artery was exposed to normal saline and served as control for imaging experiments. Exposure to $CaCl_2$ led to significant expansive remodeling over a period of 4 weeks (mean cross-sectional area: 0.095 ± 0.001 mm^2 in the control right vs. 0.33 ± 0.05 mm^2 in the aneurismal left carotid artery, n=8, p=0.001). This was associated with a 9-fold increase in MMP activity quantified by fluorimetric assay. RP782 (37 MBq) was injected intravenously to animals at 2 and 4 weeks after surgery. MicroSPECT imaging was performed at 2 hours and was followed by CT angiography to localize the carotid arteries. In vivo images demonstrated significant focal uptake of RP782 in the left, as compared to right carotid artery at 2 weeks (Left: 0.9±0.05 *vs.* Right: 0.22±0.02 counts/voxel/MBq injected, n=5, p=0.001), and 4 weeks (Left: 1.42±0.15 *vs.* Right: 0.20±0.03 counts/voxel/MBq injected, n=8, p=0.001) post-surgery. Increased tracer uptake in aneurismal arteries was confirmed by quantitative autoradiography and gamma well counting. Pretreatment with 50-fold excess of non-labeled tracer significantly reduced left carotid artery RP782 uptake at 4 weeks, validating tracer uptake specificity (1.42±0.15, n=8 *vs.* 0.12±0.10 counts/voxel/MBq, n=2, p=0.002). Next, a group of animals were serially imaged at 2 and 4 weeks in a longitudinal study. RP782 uptake at 2 weeks correlated well with the vessel area assessed by histology at 4 weeks (n=9, Spearman r=0.73, p=0.01), indicating that MMP activation detected by RP782 imaging can predict subsequent aneurism expansion in vivo. **Conclusions:** RP782 imaging can detect MMP activation in arterial aneurysm. Activated MMP imaging provides a potential tool to non-invasively predict aneurysm's propensity to expansion in vivo. **Acknowledgements:** D.S. Edwards and M. Azure are employees of Lantheus Medical Imaging. M.M. Sadeghi and A.J. Sinusas have received experimental tracers from Lantheus Medical Imaging.

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Dysregulation of Sympathetic Beta-Adrenergic and cAMP-Mediated Signaling in Type II Diabetic Rats: a Multitracer Analysis. J.T. Thackeray, M. Greene, M. Parsa-Nezhad, M. Kenk; National Cardiac PET Centre, University of Ottawa Heart Institute, Ottawa, Ontario, Canada

Objectives: Chronic diabetes and insulin resistance evoke dysregulation of the SNS characterized by persistent elevation of plasma and cardiac NE. These abnormalities in SNS signaling may represent a mechanistic link in the development of CVD in diabetes. This study assesses the effect of chronic hyperglycaemia on cardiac NE signaling in the progression of diabetic heart disease at the NE reuptake transporter (NET) using [^{11}C]*meta*-hydroxyephedrine (HED), beta-adrenoceptor (beta-AR) using [^{3}H]CGP12177 (CGP), and phosphodiesterase-4 (PDE4) as an indirect index of cAMP signal transduction using (*R*)-[^{11}C]rolipram (ROL). **Methods:** Sprague Dawley rats were rendered diabetic after 14 day high fat feeding by intraperitoneal injection of 45 mg/kg streptozotocin (STZ, n=89) or vehicle (n=48). Treated rats were stratified to hyperglycaemic (>11 mM n=45) or euglycaemic (<11 mM, n=44) subgroups. Insulin resistance was determined by euglycaemic clamp. Myocardial function and structure were assessed by echocardiography and histopathology. At 2 or 8 weeks post-STZ, rats were administered HED, CGP, and/or ROL, sacrificed and cardiac tissues counted for radioactivity. Specific retention was assessed by selective

blocking of NET, beta-AR, and PDE4, respectively. PDE4 response to acute NE elevation was tested by NET inhibitor desipramine. **Results:** High fat-fed rats treated with STZ or vehicle display insulin resistance. There was no change in tracer retention at 2 weeks post-STZ. At 8 weeks post-STZ, left ventricle retention of HED was reduced in hyperglycaemic rats (0.42±0.05 percent ID/g × body weight) compared to euglycaemic (0.53±0.08, $p<0.05$) and control rats (0.56±0.11, $p<0.05$). Similarly, CGP binding to beta-AR in hyperglycaemic rats (3.73±0.96) was significantly decreased to controls (5.60±1.60, $p<0.05$). ROL specific binding to PDE4 was similar in all groups, despite the apparent reduction in beta-AR in hyperglycaemics. All groups showed normal response to acute elevation of NE by desipramine. Systolic echocardiography parameters were unchanged and histopathology was normal. **Conclusions:** These data indicate reduced SNS innervation and beta-AR with normal systolic left ventricular function and preserved response of PDE4 to NE stimulation, suggesting compensatory maintenance of cAMP signaling in diabetes. Application of cardiac neurohormonal PET shows potential for monitoring progression of cardiac disease in type II diabetes.

SECTION 2

New Molecular Probes

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Quantification of Cardiac Sympathetic Nerve Density with 11-C-Guanyl-Meta-Octopamine. D.M. Raffel, Y.W. Jung, G. Gu, R.A. Koeppe, P.S. Sherman, C.A. Quesada; Division of Nuclear Medicine, University of Michigan, Ann Arbor, Michigan.

Objectives: Most radiotracers for imaging presynaptic cardiac sympathetic neurons, including 123-I-metaiodobenzylguanidine (MIBG) and 11-C-*meta*-hydroxyephedrine (HED) are 'flow-limited' due to rapid neuronal uptake by the norepinephrine transporter (NET). The flow-limited uptake of MIBG and HED precludes successful compartmental modeling of their myocardial kinetics. To overcome this limitation, we developed 11-C-guanyl-*meta*-octopamine (GMO), which has a slower NET transport rate and is trapped in norepinephrine storage vesicles. This study evaluated GMO's ability to provide quantitative measures of cardiac sympathetic nerve density. **Methods:** Neuronal uptake rate (Kup, mL/min/g wet) and neuronal retention time (T-1/2, h) of GMO were measured in isolated rat hearts (n = 5). MicroPET imaging in rhesus macaque monkeys assessed GMO's biodistribution and kinetics (13-39 MBq/kg; n = 3). GMO metabolites in rat plasma, heart and liver extracts were determined using radio-HPLC (t = 0.5 h, 370-740 MBq/kg, n = 5). Similarly, metabolite formation in monkey plasma was measured 5, 15, 30 and 55 min post-injection (74 MBq/kg; n = 3). GMO metabolism and microPET data from monkeys were used for compartmental modeling and Patlak graphical analysis of myocardial GMO kinetics. **Results:** In isolated rat heart, GMO's Kup = 0.30 ± 0.02 mL/min/g wet, 8-fold slower than HED. GMO's neuronal retention was very long (T-1/2 > 150 h, vs. 1.1 h for HED), indicating effectively irreversible trapping inside vesicles. In monkeys, GMO provided high quality heart images (heart/blood ratios > 7). GMO was readily metabolized in rats, with < 7% of plasma activity due to intact GMO at 0.5 h. However, activity in heart tissue extracts was 100% GMO, indicating the radiometabolites were inactive at sympathetic neurons. GMO metabolism and blood partitioning data from monkeys were used with microPET-derived LV blood pool time-activity data to prepare 'input functions' for kinetic analyses. Compartmental modeling with a 4-parameter model of the neuronal uptake and vesicular trapping of GMO (K1, k2, k3, BV) yielded stable parameter estimates, with a neuronal uptake rate constant k3 = 0.102 ± 0.024 /min. Patlak analysis yielded highly linear Patlak plots with consistent Patlak slope estimates (Ki = 0.103 ± 0.021 mL/min/g). **Conclusions:** These encouraging results in monkeys suggest that GMO could be used to quantify regional cardiac sympathetic nerve density in humans with PET.

