Published in final edited form as:

Cardiol Clin. 2009 May ; 27(2): 329–Contents. doi:10.1016/j.ccl.2008.12.008.

New Molecular Imaging Targets to Characterize Myocardial Biology

Alan R. Morrison, MD, PhD and Albert J. Sinusas, MD

Yale University School of Medicine

Synopsis

Molecular imaging represents a targeted approach to non-invasively assess biologic (both physiologic and pathologic) processes *in vivo*. Ideally the goal of molecular imaging is not just to provide diagnostic and prognostic information based on identification of the molecular events associated with a pathological process, but rather to guide individually tailored pharmacological, cell-based or genetic therapeutic regimens. This article reviews the recent advances in myocardial molecular imaging in the context of the cardiovascular processes of angiogenesis, apoptosis, inflammation and ventricular remodeling. The focus is on radiotracer-based SPECT and PET molecular imaging approaches.

Introduction

Role of Molecular Imaging

As a greater understanding of the complex molecular interactions that take place within an organism both in the physiologic as well as pathologic states develops, there is an opportunity to design more precise and effective medical therapies that carry less of a burden of side effects and invasive injuries. This evolution of truly individualized health care will require enough knowledge of an individual's genome and proteome to model molecular interactions from levels of gene expression to the complex milieu and kinetics of protein expression and post-translational modification that occur in physiologic and pathologic processes. New therapies that target specific molecular pathways can then be developed to affect outcomes before disease burden affects the health and productivity of an individual in question. To track both disease and intervention, a parallel engineering of tools to image specific molecular events must take place that allows assessment of the *in vivo* state before and after any intervention is carried out. This article will review the molecular-based nuclear imaging approaches for evaluation of critical processes within the myocardium, such as; angiogenesis, apoptosis, inflammation, and ventricular remodeling.

Approaches to Molecular Imaging

The application of imaging using biologically targeted markers (i.e. molecular imaging) has a number of requirements¹. Selection of a molecular target that adequately represents the process being studied is critical to the specificity of any imaging approach. The next requirement is a readily synthesizable probe that binds to the target molecule with a high degree of specificity.

Correspondence to: Albert J. Sinusas, M.D., FACC, FAHA, Yale University School of Medicine, Nuclear Cardiology, 3FMP, P.O. Box 208017, New Haven CT 06520-8017, Tel: (203) 785-4915 Fax: (203) 737-1026, Email: E-mail: albert.sinusas@yale.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Lastly, an imaging technology that provides the best combination of sensitivity and resolution (both spatial and temporal) to identify and localize the probe within the target organ system must be available. Molecular imaging approaches are currently being developed for most of the imaging modalities; including nuclear, magnetic resonance, X-ray computed tomography (CT), optical fluorescence, bioluminescence, and ultrasound². Though each modality carries strengths and weaknesses, the practical limitations of cost and wide-spread availability will likely be what allow any modality to be adapted for clinical use. The focus of this paper will be in the specific applications of the nuclear imaging approaches.

Nuclear Imaging

Single photon emission computed tomography (SPECT) and positron emission tomography (PET) are imaging techniques that make use of radiolabeled probes and have been used for over three decades. Radiolabeling has the unique advantage of augmenting low signal intensity objects. For example, PET can detect picomolar and nanomolar concentrations of a molecule of interest². Though SPECT offers the advantage of decreased cost and wide spread availability, PET offers the advantages of increased sensitivity with the ability to quantitate as well as repetitively image through tracers with ultra short half-lives. Traditionally, nuclear imaging modalities have been limited by attenuation artifacts from soft tissue and partial volume effects. More recent systems combining CT imaging with either SPECT or PET have allowed for attenuation correction, leading to improved imaging quantification.

The article will review several important areas of active cardiovascular research in which nuclear-based molecular imaging have been used; including the processes of angiogenesis, apoptosis, inflammation, and ventricular remodeling. Key molecular events or signaling proteins involved in each process that were identified through basic research have served as targets for imaging. Some molecular signals overlap between biological processes which emphasizes the importance of understanding the setting in which a molecular event takes place.

Processes within the Myocardium

Angiogenesis

Atherosclerotic disease can lead to chronic ischemia in areas of myocardium, which may stimulate an angiogenic response in order to restore perfusion. Angiogenesis is defined as the process of sprouting new capillaries from preexisting microvessels³. There is a great interest in understanding the processes of angiogenesis in order to design therapeutic treatments that allow revascularization through a stimulated angiogenic response. This angiogenic process often occurs in association with arteriogenesis, which represents the remodeling of larger preexisting vascular channels or collateral vessels feeding the microvascular network. The goal for any revascularization strategy would be to initiate angiogenesis and arteriogenesis in order to create an effective blood delivery systems. There is a large body of literature devoted to understanding these phenomena⁴. Angiogenesis appears to be stimulated by external processes such as ischemia, hypoxia, inflammation, and shear stress. Several cell types are involved in the process, including endothelial cells, smooth muscle cells, blood derived macrophages, circulating stem cells, and the interaction of these cells within the tissue of extracellular matrix proteins. Potential targets for molecular imaging of angiogenesis can be divided into three major categories⁵. Non-endothelial targets like molecules associated with monocytes, macrophages, and stem cells, fall into one category. The second category includes endothelial cell targets like vascular endothelial growth factor (VEGF), integrins, CD13, and syndecan-4. Extracellular matrix proteins compose the final group of molecules that might serve as targets.

Biology of Angiogenesis

Hypoxia, the imbalance between oxygen delivery and demand in a given tissue, can be a potent stimulator of angiogenesis. Hypoxic conditions such as myocardial ischemia or infarction result in upregulation of the transcriptional activator hypoxia-inducible factor 1 (HIF-1)⁶. Upregulation of HIF-1 leads to transcription of vascular endothelial growth factor (VEGF) and the VEGF receptors, Flt-1 (VEGFR-1) and FLK-1 (VEGFR-2)⁷⁻¹⁰. When VEGF binds to these receptors on the surface of endothelial cells, a signal is transduced through their tyrosine kinase activity. This initiates a series of processes that results in endothelial cell proliferation, migration, survival, and angiogenesis¹¹. The process also involves the activation molecules like integrins and matrix metalloproteinases as well as the recruitment of inflammatory cells like macrophages.

Integrins are a family of heterodimeric ($\alpha\beta$) cell-surface receptors that mediate divalent cation-dependent cell-cell and cell-matrix adhesion and signaling through tightly regulated interactions with their respective ligands¹². During angiogenesis, endothelial cells make use of integrins to adhere to one another and the extracellular matrix to construct and extend new vessels. Integrins are capable of mediating an array of cellular processes, including cell adhesion, migration, proliferation, differentiation and survival via a number of signal transduction pathways^{13;14}. Activation of c-Jun NH2-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) may lead to endothelial cell-induced remodeling of the ECM in response to mechanical stimuli. Specifically, the endothelial cell integrin, $\alpha_v\beta_3$, allows cells to interact with the ECM in a way that aids in endothelial cell migration¹⁵. Through outside-in signaling, integrin $\alpha_v\beta_3$ also plays a critical role in the survival of cells undergoing angiogenesis¹⁶.

Other molecules have been identified in this schema of endothelial cell activation. Syndecan-4 is a transmembrane heparin sulfate carrying core protein that promotes VEGF to VEGFR binding and signaling via activation of protein kinase C¹⁷. CD13 is a cell surface antigen that is expressed in endothelial cells is an aminopeptidase that serves as a membrane-bound metalloproteinase which appears to be essential for capillary tube formation¹⁸.

Molecular Targets used for Radiotracer-Based Imaging of Angiogenesis

VEGF Receptors—VEGF receptors have been targeted for imaging in models of ischemia-induced angiogenesis. Radiolabeled-VEGF₁₂₁ has been used to affectively identify angiogenesis in a rabbit model of hindlimb ischemia¹⁹. In this study, KDR and Flt-1 receptor expression was increased in the immunohistochemistry analysis of the skeletal muscle, supporting the theoretical hypoxic-driven angiogenic response. One concern of the study is the biodistribution of the radiotracer which revealed 20 fold higher levels in critical organs (liver, kidneys) compared with ischemic limb, which is presumably related to relative VEGFR density in these organ systems.