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Targeted Annexin-V Gold Nanoconjugates for the Detection of Apoptosis. S. Nune[1], N. Chanda[1], M. Swapna[1], K.V. Kattesh[1], N. Tahara[2], A. Petrov[2], S. Ohshima[2], J. Narula[2], R. Kannan[1]; 1. University of Missouri, Columbia, Missouri; 2. University of California-Irvine, Irvine, California

Objectives: Gold nanoparticle Annexin-V (GNP-AnV) conjugate is a novel X-ray CT imaging nanoprobe for use in targeting phosphatidylserine (PS) that is present in apoptotic cells. The aims of this study are, (i) to synthesize, characterize, and evaluate the binding affinity of GNP-AnV conjugate towards PS located in apoptotic cells; and (ii) to evaluate the *in vivo* CT imaging efficacy of GNPs in mice using a micro SPECT-CT system. **Methods:** Gold nanoparticles were conjugated with Annexin-V using thioctic acid as a bridging ligand. GNP-AnV conjugates were characterized using TEM and UV-Visible spectroscopic methods. Apoptotic cells were generated by adding camptothecin to Jurkat-T cells. Binding affinity of GNP-AnV conjugates toward PS was determined using an indirect assay method. In this method, increasing concentrations of GNP-AnV were added to a series of tubes containing identical concentrations of apoptotic cells, followed by addition of equal amounts of FITC-Annexin-V (fluorescent probe) and PI to each and every tube. The binding assays of GNP-AnV conjugates were evaluated by examining the solutions using a dual wavelength FACS. SEM images were recorded to confirm the binding interactions of GNP-AnV to PS present in apoptotic cells. *In vivo* CT imaging efficacy of AuNPs was evaluated in normal mice. After injecting AuNPs in mice, the CT images of heart were recorded at various time points. **Results:** The GNP-AnV conjugates synthesized exhibit a narrow size distribution of 12 ± 3 nm, and plasmon absorption maximum at 540 nm. These conjugates show excellent *in vitro* stability against various biologically relevant molecules and also in buffer solutions. GNP-AnV conjugates with concentrations of ~200μM detect more than 90% of apoptotic cells as established by indirect binding assay method. Apoptotic cells treated with GNP-AnV conjugate and non-targeted AuNPs were subjected to electron microscopic analyses. SEM images confirm the binding of GNP-AnV conjugates to apoptotic cells; whereas, non-targeted gold nanoparticles did not show any binding with PS present in apoptic cells. *In vivo* CT imaging experiments were performed in normal mice after injecting with gold nanoparticles. Images showed a significant contrast enhancement due to AuNPs, and show clear visualization of heart 1 hour after injection. Contrast persisted over 24 hours. **Conclusions:** GNP-AnV nanoconjugates were successfully synthesized and characterized. The binding affinity value of GNP-AnV conjugates toward PS present in apoptotic cells is evaluated using FACS. SEM images showed binding of GNP-AnV conjugates to PS, and non-targeted AuNP showed no binding to apoptic cells. The *in vivo* CT imaging data unequivocally demonstrated the efficiency of gold nanoparticles to serve as contrast agents to detect apoptosis. These results suggest that GNP-AnV conjugates may be highly useful in imaging apoptosis.

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Microbubble Targeting in Fast-Flow Conditions: Influence of Red Blood Cells, and Importance of Fast-Binding Ligands. S. Unnikrishnan[1], F. Guenther[2], M. Lawrence[1], K. Ley[3], A.L. Klibanov[1]; 1. University of Virginia, Charlottesville, Virginia; 2. University Clinic Freiburg, Freiburg, Germany; 3. La Jolla institute of Allergy and Immunology, La Jolla, California.

Objectives: Targeted microbubbles are widely investigated as ultrasound contrast agents for molecular imaging. At high wall shear stress (WSS) fast flow conditions, not all ligands are suitable for microbubble targeting. Fast k_{on} ligands, such as sialyl Lewis oligosaccharides, are most appropriate for targeting in fast flow. Other factors, e.g., the presence of blood components, might also influence targeting efficacy. **Methods:** We have evaluated targeting of microbubbles *in vitro* in a parallel-plate flow chamber (Glycotech) perfusion model. Flow rate was controlled by a syringe pump. One side of the flow deck was coated with the target receptor: either a model protein streptavidin, or fibrinogen-anchored ADP-activated platelets, that present P-selectin on their surface. Microbubbles used were biotinylated, or decorated with selectin-binding polymeric sialyl Lewis A. Microbubble

targeting was assessed by video microscopy. Adhesion efficacy was estimated as the adherent fraction of the microbubbles flux. **Results:** Biotinylated microbubbles were not very efficient in targeting to streptavidin-coated surface in fast flow (<0.2% adhesion efficacy at WSS=0.45 Pa) in PBS. However, presence of red blood cells (RBC) at 20% and especially 40% hematocrit resulted in more than an order of magnitude improvement of targeting ($p<0.05$). When RBCs fixed with glutaraldehyde to reduce deformability were tested, microbubble targeting was still better than in PBS. A modest reduction of microbubble flux in the immediate proximity to the target surface was observed in the presence of RBC, so the hypothetic mechanism of targeting improvement is not likely related to microbubble exclusion from the center of the vessel towards the periphery. The use of fast-binding ligands, such as sialyl Lewis A, is known to improve targeting in fast flow. We demonstrated successful attachment of targeted microbubbles to activated platelets, with significant adhesion at WSS up to 4 Pa. Leukocyte-like slow rolling of microbubbles was observed in these conditions. Control bubbles did not stick to platelets at all. **Conclusions:** The use of cell-free *in vitro* flow chamber assays performed in homogeneous medium may underestimate targeting efficacy. Therefore, studies in the presence of red blood cells at concentrations similar to those found in blood may be helpful. Selectin-targeted bubbles efficiently bind to activated platelets in RBC-free medium; we might expect further targeting improvement in blood.

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Evaluation of Multifunctional, Alpha V Beta 3 Targeted Nanoparticles for Cardiovascular Imaging. M. Shokeen[1], E.D. Pressly[2], N. Ramos[1], C.J Hawker[2], C.J. Anderson[1]; 1. Washington University School of Medicine, St. Louis, Missouri; 2. University of California-Santa Barbara, Santa Barbara, California.

Over the past decade, there has been a proliferation of nanoparticle (NP) based pharmaceuticals, largely due to the combined contributions from the fields of polymer synthetic chemistry, cell biology, imaging, and clinical medicine. In comparison to small molecules, NPs have shown superior bioavailability, biocompatibility, physical and chemical stability, and drug release kinetics at the target site. Herein, we describe the in vitro evaluation of multifunctional NPs comprised of well-defined amphiphilic graft copolymers having a controllable number of reactive functional groups for attaching moieties such as DOTA ligand for chelating Cu-64, and RGD units for targeting the integrin alpha v beta 3 (avb3), a biological marker for angiogenesis manifested in cardiovascular abnormalities such as cardiomayopathy and atherosclerosis. Tunable synthesis led to the production of NP constructs with four different RGD loadings referred to here as 5%, 10%, 20%, and 50% targeted NPs with 7, 14, 28, and 70 RGD peptides per NP respectively. The goals of the project were (1) to understand the biological behavior of the NPs with varying RGD loadings by studying the binding affinity and specificity to integrin avb3; and (2) the impact of structure/activity relationship and multivalency on integrin binding and cellular internalization. The in vitro evaluation utilized three methods: (1) plate-based integrin binding assays with NPs for their affinity toward avb3, avb5 and allbb3 integrins; (2) cellular uptake assays in avb3 positive human glioblastoma U87MG cells; and (3) cellular imaging using confocal microscopy. The isolated integrin assay results demonstrated improved binding affinity toward avb3 resulting from the increased RGD units on the NPs, with 50% RGD NP having 78-fold better affinity (1.1 nM) as compared to the 5% RGD counterpart (78.46 nM), signifying the multivalent enhancement rendered by the NP design. There was non-specific binding (>1,000 nM) to integrins avb5 (found on macrophages) and allbb3 (found on blood platelets). Cell-based assays of the Cu-64 labeled NPs showed receptor mediated endocytosis in U87MG cells with up to 35% cellular uptake per mg of protein at 30 minutes post-injection. Confocal microscopy of U87MG cells incubated with targeted NPs further validated cellular internalization. Currently, this agent is being investigated to image expression of avb3 during the re-vascularization process in a mouse model hind limb ischemia.

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High Affinity AVB3 Targeted Optical Probe in Early Atherosclerosis Detection. J. Heroux[1], A.M. Gharib[1], N.S. Danthi[2], S. Cecchini[2], J. Ohavon[3], R.I. Pettigrew[4]; 1. NIH/NIDDK, Bethesda, Maryland; 2. NIH/NHLBI, Bethesda, Maryland; 3. NIH/NHLBI, La Tronche, France; 4. NIH/NIBIB, Bethesda, Maryland

Objectives: Integrin avb3 is known to be over expressed in atherosclerosis. The present study was designed to investigate the possibility of detecting early plaque by using a high affinity integrin avb3 targeted optical probe (ITOP). This synthetic ITOP has shown 20 times higher binding affinity for avb3 receptor compared to the commercially available cyclic peptide c[RGDfv]. The first objective of the study was to test, *in vitro*, the labeling potential of the ITOP probe. The second objective of the study was to confirm *in vivo* the targeting capability of this new ITOP. **Methods:** Eight Watanabe heritable hyperlipidemic rabbits as a model of atherosclerosis and 2 New Zealand White rabbits as controls were studied. For *in vitro* experiments, Watanabe rabbits were dissected and the presence of $\alpha v\beta 3$ on the frozen aortic tissue was detected by incubating the tissue for 1hr with the ITOP. Fluorescent signal was assessed under a microscope using an ultraviolet light source (Nikon E1000). For *in vivo* experiments, two Watanabe rabbits were injected with the ITOP and dissected after 1.5 hr. Parts of the ascending and descending aortas were snap frozen, cut into 7-8μm frozen sections and mounted on slides in order to visualize the fluorescence of the ITOP (avb3 selective antagonist labeled with fluorescein isothiocyanate). A labeled antibody against avb3 was used to confirm proper targeting of avb3 receptors. **Results:** For the *in vitro* studies, sites of plaque accumulation in different regions of the ascending and descending aortas were correctly labeled and corresponded to the same sites labeled with an anti-$\alpha v\beta 3$ antibody. The signal was found principally in the adventitia and proximal intima of the vessel, but also in the media of the wall where there was some disruption. Moreover, there was a close correlation between the level of labeling with the ITOP and the degree of adventitial thickness. The *in vivo* injection demonstrated that the ITOP could reach the vascular targets inside the vessel wall and label atherosclerotic sites in Watanabe rabbits with high plasma cholesterol level. **Conclusions:** This high affinity synthetic ITOP can be used for the detection of the presence and level of avb3, which may correlate with the stage of atherosclerotic plaque development. Thus, this new probe could provide molecular imaging of a potential sensitive biomarker of plaque inflammation as its signal correlates with the degree of adventitial thickening.

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Design and Synthesis 3-Phenyl-2H-Benzo[b][1,4] Oxazine Containing PET Imaging Agent for Hypoxia. Bhaskar C. Das, S. Mani, M. Donald Blaufox; Albert Einstein College of Medicine, Bronx, NY

Objectives: The objective in this study to design and synthesize novel PET imaging agent of hypoxia. Hypoxia is a universal hallmark of tumor cells in vivo. Within the tumor microenvironment, it contributes towards resistance to radiation and chemotherapy. It is known that hypoxic cells are highly reductive in nature. Bio-reductive activation is a well-established concept for achieving selective toxicity of anti tumor agents to hypoxic cells. Keeping above objective in mind we developed novel small oxazine library to treat hypoxic cell. **Methods:** We synthesized a small library of 3-phenyl-2H-benzo[b] [1,4] oxazine derivatives and their cytotoxicity activity were tested against hypoxic and normoxic cell. We further derivatized our lead compound to synthesize 18F and 11C labeled PET imaging agent of hypoxia. **Results:** From our preliminary screening we found compound JA155 specifically inhibit hypoxic cancer cell growth (IC_{50} 10±3.7 μM) while "normoxic" cells (IC_{50} >1000μM) and compound JA153 inhibits hypoxia cancer cell growth (IC_{50} 100±2.2 μM) and normoxic cells (IC_{50} >1000μM). The absence of toxicity in normoxic cells is a crucial feature for these compounds and based on this premise, we will be able to lead optimize its potency in hypoxic conditions. Compound JA155 could further derivatized as 11C labeled and JA153 as 18F labeled PET Imaging agents. **Conclusions:** We synthesized a small library of 3-phenyl-2H-benzo[b] [1,4] oxazine derivatives and their cytotoxicity activity was tested against hypoxic and normoxic cell. Using our lead molecule, we are developing novel hypoxia PET imaging agent will communicate in near future. Alterations of

the halogen positioning or other groups in the benzyl and/or phenyl ring can yield better, perhaps more potent compounds both as therapeutic and Positron Emission Tomography (PET) or SPECT imaging agents.