A PET tracer, ⁶⁴Cu-6DOTA-VEGF₁₂₁, was recently developed for imaging angiogenesis in a rat model of myocardial infarction (Figure 1)²⁰. Rats underwent ligation of the left coronary artery and subsequent PET imaging at various time points post myocardial infarction. The investigators hypothesized that this tracer would detect early angiogenic signals because ischemia drives VEGFR expression. Co-registration of images was carried out using CT, and the zone of infarct was demonstrated using ¹⁸F-FDG uptake. The study demonstrated that ⁶⁴Cu-6DOTA-VEGF₁₂₁- specific signal was present in the infarct region and peaked on day 3 consistent with the changing levels of VEGFR expression in the tissue as analyzed by immunofluorescence microscopy.

Another type of cardiac-specific reporter has been developed as a gene expression system for use in rats with microPET imaging²¹. Briefly, the system involves adenovirus delivery of mutated thymidine kinase under the control of a cytomegalovirus promoter that drives expression in myocardial cells. The reporter probe is ¹⁸F-FHGB which crosses the myocardial membrane and gets phosphorylated by the thymidine kinase. Phosphorylation essentially traps the ¹⁸F-FHGB in the myocardium for subsequent microPET imaging. Early studies revealed that the localized site of the mutated thymidine kinase, HSV1-sr39tk, corresponded closely with that defined by postmortem autoradiography, histology, and immunohistochemistry²². Other studies have demonstrated the feasibility of utilizing a similar reporter system in pigs using a clinical PET scanner²³. The reporter system was then linked to VEGF to assess feasibility of developing an approach that links therapy and imaging²⁴. Early experiments with rat embryonic cardiomyocytes revealed a strong correlation that both the mutated thymidine kinase and VEGF were expressed in the same cells. Further studies involved injection of the VEGF/thymidine kinase reporter system in models of ischemia. Using microPET, cardiac transgene expression was assessed and the *in vivo* imaging correlated well with *ex vivo* tissue studies for gamma counting, thymidine kinase activity, and VEGF levels. There appeared to be increased capillaries and small blood vessels in the VEGF treated myocardium, however, there was no improvement in perfusion assessed by nitrogen-13 ammonia imaging or metabolism assessed with ¹⁸F-FDG imaging. These studies suggest that a reporter system can be developed to help visualize the effectiveness of delivering VEGF gene therapies for stimulation of angiogenesis.

Integrin $\alpha_v\beta_3$ —Imaging angiogenic vessels through targeting of $\alpha_v\beta_3$ integrin was first proposed through a series of magnetic resonance imaging studies using a monoclonal antibody to $\alpha_v\beta_3$ tagged with a paramagnetic contrast agent²⁵. Such studies were complicated by poor clearance of the tracer from the blood pool. Later studies made use of a number of $\alpha_v\beta_3$ antagonists that were radiolabeled^{26,27}. These studies made use of the arginine-glycine-aspartate (RGD) - binding sequence on integrins by synthesizing RGD analogues.

An ¹¹¹In-labeled quinolone (¹¹¹In-RP748) revealed high affinity and selectivity for $\alpha_v\beta_3$ integrin in adhesion assays²⁸. Subsequent studies using a cy3-labeled homologue of ¹¹¹In-RP748, demonstrated preferential binding to activated $\alpha_v\beta_3$ integrins on endothelial cells in culture, with localization to cell-cell contact points²⁹. Initial studies with this agent focused on imaging tumor angiogenesis, although the first imaging of ischemia-induced myocardial angiogenesis using ¹¹¹In-RP748 was carried out in rat and canine models of infarction³⁰. In these studies, ¹¹¹In-RP748 demonstrated favorable kinetics for *in vivo* SPECT imaging ischemia-induced angiogenesis of the heart. Relative ¹¹¹In-RP748 activity was markedly increased in the infarcted region acutely and persisted for at least 3 weeks post reperfusion^{30,31}. Therefore, targeted imaging with ¹¹¹In-RP748 has demonstrated integrin $\alpha_v\beta_3$ activation early post infarction, suggesting a role for this technique in early detection of angiogenesis as well as for detection of chronic ischemia (Figure 2)³¹.

Other experiments, utilizing a ^{99m}Tc-labeled peptide, NC100692, in the rodent model of hindlimb ischemia for targeting of $\alpha_v\beta_3$ integrin have also been carried out and support the value of integrin imaging in models of peripheral arterial disease³². The recent imaging of $\alpha_v\beta_3$ integrin by the PET imaging tracer, ¹⁸F-Galakto-RGD, in a patient with a transmural myocardial infarction two weeks prior demonstrates the feasibility of detecting angiogenesis in the myocardium in humans³³.

Apoptosis Versus Necrosis

Apoptosis is the physiological process of programmed cell death, whereby organisms selectively target cells to be eliminated when they are no longer needed. The cardiovascular

pathologies of cardiomyopathy, heart failure, myocarditis, and myocardial infarction are associated with increased levels of apoptosis, particularly in the myocyte. On the other hand, a subset of cell death that occurs as an outcome of these pathological processes outside of programmed cellular mechanisms is termed necrosis. A recent study evaluating a role of apoptosis and necrosis in the setting of acute myocardial infarction revealed a potential therapeutic role for cyclosporine³⁴. The intervention is hypothesized to minimize peri-infarct, reperfusion-related cell death that takes place in the setting of revascularization. It is estimated that 30% of cardiomyocytes in the injured myocardium become apoptotic as a result of ischemia reperfusion injury, and animal models of acute infarction demonstrate that up to 50% of the final size of the infarct can be related to lethal reperfusion injury^{35;36}. In other animal studies, inhibition of apoptosis with caspase-inhibitors is cardioprotective³⁷⁻³⁹. There is also some data to suggest early apoptosis may be the pathological substrate that leads from ischemia to necrosis⁴⁰. An ability to assess cell-death anywhere along the spectrum of apoptosis to necrosis would allow investigators to fine tune a therapeutic regimen and optimize the outcome. In targeting these pathologies for new interventions, it has become apparent that better *in vivo* imaging techniques for detection of apoptosis will be required.

Biology of Apoptosis and Necrosis

The earliest studies of apoptosis evolved around histological assessment of the cells undergoing cell death. The earliest descriptions included the microscopic visualization of chromatin condensation, dissolution of nuclear membrane, nuclear shrinkage, and formation of apoptotic bodies that were cleared by phagocytic cells⁴¹⁻⁴³. Over time it became clear that programmed cell death is central to the development and maintenance of homeostasis of a wide array of organisms⁴⁴. In addition, cell death plays a role in the pathology of various disease states⁴⁵. Depending on the initiating signals, there are two major pathways for cell death; intrinsic and extrinsic⁴⁶. The intrinsic pathway is generated from within the cell through DNA damage, mitochondrial signals, and oncogene activation, leading to activation of caspase enzymes, cysteine proteases that cleave after aspartate residues. The extrinsic pathway is initiated through extracellular signals that target cell membrane receptors like Fas, a death receptor. The culmination of this event through either pathway is the activation of a key effector, caspase-3⁴⁷.

Soon after the activation of caspase-3, the energy-dependent asymmetric distribution of phospholipids that enables the definition of various subregions within the lipid bilayer of cell membranes is lost. This leads to increased phosphatidyl serine (PS) on the outer cell membrane from its typical location on the inner cell membrane⁴⁸. In part, this is the result of increased calcium levels and decreased amounts of ATP that block the translocase enzyme responsible for maintenance of PS. The exposure of PS on the surface of the cell makes it a target for binding the protein, Annexin V⁴⁹. Annexin V binds to PS in a calcium dependent manner. This has led to an *in vitro* assay of fas-ligand initiated cell death through binding of annexin V^{48;50}.