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Phosphatidylethanolamine as a Critical Anticoagulant at the Luminal Endothelial Surface. Z. Li, M. Zhao; Medical College of Wisconsin, Milwaukee, Wisconsin

While phosphatidylethanolamine (PE) has long been known as a ubiquitous structural element in cellular membranes, emerging evidence indicates that PE is anti-thrombogenic. However, the presence of PE at the vascular luminal surface and how PE may participate in hemostasis remain uncharacterized and speculative. **Objectives:** The objective of this work was to probe for PE at the endothelial surface, with high-resolution MRI using gadolinium (Gd)-labeled Duramycin as a novel PE-specific molecular probe. **Methods:** Duramycin-DTPA was synthesized by conjugating DTPA anhydride to Duramycin. The conjugate was labeled with Gd and purified using HPLC. Duramycin derivatized with biotin was synthesized for validation purposes using histology. The aortas of the rat were dissected and rinsed to remove residual blood. A solution of Duramycin-Gd-DTPA was infused into the aorta, followed by washing and MRI using a T_1-weighted spin echo sequence on a Bruker 9.4T scanner. Validating microscopy of the aorta was acquired using Duramycin-biotin, with fluorescein-conjugated Avidin. Immunohistochemistry using an anti-PE (aPE) serum was performed to confirm the presence of PE. **Results:** While trace level of Duramycin binding was detectable along the aortic wall, prominent uptake was predominantly localized on the surface of the flow divider at the outer curvature of the aortic arch. This pattern of distribution was consistent with Duramycin-biotin detection and aPE immunohistochemistry. **Conclusions:** It is indicative that a PE pool is present on the vascular surface at the flow divider of the aortic arch. This distribution profile mirrors the high blood flow velocity, sheer stress and turbulence in the aorta. The high density of local PE is likely to play a regulatory role in the coagulate hemostasis. These findings shed light on the antithrombogenic roles of PE in the cardiovascular system and suggest a causal link between aPE and idiopathic thrombosis in aPE positive patients. The experiment serves as a proof of concept where vascular PE is detected using high-resolution MRI.

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MR Detection of Early Inflammation at the Site of Focal Catheter-Induced Vascular Injury. M. DeLeo III, M. Gounis, A. Wakhloo, A. Bogdanov; University of Massachusetts Medical School, Worcester, Massachusetts

Objectives: Catheter-induced vascular injury is not visible using standard digital biplane fluoroscopy. The molecular environment in the damaged vessel wall is largely unknown but inflammatory changes including upregulation of the key inflammatory mediator NF-kappa-B and tissue inhibitors of matrix metalloproteinases have been described. The enzyme myeloperoxidase (MPO) is known to play a significant role in the progression in various vascular pathologies. However, the role of MPO in catheter-induced vascular injury is unknown. In this study, we performed feasibility experiments in an attempt to detect areas of vascular injury using the paramagnetic MR MPO-specific imaging probe di-5-HT-GdDTPA, which has been shown to be sensitive and specific for MPO both in vitro and in vivo. **Methods:** Elastase-induced saccular aneurysms were created in NZ white rabbits (n=5). After 21 days, a 4F guide catheter was secured under fluoroscopic guidance at the neck of the aneurysm. Using 3-D roadmap, a hypotube was advanced through a microcatheter to deliver a fixed volume of normal saline (200mcL) into and around the aneurysm wall. Forty-eight hours after saline injection, animals were subjected to 3T MRI using 3D T1W-FFE sequence. Animals then received an intra-arterial injection of di-5-HT-GdDTPA (0.1mmol/kg); 3h after contrast agent injection, animals were re-scanned using the same pulse sequence. Images were then subjected to ROI analysis for calculating normalized enhancement ratios (ER). MPO activity was verified and correlated to ER of LPS-injected animals. **Results:** We were able to perform the guided interventional procedure without hemorrhagic complications. Post-contrast MRI revealed focal areas of enhancement at the dome of the aneurysm consistent with the area of vascular perforation with the hypotube. We confirmed that aneurysms demonstrated a higher enhancement ratio compared to the left carotid artery in the same animals. **Conclusions:** 1. Focal vascular injury can be visualized using the MPO-specific MR imaging probe. 2. MPO may play a role in the acute biological response to catheter-induced endothelial disruption. 3. The MPO-specific imaging probe is able to noninvasively detect very small areas of vessel injury. 4. ER can be used for predicting the extent of inflammation in aneurysms. MPO probe-assisted MRI could potentially be used to monitor catheter-induced vessel damage following endovascular interventions

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Metal Nanobeacons for X-Ray K-Edge-Based Molecular Imaging. D. Pan[1], E. Roessl[2], R. Proksa[2], J-P Schlomka[2], A. Senpan[1], S.D. Caruthers[3], M.J. Scott[1], E. Choi[1], P. Gaffney[4], S.A. Wickline[1], G.M. Lanza[1]; 1. Washington University School of Medicine. St. Louis, Missouri; 2. Philips Research Europe, Hamburg, Germany; 3. Philips Healthcare, Andover, Massachusetts; 4. St. Thomas' Hospital, London, United Kingdom

Objectives: Emerging development of x-ray K-edge based imaging (Spectral CT) is expected augment CT angiography by eliminating coronary calcium interference and detecting ruptured coronary plaque with k-edge distinctive molecular imaging agents. The goal of this study was to develop a stable, new class of colloidal nanoparticles system of "soft" nature, incorporating very high concentrations of K-edge metallic agents to permit noninvasive labeling of biologically relevant markers to detect and characterize early disease. **Methods:** K-edge metal nanobeacons of "soft nature", based on bismuth and gold have been designed and synthesized for vasculature constraint (~200nm) to enhance specificity for key markers of thrombosis (i.e., fibrin) and angiogenesis (i.e., integrin). Spectral CT nanobeacons incorporated ~50% metal content using organometallic compounds in polysorbates encapsulated by phospholipid layer. For *in vitro* demonstration of molecular imaging, fibrin-rich clots were targeted with biotinylated nanobeacons (n=3) or the control nanocolloids (n=1) using a well characterized human and fibrin-specific monoclonal antibody (NIB5F3). **Results:** The first targeted Spectral CT images were acquired uniformly for all nanobeacons treated clots; no signal was seen in the control. Typically, bismuth nanobeacon layer thickness was estimated to be ≤200 μm and the bismuth surface density was 3.5 mass%. In experiment 2, Spectral CT enhancement of the small fibrin deposits in the ruptured carotid plaque treated with bismuth nanobeacons (K-edge=90.5keV) was readily apparent, in contradistinction to the control CEA specimens, and easily differentiated from calcium attenuation (K-edge=4.03keV). As is typical of CT, poorly attenuating soft-tissue detail was lost. **Conclusions:** This study reports a novel example of Spectral CT molecular imaging agents for detection and quantification of intravascular thrombus associated with ruptured plaque. **Acknowledgements:** G.M. Lanza received funding from NIH and equipment support from Philips Medical Systems.

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The Imaging Probe Development Center—A Core Synthetic Facility Dedicated to Production of Known and Novel Molecular Imaging Probes. S.M. Cheal, A. Dulcey, C. Regino, N. Shenoy, Z-D. Shi, A. Sulima, O. Vasalatiy, H. Wu, B. Xu, K. Barbacow, S. Cofiell, C.M. Wilson, G.L. Griffiths; National Institutes of Health, National Heart, Lung, and Blood Institute, Bethesda, Maryland

Objectives: The NIH Roadmap Initiative for Medical Research recognizes the significance of imaging technologies as a key component in advancing biomedical research. The Imaging Probe Development Center (IPDC) was set up as a new synthetic chemistry core facility dedicated to fulfill the niche of production of known and novel imaging probes being unavailable commercially. IPDC is initially set up to as a resource for the NIH intramural community and anticipate providing imaging probes to the extramural scientific community. **Methods:** Imaging probes produced at the IPDC encompasses MRI, optical and radioactive modalities; several examples will be presented. We have a diverse group of organic chemists, radiochemists, biochemists and biologists that can provide services such as: peptide synthesis, custom oligonucleotide synthesis, labeling of molecules with a

variety of radionuclides, preparation of bifunctional visible and near-IR fluorescent dyes and subsequent bioconjugations, and synthesis of caged substrates. **Results:** Gadolinium-DTPA-dendrimer conjugates and iron oxide nanoparticles were prepared for MRI studies. Fluorescent agents such as analogs of fPEG-Chol have been synthesized for optical imaging of raft cholesterol in cells. Caged fluorescent dyes covalently attached to monoclonal antibodies were synthesized to be used as mapping labels in PhotoActivated Localization Microscopy (PALM). As an example of radiolabeled probes, I-125 labeled vasopressin peptide analogs for receptor binding studies will be presented. **Conclusions:** IPDC is capable of producing multiple diverse imaging probes as required by molecular imaging scientists. For more information, visit http://www.ipdc.nih.gov

SECTION 3

New Molecular Imaging Technologies

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Micro-CT Characterization of Murine Vascular Phenotype. Z.W. Zhuang[1], A.A. Lanahan[1], J. Hamilton[2], J. Shi[3], A.J. Sinusas[1], M. Simons[1]; 1. Yale University, New Haven, Connecticut; 2. Dartmouth College, Lebanon, New Hampshire; 3. M2S Company, Lebanon, New Hampshire.

Objectives: Genetically modified mice have become common models of human cardiovascular disease and this has created a demand for efficient methods to visualize and quantify the murine cardiovascular phenotype. Contrast-enhanced microscopic computed tomography (micro-CT) imaging is a potential powerful means for characterization of cardiovascular anatomy of wild-type (WT) and transgenic knockout (KO) mice, as well as for detection of arteriogenesis and remodeling of pre-existing arterioles following therapy. The aims of the current study are: (1) to develop semi-automatic 3D-software for segmentation of the arterial tree defined with microCT; (2) to compare the efficacy of two different contrast agents for the visualization and quantification of vascular development in the hindlimbs of WT mice (3) to compare arterial development in the hindlimbs of WT and myosinVI KO mice; (4) to evaluate arteriogenesis in response to hindlimb ischemia in WT and myosinVI KO mice**. Methods:** Pilot micro-CT imaging was performed in C57 BL/6J mice injected with 25% bismuth-gelatin (n = 3) or microfil (n = 3) for image optimization. After the mice were euthanized, a blunted 21G butterfly needle was cannulated into the descending aorta and contrast agent was injected at 0.75 ml/min for 0.2 ml/10 gram body weight with a syringe pump. The trimmed hindlimb was imaged with micro-CT imaging system (GE eXplore Locus SP) set to a 0.008-mm effective detector pixel size, 60-kVp x-ray tube voltage, 100-μA tube current, 3000-milli-second per frame, 1 ×1 detector binning model, 360 views, and 0.5° increments per view. This acquisition resulted in a set of contiguous axial VFF-formatted images through each hindlimb. Micro-CT imaging was performed to assess the vascular phenotype of 8-10 week old WT (n = 6) and myosinVI KO (n = 6) mice at baseline (n = 6) and 2 weeks (n = 6) following induction of hindlimb ischemia by femoral artery occlusion. After scanning, slices were reconstructed with our in-house software based on the Feldkamp algorithm and automated segmentation performed. The morphological parameters (vessel volume, relative cross-section vessel number, lumen diameter, diameter distribution) were evaluated in the surgical limb and contralateral control limb. **Results:** The automated software successfully analyzed 3D micro-CT images, separating bone from the vasculature, as well as the venous system from the arterial system, and facilitated segmentation of the purified arterial tree for quantitative analysis. Bismuth-gelatin resulted in greater attenuation than microfil on micro-CT, resulting in better visualization of arterioles and arteriogenesis than obtained using microfil. Micro-CT quantitative analysis indicated smaller arterioles in myosinVI KO mice compared to WT mice at baseline. MyosinVI KO mice also showed significantly less arteriogenesis following hindlimb ischemia, relative to WT mice. **Conclusions:** We present a novel micro-CT angiographic methodology and semi-automated analysis that characterizes the vascular phenotype in WT and myosinVI KO mice at baseline and following hindlimb ischemia, demonstrating phenotypic differences in the myosinVI KO as well as an impaired angiogenic response to ischemia. The semi-automated analysis easily distinguishes phenotypic differences between different mouse models and has implications for the evaluation of therapeutic angiogenesis/arteriogenesis.

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High-Resolution Ultrasound Assessment of Infarct Size and Cardiac Function in Mice Early After Myocardial Infarction. L.J. Yuan[1], T. Wang[1], M.L. Kahn[2], V.A. Ferrari[2]; 1. Penn Cardiovascular Institute; 2. Division of Cardiovascular Medicine, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

Objectives: Few non-invasive techniques exist for high-throughput study of efficacy of novel therapies for post-infarction heart failure therapy post myocardial infarction (MI). We developed a simple, rapid and accurate echocardiographic method protocol for simultaneously assessment of the infarct size (IS) and cardiac dysfunction in an acute mouse infarct model. **Methods:** We studied 18 normal and infarcted C57BL/6J mice (n=18) pre- and 48 hours after following permanent left anterior descending coronary artery ligation using a high-resolution (30 MHz) Vevo 770 ultrasound system (VisualSonic Inc., Canada). Four equally-spaced short-axis and one apical 2-chamber long-axis views of the left ventricle (LV) cine-loop clips were recorded in ultrahigh-frame-rate EKV™ (ECG-based kHz visualization) acquisition mode. The echo-derived IS assessment was compared and validated with three different histological measurements (area-, mass- and angle-based [AB] approach) defined by triphenyltetrazolium chloride staining. LV systolic and diastolic function was evaluated by 4-plane modified Simpson's method and pulsed-wave Doppler, respectively. **Results:** MI reduced LV ejection fraction (EF) to 36.6±6.0% compared with baseline 71.1±7.9%, p=0.014. Correlation and Bland-Altman analysis by slice-by-slice comparison in 4 LV short-axis views revealed excellent agreement between high-resolution echocardiographic assessment and AB of IS (r=0.82, P< 0.001), but not with area- and mass-based measurements, likely due to differences in area selection. IS by echo and AB strongly correlated with Tei index (r=-0.82, p=0.0006 and r=0.74, P=0.0035). IS by AB correlated well with Tei index (r=0.74, P=0.0035), EF (r=-0.057, P=0.048), fractional area change (r= -0.75, P=0.005), stroke volume (r=-0.62, P=0.033) and isovolumic relaxation time (r=0.76, P=0.046). In contrast, mass- and area-based measurement of IS and echo-derived functional parameters correlated poorly. **Conclusions:** A straightforward high-resolution echo protocol rapidly and accurately depicts myocardial infarction size and cardiac dysfunction in vivo.