The first application of annexin binding to identify phosphatidyl serine on the surface of cells in a cardiovascular model came from a mouse model of acute myocardial infarction⁵¹. In this study, the left anterior descending coronary artery of a series of mice was ligated shortly after the injection of biotinylated annexin V. Immunohistochemical analysis of the tissue distal to the site of ligation revealed annexin A5 binding in an area of cell death. DNA laddering confirmed programmed cell death to be occurring in the same region as the annexin A5 binding.

As myocardial ischemia or infarction persists, cells move from early apoptotic signals to complete necrosis. Breakdown of mitochondrial respiration and loss membrane potential lead to the accumulation of calcium in the mitochondria of infarcted or severely injured myocardium^{52;53}. With loss of membrane potential cellular structures also begin to dissipate.

Positively charged histones and other organelle proteins are exposed from the protection of their membrane barriers. These changes in early necrotic tissue have been utilized for imaging techniques that seek to identify early necrosis in acute myocardial infarctions and are discussed in more detail below.

Molecular Targets used for Radiotracer-Based Imaging in Apoptosis and Necrosis

Annexin V—Initial studies utilizing annexin A5 for imaging purposes involved ^{99m}Tc labeling. The goal was to image the distribution of cells expressing PS noninvasively with a standard gamma camera. Radiolabeling involved derivatization of annexin A5 with hydrazinonicotinamine (HYNIC), which binds to reduced ^{99m}Tc ⁵⁴. The initial studies were carried out in mice with fulminant hepatic apoptosis through the injection of an anti-Fas antibody, which initiates an apoptotic cascade, particularly in hepatocytes⁵⁵. Concomitant TUNEL studies confirmed localization of annexin A5 with apoptotic cells.

^{99m}Tc -labeled annexin V was utilized in humans to detect *in vivo* cell death in patients presenting with myocardial infarction (Figure 3)⁵⁶. Patients presenting with their first myocardial infarction within 6 hours of symptom onset underwent standard revascularization with percutaneous intervention. Within 2 hours of revascularization, SPECT imaging was performed utilizing ^{99m}Tc -labeled annexin V. This was followed by perfusion imaging 6-8 weeks after discharge using ^{99m}Tc -sestamibi. Regional retention of ^{99m}Tc -labeled annexin V correlated with the perfusion defect identified 6-8 weeks after discharge, providing a proof of concept that Annexin-V imaging can be utilized for noninvasive detection of myocardial cell death.

Annexin V imaging has also been used to differentiate between benign and malignant cardiac tumors given the high proliferation and cell death rates associated with malignancy⁵⁷.

Heart transplant rejection is characterized by perivascular and interstitial mononuclear inflammatory infiltrates associated with myocyte apoptosis and necrosis⁵⁸. In a study of 18 patients undergoing apoptotic imaging within 1 year of cardiac transplantation, Annexin-V retention correlated with the severity of rejection⁵⁹. Patients with a negative scan had a concomitant negative biopsy. Of the five patients with a positive scan, three patients demonstrated regional uptake while two patients demonstrated diffuse uptake. The Annexin-V scans correlated to the degree of severity of rejection by biopsy specimens. The authors suggested that serial annexin V imaging for apoptotic cells could be used as a surrogate for detection of allograft rejection in place of serial biopsies in patients following heart transplantation.

Myocarditis is another area of pathological condition where apoptosis is known to occur⁶⁰. In a rat model of autoimmune myocarditis, ^{99m}Tc labeled annexin V retention corresponded to histological TUNEL staining for areas of myocardial apoptosis. Interestingly, these areas could be differentiated from areas of inflammation by ^{14}C -labeled deoxyglucose that corresponded to foci of inflammation. This suggests that one could differentiate between inflammation and active apoptosis with molecular imaging. To date, no studies have attempted to employ this technique in conjunction with FDG-PET in human cases of myocarditis.

Caspase Inhibitors—Because phosphatidyl serine can be exposed on the surface of cells in physiologic conditions other than apoptosis, there is interest in developing more specific apoptosis tracers. Recently, caspase-3 inhibitors have been synthesized and labeled with ^{18}F as potential PET tracers for *in vivo* imaging of apoptosis (Figure 3)^{61;62}. These caspase-3 targeted tracers have shown favorable biodistribution and clearance. MicroPET imaging in a murine model of hepatic apoptosis has shown specificity of the tracer to the liver, however, more studies are needed to assess binding relative to activated caspase density. In addition,

further analysis in cardiovascular models will be necessary to determine feasibility of utilizing this new class of tracers for cardiac applications in human.

Pyrophosphate and Glucarate—The *in vivo* noninvasive detection myocardial infarction will allow for early diagnosis and treatment in patients when electrocardiographic changes may not be evident or when biomarkers may not distinguish between ischemic injury associated with acute plaque rupture that may be treatable with mechanical revascularization versus unstable angina and demand related ischemia. In addition to visualizing apoptosis, several studies have demonstrated that certain agents allow for the visualization of ongoing myocardial necrosis as a mechanism of identifying acute infarction potentially even in the presence of prior myocardial infarction. ^{99m}Tc -labeled pyrophosphate has been shown to bind to areas of necrosis and is thought to bind exposed mitochondrial calcium^{52;53}. ^{99m}Tc -pyrophosphate has a moderate degree of sensitivity for acute infarction depending on the presence of Q wave infarction or a non ST elevation infarction⁶³. The specificity for acute myocardial infarction is considered to be between 60% to 80%. The primary reason why ^{99m}Tc -pyrophosphate has not gained widespread clinical use is the limitation in the detection of early infarction. In fact, depending of the residual degree of perfusion to the infarct zone, the test may not be positive for the first 24 hours.

^{99m}Tc -glucarate imaging provides an alternative to ^{99m}Tc -pyrophosphate imaging for the detection of acute infarction⁵³. ^{99m}Tc -glucarate enters the necrotic cells by passive diffusion following breakdown of the sarcolemma, and binds to exposed histones in the nucleus of the myocytes. Canine models for ischemia and infarction reveal a high affinity of ^{99m}Tc -glucarate for necrotic tissue over ischemic but viable myocardium⁶⁴. In a rabbit model of infarction, ^{99m}Tc -glucarate did not accumulate in areas of ischemia and could be imaged in areas of infarction as early as 10 minutes post reperfusion and within 30-60 minutes in non-reperfused zones⁶⁵. Initial data in patients revealed that ^{99m}Tc -glucarate is able to noninvasively diagnose myocardial infarction in patients presenting with chest pain with a sensitivity that is dependent on the onset of symptoms, specifically within the first 9 hours of symptom onset⁶⁶. ^{99m}Tc -glucarate does have a rapid blood clearance and good target to background signal. ^{99m}Tc -glucarate imaging is currently under investigation as a tool to detect early infarction in a number of clinical trials.

Inflammation

Inflammation plays an important role in many cardiovascular processes including myocardial infarction, reperfusion injury, angiogenesis, apoptosis, cardiac allograft rejection, and myocarditis. Radiolabeling leukocytes with ^{99m}Tc or ^{111}In has been carried out in the past and requires removal of blood and *in vitro* labeling techniques prior to reinjection for imaging^{67;68}. These techniques carry concern for nonspecific activation of the cells that may interfere with the localization *in vivo*. ^{67}Ga -citrate has also been used but has been shown to be relatively nonspecific⁶⁹. ^{18}F -labeled deoxyglucose (FDG) PET imaging takes advantage of increased metabolic activity of inflammatory cells but can also be relatively nonspecific as a change in glucose uptake can be associated with other tissues and disease processes including tumors^{70;71}. There is tremendous interest in developing more specific noninvasive imaging techniques to detect inflammation in myocardium.