SECTION 4

Preclinical Applications of Molecular Imaging

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Role of Ponsin in Myocardial Glucose Metabolism and Function: Preliminary Observations with Molecular Imaging. K. Kotak, S. Tavakoli, H. Jadvar, M. Pashmforoush; University of Southern California, Los Angeles, California

Objectives: Ponsin is a member of SoHo family of multidomain adaptor 1molecules that has been identified as a component of a PI3K-independent signaling pathway for regulation of glucose metabolism and is essential for translocation of GLUT4 in response to insulin stimulation in adipocytes. However, the exact role of ponsin in cardiac function and glucose metabolism is unknown. **Methods:** We generated a mouse knockout model of Ponsin ($Sorbs1^{-/-}$) and then performed pilot echocardiography and dynamic micro-positron emission tomography (microPET) imaging studies after tail vein administration of 200 uCi [F-18]-fluorodeoxyglucose (FDG) in both $Sorbs1^{-/-}$ and wild-type mice. Plasma insulin was also measured after glucose tolerance test. **Results:** $Sorbs1^{-/-}$ mice demonstrated insulin sensitivity and protection against high-fat diet induced insulin resistance as well as a significant decrease in plasma insulin after glucose tolerance test. Ponsin null mice also showed dilated cardiomyopathy on echocardiography and an initially rapid rise in cardiac FDG accumulation followed by a slower uptake rate with an overall lower myocardial uptake in comparison to the

wild type controls. **Conclusions:** Our pilot proof-of-concept study suggests a modulatory role of Ponsin in myocardial glucose metabolism and function. Additional studies are currently underway to further elucidate the role of Ponsin in cardiac glucose metabolism.

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Messenger RNA Transfection of Human Sodium Iodide Symporter Reporter Gene Into Stem Cells for Nuclear Imaging. F. See[1], T. Seki[2], Y. Tekabe[2], M.R. Abraham[3], S. Itescu[2], L.L. Johnson[2]; 1. Columbia University and The University of Melbourne, New York, New York and Melbourne, Australia; 2. Columbia University, New York, New York; 3. Johns Hopkins University, Baltimore, Maryland

Objectives: To determine the feasibility of mRNA transfection as a method to induce functional human sodium iodide (hNIS) reporter gene expression in adult human bone marrow-derived mesenchymal progenitor cells (hMPCs: classified as an investigational drug by the FDA) for nuclear imaging. **Methods:** hNIS mRNA was synthesized *in vitro* by PCR amplification of hNIS cDNA using primers containing the T7 promoter, followed by transcription using T7 RNA polymerase and stabilization by the addition of a polyA tail. hMPCs were transfected with hNIS mRNA using Lipofectamine. Transfection efficiency was determined at various time points by flow cytometric analysis of cell-surface expression of hNIS. The functionality and specificity of the symporter protein expressed by transfected hMPCs was evaluated *in vitro* by counting the gamma emission of samples of known numbers of hNIS-transfected cells pulsed with ^{99m}Tc in the presence or absence of sodium perchlorate. **Results:** Flow cytometric analysis of hMPC cultures transfected with hNIS mRNA demonstrated cell-surface expression of hNIS protein at 48 h (38.7%), 72 h (39.8%) and 7 days (40.4%) following transfection. In contrast, hNIS was not detected in non-transfected hMPC controls. Brief incubation of hNIS-transfected hMPCs with ^{99m}Tc resulted in cell number-dependent gamma signals, which were corrected for background (Corrected ^{99m}Tc cpm: 125,000 hMPCs: 865.1±85.2; 50,000 hMPCs: 506.5±184.4; 10,000 hMPCs: 374.3±5.2). ^{99m}Tc-binding to hNIS-transfected cells was completely inhibited in the presence of sodium perchlorate. **Conclusions:** Together these data suggest that hNIS mRNA transfection is a feasible approach to expressing functional hNIS protein at the cell surface of hMPCs for up to 7 days. mRNA transfection of hNIS reporter gene into hMPCs may represent a clinically applicable method for labeling these cells to enable tracking of their distribution and fate by nuclear imaging following administration *in vivo.* **Acknowledgements:** Author S. Itescu is affiliated with Angioblast Systems.

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Non-Invasive Assessment of Myocardial Glucose Metabolism, LV Structure and Function in a Murine Model of Left Ventricular Hypertrophy. B.K. Kundu , L.W. Locke, G.P. Matherne, F.H. Epstein, S.S. Berr, D.K. Glover; University of Virginia, Charlottesville, Virginia

Objectives: Assessing changes in myocardial metabolism that occur during progression to heart failure with left ventricular hypertrophy (LVH) is now possible due to recent technological advances in non-invasive imaging of the mouse heart. MicroPET and MR imaging were performed to evaluate the relationship between alterations in myocardial glucose uptake and structural and functional changes in the heart associated with LVH. **Methods:** LVH was induced in seven 8-9 week old C57BL6 male mice, by transverse aortic constriction (TAC) over 4 weeks. In 3 of these mice, propranalol treatment (1mg/kg/day) was initiated, in mini-pumps subcutaneously, 2 weeks post TAC (50% LVH) and continued for an additional 2 weeks. A third group of 4 sham-operated mice were also studied for comparison. 18F-FDG PET and MR imaging was performed at baseline, and at 2 and 4 weeks post TAC. Semi-quantitative measurements of the myocardial glucose standardized uptake value (SUV) were made at each time point. Quantitative measurements were also performed using dynamic PET imaging and 3-compartment model, accounting for the effects of Partial Volume (PV) and attenuation on the rates of myocardial glucose uptake (Ki) and utilization (rMGU). Ejection fraction (EF), LV wall thickness and heart mass were measured in-vivo by MR and heart weight-to-body weight ratios (HW/BW) were calculated. Septal wall strain was also measured by spiral DENSE MR. **Results:** TAC resulted in increased LV wall thickness (↑ factor of 1.4), septal wall strain (↑ factor of 4) and HW/BW ratios (↑ factor of 1.7) with decreased EF (↓ factor of 1.8) at 4 weeks as compared to shams ($p<0.01$). Glucose SUV increased by a factor of 4.4, whereas by compartmental analysis, Ki and rMGU increased by a factor of 6.9 in TAC vs. sham-operated mice ($p<0.001$). With propranalol treatment, there was an improvement in EF by a factor of 1.4 ($p<0.05$) and a reduction in HW/BW ratios (↓ factor of 1.6) and septal wall strain (↓ factor of 3.7) while Ki decreased by a factor of 5.5 as compared to TAC mice ($p<0.005$). **Conclusions:** Quantitative PET and MR imaging demonstrated increased glucose metabolism that preceded structural and functional changes in a murine model of pressure overload LVH. Compartmental analysis provided a more sensitive means of detecting changes in metabolism over standard SUV measurements. Propranolol treatment resulted in lower glucose utilization with improvement in LV structure and function.