Molecular Targets used for Radiotracer-Based Imaging of Inflammatory Mediated Processes

Antimyosin Antibody—Injury to myocytes in the setting of inflammation leads to the disruption of cellular membranes and the release of myosin heavy chain. In order to take advantage of this extracellular exposure of myosin in the setting of inflammation and necrosis, monoclonal antibody to myosin was generated in hopes of applying this for imaging. Early attempts to visualize myosin utilized ^{111}In -labeled antimyosin antibodies to visualize myocyte

damage in myocardial infarction⁷². Other studies utilized ^{99m}Tc-labeled monoclonal antibody fragments to quantitate the degree of myosin exposure in patients in the setting of acute myocardial infarction and correlate it with necrosis⁷³. Inflammation associated with myocarditis, an inflammatory process not associated with ischemia, were also carried out using ¹¹¹In-antimyosin antibodies^{74;75}. Utilizing antimyosin antibody imaging, patients with dilated cardiomyopathy and lower ejection fractions revealed positive studies, suggesting a role for this for stratifying appropriate patients for cardiac transplantation. Though these initial studies showed promise, the background antibody binding to necrotic debris in the cell was high, and therefore the specificity of ¹¹¹In-labeled antimyosin antibody turned out to be very low (25-50%)⁷⁶.

Antitenascin-C antibody—Another monoclonal antibody against an extracellular matrix protein tenascin-C, which appears to be involved in wound healing and inflammation, has been identified as another potential imaging agent. In rodent models of myocarditis, ¹¹¹In-labeled antitenascin-C localizes to the sites of myocardial inflammation⁷⁷. Using a dual isotope SPECT approach with ¹¹¹In-antitenascin-C and ^{99m}Tc-sestamibi, the antibody localized to the injured septal wall by *in vivo* imaging.

LTB₄ Receptor—LTB₄ is a lipid mediator synthesized from arachidonic acid and secreted by neutrophils, macrophages, and endothelial cells as a potent chemotactic agent^{78;79}. The LTB₄ receptor can be found on neutrophils and signaling through this receptor stimulates endothelial adhesion and superoxide production. Recently, a radiolabeled LTB₄ receptor antagonist, ^{99m}Tc-RP517, was developed for *in vivo* imaging of inflammation^{80;81}. ^{99m}Tc-RP517 localized to sites of inflammation induced by *S. aureas* and *E. coli* infection, and chemical (phosphorbol-ester)-induced bowel inflammation.

When prepared with human peripheral whole blood *in vitro*, fluorinated RP517 localized to neutrophils by fluorescence activated cell sorter (FACs) analysis⁸². This confirmed the potential to label human blood neutrophils with ^{99m}Tc-labeled RP517. In an attempt to characterize the *in vivo* imaging ability of ^{99m}Tc-RP517, a canine model of post ischemic myocardial inflammation was utilized. ^{99m}Tc-RP517 was injected into open chest dogs before occlusion and reperfusion. There was an inverse relationship between radiotracer uptake and occlusion flow, suggesting localization of the imaging agent to the site of ischemic inflammation (Figure 4). *Ex vivo* segment analysis revealed that ^{99m}Tc-RP517 correlated with the neutrophil enzyme, myeloperoxidase. Intramyocardial injection of TNF α also correlated with ^{99m}Tc-RP517 uptake and concomitant myeloperoxidase activity, again supporting localization to the site of inflammation. One concern regarding the application of ^{99m}Tc-RP517 is the lipophilic nature of the molecule, resulting in high hepatobiliary clearance and thus large amounts of gastrointestinal uptake. To overcome this, alternative constructs of the LTB₄ antagonist are currently being evaluated⁸³.

Ventricular Remodeling

Ventricular remodeling is a complex biological process that involve inflammation, repair, and healing with specific biochemical and structural alterations in the myocardial infarct and peri-infarct regions as well as remote regions^{84;85}. The process is one of adaptation to form a scar that allows a degree of mechanical stability. The remodeling process involves several key cell types and structural elements, including myocardial cells, endothelial cells, inflammatory cells and the extracellular matrix.

Biology of Myocardial Remodeling

Early in the first weeks after a myocardial infarction, an innate immune response initiates a complex process of wound healing in the necrotic tissue. This process evolves into a more

chronic remodeling process that can involve hypertrophy, chamber dilation and, depending on the success of healing, heart failure⁸⁶.

Matrix metalloproteinases (MMPs) are a family of zinc-containing enzymes that play a key role in ventricular remodeling by degradation of the extracellular matrix^{87;88}. MMPs play an integral role in infarct expansion and left ventricular dilation. Gene deletion of MMPs has been demonstrated to have some cardioprotective effects from ventricular dilation and rupture post-infarct⁸⁹. Pharmacologic inhibition of MMPs has also been shown to decrease left ventricular dilation in infarct models⁹⁰⁻⁹².

Factor XIII has been shown to be crucial in organizing the new matrix of the scar by involvement with extracellular matrix turnover and regulation of inflammatory cascades^{93;94}. Mice with decreased levels of factor XIII demonstrate increased ventricular dilation and post infarct rupture. Patients with infarct rupture were demonstrated to have lower levels of factor XIII in their myocardium. Factor XIII is activated by thrombin and often decreased in the setting of acute myocardial infarction in part because of therapeutic inhibition of thrombin. It is hypothesized that supplementing Factor XIII activity may have a beneficial role in post infarct remodeling.

A critical system that is locally activated during remodeling and contributes to the progression to heart failure is the rennin-angiotensin system⁹⁵. As healing and remodeling are unsuccessful in the failing heart, there is increased expression of prorenin, renin, and angiotensin-converting enzyme (ACE). Activation of this system through signaling pathways mediated by the angiotensin II type I receptor (AT1) leads to myocyte hypertrophy, interstitial and perivascular collagen deposition, and myocyte apoptosis⁹⁶. Inhibition of this pathway has been demonstrated to reverse the functional abnormalities associated with this negative remodeling.

Molecular Targets used for Radiotracer-Based Imaging of Left Ventricular Remodeling

MMPs—By radiolabeling molecules that target MMPs like pharmacological inhibitors that specifically bind to the catalytic domain, MMP activation post infarct can be visualized *in vivo* (Figure 5)⁹⁷. Initial studies involved non imaging techniques with ¹¹¹In-labeled broad-spectrum MMP inhibitor (RP782), a molecule that selectively targets activated MMPs. This MMP targeted agent demonstrated a favorable biodistribution in a murine model of myocardial infarction. One week after myocardial infarction, ¹¹¹In-RP782 localized primarily within the infarct region, although a lesser increase in retention was seen in the remote non-infarcted regions of the heart, consistent with global MMP activation and remodeling.

Further imaging studies were carried out utilizing ^{99m}Tc-labeled analog of RP782 (^{99m}Tc-RP805) and hybrid SPECT/CT imaging with a dual isotope protocol involving ^{99m}Tc-RP805 imaging and adjunctive ²⁰¹Tl- perfusion imaging. The dual isotope imaging studies revealed MMP activation within the perfusion defect region. This suggests that MMP activation is taking place primarily within the sites of injury and is proof of concept that molecules that target MMP activation might be utilized to evaluate ventricular remodeling, and therapeutic interventions directed at inhibition of MMP activation.

Factor XIII—¹¹¹In-DOTA-FXIII is a radiolabeled glutaminase factor XIII substrate analogue that Factor XIII recognizes and cross-links to extracellular matrix proteins (Figure 6)⁹⁴. ¹¹¹In-DOTA-FXIII accumulates in areas of increased factor XIII activity. In a murine model of myocardial infarction, this ¹¹¹In-labeled peptide substrate was demonstrated to be decreased in infarcts of animals treated with the direct thrombin inhibitor, dalteparin. Moreover, dalteparin treatment increased the risk of infarct rupture. Conversely, mice treated with factor XIII intravenously exhibited increased factor XIII activity in the infarct zone and demonstrated more rapid inflammatory turnover of neutrophils and increased recruitment of

macrophages to the site of infarction. There was also increased collagen synthesis and capillary density in the factor XIII-treated animals, suggesting improved healing post infarction.

ACE Inhibitors and AT1 Antagonists—A number of ACE inhibitors and AT1 antagonists have been radiolabeled for molecular imaging techniques⁹⁵. In a study of explanted hearts from patients with ischemic cardiomyopathy, ¹⁸F-fluorobenzoyl-lisinopril was used to assess ACE levels in infarcted myocardium and fibrosed tissue⁹⁸. The study demonstrated that the radiolabeled ACE inhibitor bound with some degree of specificity to areas adjacent to the infarct. Other studies using AT1 antagonists have demonstrated a differential between ACE activity and AT1 levels⁹⁹. In an ovine model of heart failure, ACE activity was primarily in the vascular endothelium while AT1 was upregulated in the myofibroblasts of the infarct region. In a murine model of acute myocardial infarction, a ^{99m}Tc-labeled AT1 receptor peptide analogue was developed and demonstrated specificity to the myofibroblasts that localized to the infarct region in the weeks following the infarction. These early studies suggest that the neurohormonal changes that take place within an infarction may be utilized to identify those at risk for developing significant heart failure after myocardial infarction. Much more work is needed to assess the feasibility of these agents for imaging of post-infarction remodeling in clinical trials.

Conclusions

In summary, molecular imaging represents a targeted approach to non-invasively assess biological processes *in vivo*. The goal of molecular imaging is to develop an approach to studying not only the disease process but more importantly the efficacy of an individually tailored therapeutic regimen. The relatively high sensitivity of radiotracer-based imaging approaches such as SPECT and PET have been of great use in the practical application of molecular imaging techniques. Research has demonstrated the feasibility of specific targeted imaging approaches in the evaluation of angiogenesis, apoptosis, inflammation, and ventricular remodeling. Combining nuclear (SPECT or PET) and CT imaging modalities should help overcome issues of attenuation or partial volume effects and improve quantitative accuracy. The evolution of hybrid imaging systems and imaging protocols that include application of dual isotopes for monitoring physiological parameters (metabolism or perfusion) with targeted molecular probes shows promise in the areas of myocardial infarction and angiogenesis. Tailoring gene therapy with PET reporter constructs should allow for optimization of therapeutic efficacy in the areas of angiogenesis. The role for apoptotic imaging in understand reperfusion injury and the effects of therapeutic interventions, or in identifying the presence and severity of graft rejection following cardiac transplantation without the need for biopsy shows some promise. Imaging the activation of matrix metalloproteinases, active factor XIII or the levels of neurohormonal activation during ventricular remodeling may guide therapeutic regimens that could help positively influence outcomes post infarction. In conclusion, targeted radiotracer-based molecular imaging is clearly feasible and may play an important role in the evaluation and management of cardiovascular disease, including the future investigation of novel genetic or cell based therapeutic interventions.

References

1. Pichler, A.; Piwnica-Worms, D. Overview of Cardiovascular Molecular Imaging. In: Gropler, RJ.; Glover, DK.; Sinusas, AJ., et al., editors. Cardiovascular Molecular Imaging. New York: Informa Healthcare U S A. Inc; 2007. p. 1-8.
2. Sinusas AJ, Bengel F, Nahrendorf M, et al. Multimodality Cardiovascular Molecular Imaging, Part I. Circulation: Cardiovascular Imaging 2008;1:244–256.
3. Fam NP, Verma S, Kutryk M, et al. Clinician guide to angiogenesis. Circulation 2003;108:2613–2618. [PubMed: 14638526]

4. Sasayama S, Fujita M. Recent insights into coronary collateral circulation. *Circulation* 1992;85:1197–1204. [PubMed: 1371432]
5. J Nucl Cardiol; Lake Tahoe invitation meeting 2002; 2003. p. 223–257.
6. Lee SH, Wolf PL, Escudero R, et al. Early expression of angiogenesis factors in acute myocardial ischemia and infarction. *N Engl J Med* 2000;342:626–633. [PubMed: 10699162]
7. Shweiki D, Itin A, Soffer D, et al. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* 1992;359:843–845. [PubMed: 1279431]
8. Brogi E, Schatteman G, Wu T, et al. Hypoxia-induced paracrine regulation of vascular endothelial growth factor receptor expression. *J Clin Invest* 1996;97:469–476. [PubMed: 8567969]
9. Banai S, Jaklitsch MT, Shou M, et al. Angiogenic-induced enhancement of collateral blood flow to ischemic myocardium by vascular endothelial growth factor in dogs. *Circulation* 1994;89:2183–2189. [PubMed: 7514110]
10. Li J, Brown LF, Hibberd MG, et al. VEGF, flk-1, and flt-1 expression in a rat myocardial infarction model of angiogenesis. *Am J Physiol* 1996;270:H1803–H1811. [PubMed: 8928889]
11. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med* 2003;9:669–676. [PubMed: 12778165]
12. Xiong JP, Stehle T, Diefenbach B, et al. Crystal structure of the extracellular segment of integrin alpha Vbeta3. *Science* 2001;294:339–345. [PubMed: 11546839]
13. Schwartz MA, Schaller MD, Ginsberg MH. Integrins: emerging paradigms of signal transduction. *Annu Rev Cell Dev Biol* 1995;11:549–599. [PubMed: 8689569]
14. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell* 2002;110:673–687. [PubMed: 12297042]
15. Clyman RI, Mauray F, Kramer RH. Beta 1 and beta 3 integrins have different roles in the adhesion and migration of vascular smooth muscle cells on extracellular matrix. *Exp Cell Res* 1992;200:272–284. [PubMed: 1374036]
16. Brooks PC, Montgomery AM, Rosenfeld M, et al. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell* 1994;79:1157–1164. [PubMed: 7528107]
17. Li J, Brown LF, Laham RJ, et al. Macrophage-dependent regulation of syndecan gene expression. *Circ Res* 1997;81:785–796. [PubMed: 9351452]
18. Bhagwat SV, Lahdenranta J, Giordano R, et al. CD13/APN is activated by angiogenic signals and is essential for capillary tube formation. *Blood* 2001;97:652–659. [PubMed: 11157481]
19. Lu E, Wagner WR, Schellenberger U, et al. Targeted in vivo labeling of receptors for vascular endothelial growth factor: approach to identification of ischemic tissue. *Circulation* 2003;108:97–103. [PubMed: 12821549]
20. Rodriguez-Porcel M, Cai W, Gheysens O, et al. Imaging of VEGF receptor in a rat myocardial infarction model using PET. *J Nucl Med* 2008;49:667–673. [PubMed: 18375924]
21. Wu JC, Inubushi M, Sundaresan G, et al. Positron emission tomography imaging of cardiac reporter gene expression in living rats. *Circulation* 2002;106:180–183. [PubMed: 12105155]
22. Inubushi M, Wu JC, Gambhir SS, et al. Positron-emission tomography reporter gene expression imaging in rat myocardium. *Circulation* 2003;107:326–332. [PubMed: 12538436]
23. Bengel FM, Anton M, Richter T, et al. Noninvasive imaging of transgene expression by use of positron emission tomography in a pig model of myocardial gene transfer. *Circulation* 2003;108:2127–2133. [PubMed: 14530205]
24. Wu JC, Chen IY, Wang Y, et al. Molecular imaging of the kinetics of vascular endothelial growth factor gene expression in ischemic myocardium. *Circulation* 2004;110:685–691. [PubMed: 15302807]
25. Sipkins DA, Cheresch DA, Kazemi MR, et al. Detection of tumor angiogenesis in vivo by alphaVbeta3-targeted magnetic resonance imaging. *Nat Med* 1998;4:623–626. [PubMed: 9585240]
26. Haubner R, Wester HJ, Weber WA, et al. Noninvasive imaging of alpha(v)beta3 integrin expression using 18F-labeled RGD-containing glycopeptide and positron emission tomography. *Cancer Res* 2001;61:1781–1785. [PubMed: 11280722]