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Molecular Imaging of VEGF Receptors in Remodeling Human Coronary Arteries. J. Zhang[1], M. Razavian[1], L. Nie[1], S. Tavakoli[1], J.M. Backer[2], M.V. Backer[2], M.M. Sadeghi[1]; 1. Cardiovascular Molecular Imaging Laboratory, Section of Cardiovascular Medicine, Yale University School of Medicine, and VA Connecticut Healthcare System, New Haven, Connecticut and West Haven, Connecticut; 2. SibTech, Inc., Brookfield, Connecticut

Objectives: Vascular endothelial growth factor (VEGF) plays a causal role in many forms of vascular remodeling, including graft arteriosclerosis (GA). GA is characterized by neointima formation and progressive loss of lumen in allograft arteries, and is the major cause of late organ failure after transplantation. While expression of VEGF in GA is well established, the data on VEGF receptor expression are non-conclusive. In this study, we used an engineered Cy5.5-labeled single-chain VEGF (scVEGF/Cy) to assess VEGF receptor expression by molecular imaging in GA. **Methods and Results:** Segments of human coronary arteries were transplanted into the abdominal aorta of severe combined immunodeficient (SCID) mice. Adoptive transfer of allogeneic human peripheral blood mononuclear cells (PBMCs) after transplantation led to prominent neointima formation and expansive remodeling over a period of 4 weeks. The intimal and total vessel areas increased from 0.062 ± 0.025 mm^2 and 0.368 ± 0.070 mm^2 in the absence of PBMCs to 0.551 ±0.135 mm^2 and 1.054 ±0.177 mm^2 at 4 weeks after PBMC transfer ($n= 5$, $p<0.05$ for both values). VEGF receptor expression in coronary arteries was confirmed by immunostaining. scVEGF/Cy (10μg/mouse) was injected intravenously to transplant recipients 4 weeks after PBMC transfer (n=3). Transplant recipients without PBMC transfer were used as control. Near-infrared imaging at 24 hours demonstrated focal uptake of scVEGF/Cy in the transplanted coronary arteries post-PBMC transfer (mean fluorescence intensity 158.51±55.91 after PBMC transfer vs. 24.10±7.98 without PBMC transfer, $p<0.05$). Uptake specificity was demonstrated using inactivated scVEGF/Cy in transplant recipients 4 weeks after PBMC transfer (mean fluorescence intensity: 25.25±20.51, n=2). **Conclusions:** VEGF receptors are upregulated in conjunction with neointima formation and expansive remodeling in transplanted human coronary arteries. Molecular imaging of VEGF receptors may provide a non-invasive tool for detection of GA in solid organ transplantation. **Acknowledgements:** J.M. Backer and M.V Backer are employees of SibTech, Inc. M.M. Sadeghi received experimental tracers from SibTech, Inc.

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Overexpression of TEAD-1 in Transgenic Mouse Heart Produces Progressive Myocardial Hypertophy. L. Ma, B. Morgan, C. Schramm, R. Tsika; University of Missouri, Columbia, Missouri

Objectives: TEA domain (TEAD) transcription factors serve important functional roles during embryonic development and in striated muscle gene expression. Previous work has implicated a role for TEAD-1 in the fast-to-slow fiber-type transition in response to mechanical overload. In this work, we aim to investigate whether TEAD-1 regulates heart gene expression *in vivo*, and whether TEAD-1 overexpression has a role in causing myocardial remodeling and cardiomyopathy. **Methods:** Transgenic mice were generated

by expressing hemagglutinin (HA)-tagged TEAD-1 under the control of the muscle creatine kinase promoter. High resolution gel electrophoresis was performed to analyze gene expression in isolated heart tissues. In vivo cardiac magnetic resonance imaging (MRI) was carried out on 2, 6 and 11 month-old TEAD-1 transgenic (Tg) mice and the age-matched wild-type (WT) mice. **Results:** Myocardial structural remodeling was detected in the left and right ventricles for the TEAD-1 Tg hearts at as early as 2 month-old. For example, septum wall thickness was 1.13 ± 0.21 mm for the Tg mice and 0.80 ± 0.16 mm for the WT mice. At early ages (2 and 6 months), there were no significant dysfunctions in the Tg hearts. However, the hearts of the 11 month-old Tg mice had significantly decreased myocardial contractility and function. For example, left ventricular (LV) end-systolic volume was significantly increased for the Tg group (40.35 ± 19.61 μL) compared to the control (20.93 ± 8.07 μL). LV ejection fraction was decreased to 48.78 ± 15.19% (Tg) versus 70.32 ± 5.82% (WT). LV peak ejection rate was significantly decreased from 1.16 ± 0.29 μL/ms (WT) to 0.70 ± 0.25 μL/ms (Tg). Peak filling rate decreased from 1.22 ± 0.27 μL/ms (WT) to 0.79 ± 0.28 μL/ms (Tg). Further, comparative high resolution gel electrophoresis analysis of myofibrillar protein isolated from either WT or TEAD-1 Tg heart demonstrated a shift of a motor protein, myosin heavy chain (MyHC) protein isomers, in the Tg heart. **Conclusions:** We show that overexpression of TEAD-1 produces a progressive hypertrophic cardiomyopathy. We also show that a persistent increase in TEAD-1 protein induced a change in MyHC protein expression pattern in heart. The present study provides the first *in vivo* evidence that TEAD-1 participates in gene regulation and myocardial remodeling. The work was supported by the National Institute of Health, the University of Missouri and the Harry S. Truman VA Hospital-Columbia, Missouri.

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Injection of Murine ES-Derived Cardiomyocytes Improves Cardiac Function in Infarcted Myocardium but Forms Few Grafts. H. Qiao, H.L. Zhang, R. Zhou, V.A. Ferrari; University of Pennsylvania, Philadelphia, Pennsylvania

Background: Embryonic stem cells (ESC) readily differentiate and follow a cardiac lineage, making them a potential source of transportable cells for myocardial regeneration. However, low yields of ESC-derived cardiomyocytes (ESC-CMs) using conventional differentiation methods makes it difficult to perform in vivo studies, and low enrichment of CMs leads to concerns for teratoma formation. **Methods:** We evaluated the effect of generating ESC-CMs using a murine ESC line containing a puromycin resistance gene controlled by a cardiac specific promoter, sodium calcium exchanger (NCX), on CM yield and enrichment. We also studied the effect of peri-infarct injection of ESC-CMs on post-infarction LV function. ESC-CMs were labeled with superparamagnetic iron-oxide nanoparticles for MRI detection. Reperfused myocardial infarction was induced in athymic rats following a 45 min occlusion. Infarction size (IS) was estimated by MRI on post-op day 1 to exclude animals with IS <10% or >35% of the LV volume. On post-op day 7, 5-10 million labeled ESC-CMs were injected into the peri-infarction region, while the Control group was injected with vehicle. MRI scans were performed on post-op day 8 to confirm successful CM cell transplantation. Global cardiac function in ESC-CM and vehicle treated animals was assessed by MRI at 1 and 2 months post-op. Immunohistology staining and electrophysiology were performed on postmortem hearts and ESC-CMs, respectively. **Results:** A high yield of ESC-CMs was achieved with positive cardiac specific alpha-actinin in >90% of cells. The low proliferative capacity of ESC-CMs permits SPIO retention for serial MRI tracking. LV ejection fraction (LVEF) in ESC-CM treated rats at 1 and 2 months was significantly greater than that of Controls. Immunohistochemistry demonstrated graft formation in the host myocardium and gap junctions between injected ESC-CMs and host CMs, however, these grafts were few in number. The rats treated with ESC-CMs showed a much greater increase in LVEF than the control rats both at 1 month ($p=0.0003$) and 2 months ($p<0.0001$), while the infarction size on day 1 post-op was similar between two groups (20.02 ±3.11 vs. 19.09 ±4.72, $p=0.65$). **Conclusions:** Large numbers of highly pure ESC-CMs were obtained using an NCX cardiac specific promoter. While ESC-CMs form grafts and improve LV function in the infarcted hearts, the predominant mechanism of improved LVEF may be a paracrine or other effect rather than extensive ESC-CM grafting.