27. Haubner R, Wester HJ, Burkhart F, et al. Glycosylated RGD-containing peptides: tracer for tumor targeting and angiogenesis imaging with improved biokinetics. *J Nucl Med* 2001;42:326–336. [PubMed: 11216533]
28. Harris TD, Kalogeropoulos S, Nguyen T, et al. Design, synthesis, and evaluation of radiolabeled integrin alpha v beta 3 receptor antagonists for tumor imaging and radiotherapy. *Cancer Biother Radiopharm* 2003;18:627–641. [PubMed: 14503959]
29. Sadeghi M, Krassilnikova S, Zhang J, et al. Imaging of avb3 integrin in vascular injury: Does this reflect increased integrin expression or activation? *Circulation* 2008;108:404.
30. Meoli DF, Sadeghi MM, Krassilnikova S, et al. Noninvasive imaging of myocardial angiogenesis following experimental myocardial infarction. *J Clin Invest* 2004;113:1684–1691. [PubMed: 15199403]
31. Kalinowski L, Dobrucki LW, Meoli DF, et al. Targeted imaging of hypoxia-induced integrin activation in myocardium early after infarction. *J Appl Physiol* 2008;104:1504–1512. [PubMed: 18356482]
32. Su H, Hu X, Bourke B, et al. Detection of myocardial angiogenesis in chronic infarction with a novel technetium-99m labeled peptide targeted at avb3 integrin. *Circulation* 2003;108:278–279.
33. Makowski MR, Ebersberger U, Nekolla S, et al. In vivo molecular imaging of angiogenesis, targeting alphavbeta3 integrin expression, in a patient after acute myocardial infarction. *Eur Heart J* 2008;29:2201. [PubMed: 18375397]
34. Piot C, Croisille P, Staat P, et al. Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *N Engl J Med* 2008;359:473–481. [PubMed: 18669426]
35. Fliss H, Gattlinger D. Apoptosis in ischemic and reperfused rat myocardium. *Circ Res* 1996;79:949–956. [PubMed: 8888687]
36. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med* 2007;357:1121–1135. [PubMed: 17855673]
37. Yaotani H, Ogawa K, Maehara K, et al. Attenuation of ischemia/reperfusion injury in rats by a caspase inhibitor. *Circulation* 1998;97:276–281. [PubMed: 9462530]
38. Dumont EA, Reutelingsperger CP, Smits JF, et al. Real-time imaging of apoptotic cell-membrane changes at the single-cell level in the beating murine heart. *Nat Med* 2001;7:1352–1355. [PubMed: 11726977]
39. Hayakawa Y, Chandra M, Miao W, et al. Inhibition of cardiac myocyte apoptosis improves cardiac function and abolishes mortality in the peripartum cardiomyopathy of Galpha(q) transgenic mice. *Circulation* 2003;108:3036–3041. [PubMed: 14638549]
40. Thimister PW, Hofstra L, Liem IH, et al. In vivo detection of cell death in the area at risk in acute myocardial infarction. *J Nucl Med* 2003;44:391–396. [PubMed: 12621005]
41. Wyllie AH. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 1980;284:555–556. [PubMed: 6245367]
42. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 1972;26:239–257. [PubMed: 4561027]
43. Wyllie AH, Kerr JF, Currie AR. Cell death: the significance of apoptosis. *Int Rev Cytol* 1980;68:251–306. [PubMed: 7014501]
44. Danial NN, Korsmeyer SJ. Cell death: critical control points. *Cell* 2004;116:205–219. [PubMed: 14744432]
45. Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. *Science* 2004;305:626–629. [PubMed: 15286356]
46. Riedl SJ, Shi Y. Molecular mechanisms of caspase regulation during apoptosis. *Nat Rev Mol Cell Biol* 2004;5:897–907. [PubMed: 15520809]
47. Tait JF. Imaging of apoptosis. *J Nucl Med* 2008;49:1573–1576. [PubMed: 18794267]
48. Martin SJ, Reutelingsperger CP, McGahon AJ, et al. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. *J Exp Med* 1995;182:1545–1556. [PubMed: 7595224]

49. Koopman G, Reutelingsperger CP, Kuijten GA, et al. Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. *Blood* 1994;84:1415–1420. [PubMed: 8068938]
50. van Engeland M, Ramaekers FC, Schutte B, et al. A novel assay to measure loss of plasma membrane asymmetry during apoptosis of adherent cells in culture. *Cytometry* 1996;24:131–139. [PubMed: 8725662]
51. Dumont EA, Hofstra L, van Heerde WL, et al. Cardiomyocyte death induced by myocardial ischemia and reperfusion: measurement with recombinant human annexin-V in a mouse model. *Circulation* 2000;102:1564–1568. [PubMed: 11004148]
52. Khaw BA. The current role of infarct avid imaging. *Semin Nucl Med* 1999;29:259–270. [PubMed: 10433340]
53. Flotats A, Carrio I. Non-invasive in vivo imaging of myocardial apoptosis and necrosis. *Eur J Nucl Med Mol Imaging* 2003;30:615–630. [PubMed: 12638039]
54. Blankenberg FG, Katsikis PD, Tait JF, et al. In vivo detection and imaging of phosphatidylserine expression during programmed cell death. *Proc Natl Acad Sci U S A* 1998;95:6349–6354. [PubMed: 9600968]
55. Ogasawara J, Watanabe-Fukunaga R, Adachi M, et al. Lethal effect of the anti-Fas antibody in mice. *Nature* 1993;364:806–809. [PubMed: 7689176]
56. Hofstra L, Liem IH, Dumont EA, et al. Visualisation of cell death in vivo in patients with acute myocardial infarction. *Lancet* 2000;356:209–212. [PubMed: 10963199]
57. Hofstra L, Dumont EA, Thimister PW, et al. In vivo detection of apoptosis in an intracardiac tumor. *JAMA* 2001;285:1841–1842. [PubMed: 11308396]
58. Laguens RP, Meckert PM, Martino JS, et al. Identification of programmed cell death (apoptosis) in situ by means of specific labeling of nuclear DNA fragments in heart biopsy samples during acute rejection episodes. *J Heart Lung Transplant* 1996;15:911–918. [PubMed: 8889987]
59. Narula J, Acio ER, Narula N, et al. Annexin-V imaging for noninvasive detection of cardiac allograft rejection. *Nat Med* 2001;7:1347–1352. [PubMed: 11726976]
60. Tokita N, Hasegawa S, Tsujimura E, et al. Serial changes in 14C-deoxyglucose and 201Tl uptake in autoimmune myocarditis in rats. *J Nucl Med* 2001;42:285–291. [PubMed: 11216528]
61. Faust A, Wagner S, Law MP, et al. The nonpeptidyl caspase binding radioligand (S)-1-(4-(2-[18F] Fluoroethoxy)-benzyl)-5-[1-(2-methoxymethylpyrrolidinyl)s ulfonyl]isatin ([18F]CbR) as potential positron emission tomography-compatible apoptosis imaging agent. *Q J Nucl Med Mol Imaging* 2007;51:67–73. [PubMed: 17372575]
62. Zhou D, Chu W, Rothfuss J, et al. Synthesis, radiolabeling, and in vivo evaluation of an 18F-labeled isatin analog for imaging caspase-3 activation in apoptosis. *Bioorg Med Chem Lett* 2006;16:5041–5046. [PubMed: 16891117]
63. Corbett JR, Lewis M, Willerson JT, et al. 99mTc-pyrophosphate imaging in patients with acute myocardial infarction: comparison of planar imaging with single-photon tomography with and without blood pool overlay. *Circulation* 1984;69:1120–1128. [PubMed: 6325039]
64. Orlandi C, Crane PD, Edwards DS, et al. Early scintigraphic detection of experimental myocardial infarction in dogs with technetium-99m-glucaric acid. *J Nucl Med* 1991;32:263–268. [PubMed: 1992031]
65. Narula J, Petrov A, Pak KY, et al. Very early noninvasive detection of acute experimental nonreperfused myocardial infarction with 99mTc-labeled glucarate. *Circulation* 1997;95:1577–1584. [PubMed: 9118528]
66. Mariani G, Villa G, Rossettin PF, et al. Detection of acute myocardial infarction by 99mTc-labeled D-glucaric acid imaging in patients with acute chest pain. *J Nucl Med* 1999;40:1832–1839. [PubMed: 10565778]
67. Peters AM, Danpure HJ, Osman S, et al. Clinical experience with 99mTc-hexamethylpropylene-amineoxime for labelling leucocytes and imaging inflammation. *Lancet* 1986;2:946–949. [PubMed: 2877132]
68. Peters AM, Saverymuttu SH. The value of indium-labelled leucocytes in clinical practice. *Blood Rev* 1987;1:65–76. [PubMed: 3332088]