SECTION 5

Clinical Applications

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Cardiac FDG Uptake, an Indicator of Response to Therapy in Cancer Patients. H.M. Elsamaloty[1], M.S. Elzawawi[2], N.A. Herial[1], H.B. Semaan[1]; 1. University of Toledo Medical Center, Toledo, Ohio; 2. Menoufyia University, Menoufyia, Egypt.

Objectives: To evaluate the relationship between the myocardial FDG uptake during PET/CT studies and the response to therapy in cancer patients. **Materials and Methods:** Institutional review board approval was obtained. A retrospective study was conducted on 95 patients pathologically diagnosed with various types of cancer, who presented to the Radiology Department for periodic follow-up PET/CT scans as part of staging / restaging process and to assess response to therapy. The maximum standard uptake values (SUV) of the myocardium were measured at the lateral wall of the left ventricle during the initial and follow-up scans and the changes were correlated with patient's response to therapy. **Results:** Decreasing tumor SUVs were associated with increasing myocardial SUVs. A significant negative linear correlation was observed between the tumor SUVs and the myocardial SUVs ($r= - 0.49$, $p<0.001$). This was particularly evident in lymphomas ($r = -0.53$, $p=0.002$). Changes in the myocardial SUVs were also correlated with the clinical outcome. Average change in the myocardial SUVs was significantly different between the clinical outcome groups ($F=7.91$, $p<0.001$). Adjusting for age, gender, and tumor type, patients with improved clinical outcome (mild, moderate or marked), compared to those with a stationary or deteriorating clinical course were more likely to have increased myocardial SUVs (OR=15.5, 95% CI=5.6-43.5). **Conclusions:** This study suggests a significant association between myocardial SUV and the clinical outcome. Cardiac FDG uptake could be used as an indirect sign of response to therapy in cancer patients. Increasing change in myocardial SUVs was associated with positive response to therapy and decreasing change was associated with a negative response. Future larger scale studies could further assess this relationship and its value in different types of malignancy.

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Coronary Calcium Scores on Gated Contrast-Enhanced CT Angiograms Underestimate but Closely Correlate to Non-Contrast Coronary Calcium Scores. A.D. Morris[1], B.J. McKee[1], C. Wald[1], S. Flacke[1], A. Ulano[2]; 1. Lahey Clinic, Burlington, Massachusetts; 2. Tufts University School of Medicine, Boston, Massachusetts

Objectives: To develop a method for coronary calcium scoring on contrast enhanced CT angiograms and to compare this method to the non-enhanced gold standard. **Method and Materials:** 55 consecutive patients (age range 28-81) were enrolled into this study. All patients underwent a non-contrast enhanced calcium score prior to performance of a contrast enhanced CT angiogram for the evaluation of CAD. Data were acquired on a Siemens Definition dual-source scanner and processed on a Leonardo workstation (Siemens Medical Solutions). Post contrast calcium scores were calculated using the 70% phase three ways. The lower threshold value of the standard non-contrast calcium scoring algorithm (130 HU) was set to 150% and 175% of the mean and 115% of the maximum HU value of a 1 sq cm ROI of the aorta within 1 cm of the superior coronary ostia. Patient with maximum aortic HU greater than 700 were excluded as none of the proposed methods reliably identified calcium. The resulting post-contrast calcium scores of the three different methods were compared to the gold standard non-contrast calcium scores using regression analysis. **Results:** Non-contrast calcium scores ranged from 0 to 2701 with at least 10 studies in each of the standard scoring intervals: <10, 11-100, 101-400, >400. 5 patients were excluded with maximum aortic HU greater than 700. The correlation coefficient between contrast and non-contrast calcium scores using 115% of maximum aorta HU threshold (115Max) was 0.94. Using 150% and 175% of mean aorta HU as thresholds the correlation coefficients were 0.91 and 0.92 respectively. A 115Max score over 10 always predicted a positive non-contrast calcium score. A 115Max score less than 27.4

predicted a non-contrast calcium score less than 100. A 115Max score between 35.5 and 106.8 predicted a calcium score between 100 and 400. A 115 score over 127.8 predicted a non-contrast calcium score greater than 400. The115Max score always underestimated non-contrast calcium scores greater than 10 while the cores using 150% and 175% of mean aorta HU thresholds occasionally exceeded the non-contrast score. **Conclusions:** A calcium score calculated from a contrast enhanced CTA using 115% of the maximum HU value in the aorta closely correlates to the non-contrast score. A calcium score derived from a contrast-enhanced study allows estimation of calcium burden without additional radiation in studies such as triple rule out or pulmonary vein assessment.

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Automatic Attenuation and Emission Alignment in Cardiac PET/CT Based on Consistency Conditions. A.M. Alessio, J.H. Caldwell; University of Washington, Seattle, Washington

Objectives: In cardiac PET and PET/CT imaging, misaligned attenuation correction factors can cause erroneous perfusion defects and quantification. Previous studies have shown that misalignment between cardiac PET and helical CT acquisitions can cause moderate to severe perfusion artifacts in roughly 40% of cardiac PET/CT studies. At present, the only available option for correction of this misalignment is a subjective, manual realignment. We propose and evaluate a reproducible, automatic method for realignment and the reduction of artifacts. **Methods:** We propose an alignment process derived from work by Welch et al. (1998) for stand-alone PET imaging. The CT-based attenuation map is iteratively transformed until the attenuation corrected emission data minimizes an objective function based on the Radon consistency conditions. From a cohort of a total of 42 attenuation-emission combinations (patients: 86-140kg), we selected 8 studies with, by visual inspection, clearly aligned attenuation maps and 8 studies with misaligned attenuation maps. First, the originally aligned attenuation maps where transformed out of alignment by a known amount. The proposed algorithm attempted to realign the data and reduce the known transformation. Secondly, the originally misaligned maps where automatically aligned with the proposed method and two trained, independent, blinded readers reviewed the original alignment and the proposed alignment, selecting the attenuation-emission combination with less misalignment. **Results:** In all patient combinations representing a wide range of patient sizes and noise levels, the proposed method reproducibly converged to single solutions of aligned attenuation maps. For the combinations in which we induced a known transformation, the proposed method reduced the error in the transformation by on average 43%+-8%. For the combinations with original misalignment, the observers preferred the combination after the proposed algorithm in all cases, except one in which a single observer was unable to see a significant difference between the original and proposed. **Conclusions:** The proposed alignment procedure offers a reproducible, automatic method to reduce attenuation correction artifacts in cardiac PET/CT and offers a viable alternative to the subjective, manual realignment tools currently used in practice. The proposed method could also be applied to ensure alignment of dual-modality functional and anatomical image volumes.