69. Lavender JP, Lowe J, Barker JR, et al. Gallium 67 citrate scanning in neoplastic and inflammatory lesions. *Br J Radiol* 1971;44:361–366. [PubMed: 5108360]
70. Mochizuki T, Tsukamoto E, Kuge Y, et al. FDG uptake and glucose transporter subtype expressions in experimental tumor and inflammation models. *J Nucl Med* 2001;42:1551–1555. [PubMed: 11585872]
71. Kubota R, Yamada S, Kubota K, et al. Intratumoral distribution of fluorine-18-fluorodeoxyglucose in vivo: high accumulation in macrophages and granulation tissues studied by microautoradiography. *J Nucl Med* 1992;33:1972–1980. [PubMed: 1432158]
72. Johnson LL, Seldin DW, Becker LC, et al. Antimyosin imaging in acute transmural myocardial infarctions: results of a multicenter clinical trial. *J Am Coll Cardiol* 1989;13:27–35. [PubMed: 2642491]
73. Khaw BA, Gold HK, Yasuda T, et al. Scintigraphic quantification of myocardial necrosis in patients after intravenous injection of myosin-specific antibody. *Circulation* 1986;74:501–508. [PubMed: 3017604]
74. Yasuda T, Palacios IF, Dec GW, et al. Indium 111-monoclonal antimyosin antibody imaging in the diagnosis of acute myocarditis. *Circulation* 1987;76:306–311. [PubMed: 3608120]
75. Dec GW, Palacios I, Yasuda T, et al. Antimyosin antibody cardiac imaging: its role in the diagnosis of myocarditis. *J Am Coll Cardiol* 1990;16:97–104. [PubMed: 2358612]
76. Narula J, Khaw BA, Dec GW, et al. Diagnostic accuracy of antimyosin scintigraphy in suspected myocarditis. *J Nucl Cardiol* 1996;3:371–381. [PubMed: 8902668]
77. Sato M, Toyozaki T, Odaka K, et al. Detection of experimental autoimmune myocarditis in rats by 111In monoclonal antibody specific for tenascin-C. *Circulation* 2002;106:1397–1402. [PubMed: 12221059]
78. Ford-Hutchinson AW. Regulation of leukotriene biosynthesis. *Cancer Metastasis Rev* 1994;13:257–267. [PubMed: 7712588]
79. Yokomizo T, Izumi T, Shimizu T. Leukotriene B4: metabolism and signal transduction. *Arch Biochem Biophys* 2001;385:231–241. [PubMed: 11368003]
80. Serhan CN, Prescott SM. The scent of a phagocyte: Advances on leukotriene b(4) receptors. *J Exp Med* 2000;192:F5–F8. [PubMed: 10934235]
81. Brouwers AH, Laverman P, Boerman OC, et al. A 99Tcm-labelled leukotriene B4 receptor antagonist for scintigraphic detection of infection in rabbits. *Nucl Med Commun* 2000;21:1043–1050. [PubMed: 11192710]
82. Riou LM, Ruiz M, Sullivan GW, et al. Assessment of myocardial inflammation produced by experimental coronary occlusion and reperfusion with 99mTc-RP517, a new leukotriene B4 receptor antagonist that preferentially labels neutrophils in vivo. *Circulation* 2002;106:592–598. [PubMed: 12147542]
83. van Eerd JE, Oyen WJ, Harris TD, et al. A bivalent leukotriene B(4) antagonist for scintigraphic imaging of infectious foci. *J Nucl Med* 2003;44:1087–1091. [PubMed: 12843226]
84. Weber KT. Extracellular matrix remodeling in heart failure: a role for de novo angiotensin II generation. *Circulation* 1997;96:4065–4082. [PubMed: 9403633]
85. Sutton MG, Sharpe N. Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. *Circulation* 2000;101:2981–2988. [PubMed: 10869273]
86. Sutton MG, Sharpe N. Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. *Circulation* 2000;101:2981–2988. [PubMed: 10869273]
87. Creemers EE, Cleutjens JP, Smits JF, et al. Matrix metalloproteinase inhibition after myocardial infarction: a new approach to prevent heart failure? *Circ Res* 2001;89:201–210. [PubMed: 11485970]
88. Spinale FG. Matrix metalloproteinases: regulation and dysregulation in the failing heart. *Circ Res* 2002;90:520–530. [PubMed: 11909815]
89. Ducharme A, Frantz S, Aikawa M, et al. Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction. *J Clin Invest* 2000;106:55–62. [PubMed: 10880048]
90. Rohde LE, Ducharme A, Arroyo LH, et al. Matrix metalloproteinase inhibition attenuates early left ventricular enlargement after experimental myocardial infarction in mice. *Circulation* 1999;99:3063–3070. [PubMed: 10368126]

91. Lindsey ML, Gannon J, Aikawa M, et al. Selective matrix metalloproteinase inhibition reduces left ventricular remodeling but does not inhibit angiogenesis after myocardial infarction. *Circulation* 2002;105:753–758. [PubMed: 11839633]
92. Yarbrough WM, Mukherjee R, Escobar GP, et al. Selective targeting and timing of matrix metalloproteinase inhibition in post-myocardial infarction remodeling. *Circulation* 2003;108:1753–1759. [PubMed: 12975256]
93. Nahrendorf M, Hu K, Frantz S, et al. Factor XIII deficiency causes cardiac rupture, impairs wound healing, and aggravates cardiac remodeling in mice with myocardial infarction. *Circulation* 2006;113:1196–1202. [PubMed: 16505171]
94. Nahrendorf M, Aikawa E, Figueiredo JL, et al. Transglutaminase activity in acute infarcts predicts healing outcome and left ventricular remodeling: implications for FXIII therapy and antithrombin use in myocardial infarction. *Eur Heart J* 2008;29:445–454. [PubMed: 18276618]
95. Shirani J, Dilsizian V. Imaging left ventricular remodeling: targeting the neurohumoral axis. *Nat Clin Pract Cardiovasc Med* 2008;5:S57–S62. [PubMed: 18641608]
96. Aras O, Messina SA, Shirani J, et al. The role and regulation of cardiac angiotensin-converting enzyme for noninvasive molecular imaging in heart failure. *Curr Cardiol Rep* 2007;9:150–158. [PubMed: 17430683]
97. Su H, Spinale FG, Dobrucki LW, et al. Noninvasive targeted imaging of matrix metalloproteinase activation in a murine model of postinfarction remodeling. *Circulation* 2005;112:3157–3167. [PubMed: 16275862]
98. Dilsizian V, Eckelman WC, Loreda ML, et al. Evidence for tissue angiotensin-converting enzyme in explanted hearts of ischemic cardiomyopathy using targeted radiotracer technique. *J Nucl Med* 2007;48:182–187. [PubMed: 17268012]
99. Shirani J, Narula J, Eckelman WC, et al. Early imaging in heart failure: exploring novel molecular targets. *J Nucl Cardiol* 2007;14:100–110. [PubMed: 17276312]

Figure 1.

Myocardial ^{64}Cu -DOTA-VEGF₁₂₁ and ^{18}F -FDG PET-CT imaging after MI. Upper images demonstrate co-registered images of microCT (left), PET (right), and fused PET/CT image (center) in a representative animal after myocardial infarction. The ^{64}Cu -DOTA-VEGF₁₂₁ signal is detected by PET in the anterolateral myocardium (PET and fused images, red arrow). Intercostal muscle layer is designated on microCT image with a white arrow. There is some increased uptake in area of surgical wound (PET image, arrowhead). Lower images demonstrate ^{64}Cu -DOTA-VEGF₁₂₁ (left), ^{18}F -FDG (right), and ^{64}Cu -DOTA-VEGF₁₂₁/ ^{18}F -FDG fused image (middle). ^{18}F -FDG scan shows that coronary artery ligation resulted in development of a scar by lack of ^{18}F -FDG uptake (yellow arrow) and that uptake of ^{64}Cu -DOTA-VEGF₁₂₁ occurs in the region of that scar (turquoise arrow). Fusion of both scans results in complementation of ^{18}F -FDG and ^{64}Cu -DOTA-VEGF₁₂₁ signals. Again, increased uptake in area of surgical wound is designated with an arrowhead. (Reprinted with permission from Rodriguez-Porcel M, Cai W, Gheysens O, et al: Imaging of VEGF receptor in a rat myocardial infarction model using PET. *J Nucl Med.* 49:667-673, 2008.)²⁰

Figure 2.

In vivo and ex vivo $^{111}\text{In-RP748}$ and $^{99\text{m}}\text{Tc-sestamibi}$ ($^{99\text{m}}\text{Tc-MIBI}$) images from dogs with chronic infarction. Serial in vivo $^{111}\text{In-RP748}$ SPECT short axis, vertical long axis (VLA), and horizontal long axis (HLA) images in a dog 3 wks post LAD infarction at 20 min, and 75 min post injection in standard format (figure 2a). $^{111}\text{In-RP748}$ SPECT images were registered with $^{99\text{m}}\text{Tc-MIBI}$ perfusion images (third row). The 75 min $^{111}\text{In-RP748}$ SPECT images were colored red and fused with MIBI images (green) to better demonstrate localization of $^{111}\text{In-RP748}$ activity within the heart (color fusion, bottom row). Right ventricular (RV) and left ventricular (LV) blood pool activity are seen at 20 min. Filled arrows indicate region of increased $^{111}\text{In-RP748}$ uptake in anterior wall. This corresponds to the anteroapical $^{99\text{m}}\text{Tc-sestamibi}$ perfusion defect (open arrow). Sequential $^{99\text{m}}\text{Tc-sestamibi}$ (top row) and $^{111}\text{In-RP748}$ in vivo SPECT HLA images at 90 min post injection (middle row) from a dog at 8 hrs (Acute), and 1 and 3 wks post LAD infarction (figure 2b). Increased myocardial $^{111}\text{In-RP748}$ uptake is seen in anteroapical wall at all three time points, although appears to be maximal at 1 wk post infarction. Color fusion $^{99\text{m}}\text{Tc-MIBI}$ (green) and $^{111}\text{In-RP748}$ (red) images (bottom row) demonstrate $^{111}\text{In-RP748}$ uptake within $^{99\text{m}}\text{Tc-MIBI}$ perfusion defect. Ex vivo $^{99\text{m}}\text{Tc-}$

sestamibi (left) and ^{111}In -RP748 (center) images of myocardial slices from a dog 3 wks post LAD occlusion, with color fusion image on right (figure 2c). Short axis slices are oriented with anterior wall on top, RV on left. Open arrows indicate anterior location of non-transmural perfusion defect region, and filled arrows indicate corresponds area of increased ^{111}In -RP748 uptake. (Reprinted with permission from Meoli DF, Sadeghi MM, Krassilnikova S, et al: Noninvasive imaging of myocardial angiogenesis following experimental myocardial infarction. *J. Clin Invest* 113: 1684-1691, 2004.)³⁰

Figure 3.

In vivo imaging of apoptosis. A, B (Upper panels): Transverse tomographic images of acute anteroseptal infarction in a patient. A: Arrow shows increased ^{99m}Tc -labelled annexin-V uptake in the anteroseptal region 22 h after reperfusion. B: Perfusion scintigraphy with sestamibi 6–8 weeks after discharge shows an irreversible perfusion defect which coincides with the area of increased ^{99m}Tc -labelled annexin-V uptake (arrow). (Reprinted with permission from *Lancet* 359: 209-212, 2000.)⁵⁶ C (Lower panel): Whole-body MicroPET images of caspase-3 specific inhibitor, ^{18}F -WC-II-89, distribution in a control rat (left) and cycloheximide-treated rat (right). Images were summed from 10 to 60 min after intravenous injection of approximately 150 μCi of ^{18}F -WC-II-89. (Reprinted with permission from Zhou D, Chu W, Rothfuss J et al: Synthesis, radiolabeling, and in vivo evaluation of an ^{18}F -labeled isatin analog for imaging caspase-3 activation in apoptosis. *Bioorg. Med. Chem. Lett* 16: 5041-5046, 2006.)⁶²

Figure 4.

Imaging ischemic inflammation with the LTB_4 receptor antagonist, RP517. TTC-stained heart slice (A) and *ex vivo* ^{99m}Tc -RP517 image (B) of the same heart slice. C, Raw (left) and background subtracted (right) *in vivo* ^{99m}Tc -RP517 images acquired from a dog 60 minutes after reperfusion. Background subtraction was performed to eliminate the surgically related tracer uptake in the field of view. The shadow on the raw image denoted by an asterisk is the metal rib spreader. Note that focal ^{99m}Tc -RP517 uptake was readily observed in the inflamed antero-septal region of the heart on both *ex vivo* and *in vivo* images. Tracer uptake was negligible in the normal, posterior wall. (Reprinted with permission from Riou LM, Ruiz M, Sullivan GW, et al: Assessment of myocardial inflammation produced by experimental coronary occlusion and reperfusion with ^{99m}Tc -RP517, a new leukotriene B₄ receptor antagonist that preferentially labels neutrophils in vivo. *Circulation* 106: 592-598, 2002)⁸²

Figure 5.

Imaging of matrix metalloproteinase (MMP) activity postinfarction. Hybrid Micro-SPECT/CT reconstructed short-axis images were acquired without x-ray contrast (A) in control sham-operated mouse (left) and selected mice at 1 week (middle) and 3 weeks (right) after MI, after injection of ^{201}Tl (top row, green) and $^{99\text{m}}\text{Tc}$ -RP805 (middle row, red). A black-and-white (B&W) and multicolor fusion image is shown on bottom. Control heart demonstrates normal myocardial perfusion and no focal $^{99\text{m}}\text{Tc}$ -RP805 uptake within the heart, although some uptake is seen in chest wall at the thoracotomy site (dashed arrows). All post-MI mice have a large anterolateral ^{201}Tl perfusion defect (yellow arrows) and focal uptake of $^{99\text{m}}\text{Tc}$ -RP805 in defect area. A dashed circle is drawn around the heart to demonstrate localization of $^{99\text{m}}\text{Tc}$ -RP805,

the MMP radiotracer, within the infarcted area of the heart. Some activity is also seen in the peri-infarct border zone. Additional micro-SPECT/CT images were acquired by use of a higher-resolution SPECT detector after the administration of x-ray contrast, at 1 week (B) and 3 weeks (C) after MI. The contrast agent permitted better definition of the LV myocardium, which is highlighted by white dotted line. Representative short-axis (SA), horizontal long-axis (HLA), and vertical long-axis (VLA) images are shown. Focal uptake of ^{99m}Tc -RP805 is seen within the central infarct and peri-infarct regions, which again corresponds to ^{201}Tl perfusion defect. (Reprinted with permission from Su H, Spinale FG, Dobrucki LW et al: Noninvasive targeted imaging of matrix metalloproteinase activation in a murine model of postinfarction remodeling. *Circulation* 112: 3157-3167, 2005.)⁹⁷

Figure 6.

In vivo molecular imaging of transglutaminase factor XIII (FXIII) activity predicts survival and evolution of heart failure. (A–I) Longitudinal imaging study (MRI day 2, SPECT-CT day 3, second MRI day 21); on day 2 (A, D, G), late enhancement MRI showed similar infarct size in all groups. FXIII-treatment led to higher SPECT signal (H, K). In dalteparin-treated mice, the SPECT signal was lower (E, K). Serial MRI showed attenuated left ventricular (LV) dilation in FXIII-treated mice (L). Due to reduced survival in dalteparin (DP)-treated mice, the second MRI on day 21 was not acquired (F). * $P < 0.05$, ** $P < 0.001$. (Reprinted with permission from Nahrendorf M, Aikawa E, Figueiredo JL, et al: Transglutaminase activity in acute infarcts predicts healing outcome and left ventricular remodelling: implications for FXIII therapy and antithrombin use in myocardial infarction. *Eur Heart J* 29: 445-454, 2008)⁹⁴