Evaluation of LMI1195, a Novel $^{18}$F Labeled Cardiac Neuronal PET Imaging Agent, in Cells and Animal Models

Yu et al: LMI1195 for Cardiac Neuronal PET Imaging

Ming Yu, MD, PhD; Jody Bozek, BS; Melanie Lamoy, BS; Mary Guaraldi, MS; Paula Silva, MS; Mikhail Kagan, MS; Padmaja Yalamanchili, PhD; David Onthank, PhD; Mahesh Mistry, PhD; Joel Lazewatsky, PhD; Matthias Broekema, MS; Heike Radeke, PhD; Ajay Purohit, PhD; Michael Azure, PhD; Richard Cesati, PhD; David Casebier, PhD; Simon P. Robinson, PhD

Correspondence to:
Ming Yu, MD, PhD
Discovery Research
Lantheus Medical Imaging
331 Treble Cove Rd
N. Billerica, MA 01862
Telephone: 978-671-8142
Fax: 978-667-3926
Email: ming.yu@lantheus.com

Journal Subject Codes: 32, 124, 110
Abstract

**Background**—Heart failure (HF) has been associated with impaired cardiac sympathetic neuronal function. Cardiac imaging with radiolabeled agents that are substrates for the neuronal norepinephrine transporter (NET) has demonstrated the potential to identify individuals at risk of cardiac events in HF patients. LMI1195 is a newly developed $^{18}$F labeled NET substrate designed to allow cardiac neuronal imaging with the high sensitivity, resolution and quantification afforded by positron emission tomography (PET).

**Methods and Results**—LMI1195 was evaluated in comparison with norepinephrine (NE) *in-vitro* and $^{123}$I-meta-iodobenzylguanidine (MIBG) *in-vivo*. The affinity (K$i$) of LMI1195 for NET was 5.16±2.83 μM, similar to that of NE (3.36±2.77 μM) in a cell membrane binding assay. Similarly, LMI1195 uptake kinetics examined in a human neuroblastoma cell line had $K_m$ and $V_{max}$ values of 1.44±0.76 μM and 6.05±3.09 pmol/million cells/min, comparable to NE (2.01±0.85 μM and 6.23±1.52 pmol/million cells/min). In rats, LMI1195 heart uptake at 15 and 60 minutes after iv administration was 2.36±0.38 and 2.16±0.38 % injected dose per g tissue (%ID/g), similar to $^{123}$I-MIBG (2.14±0.30 and 2.19±0.27 %ID/g). However, the heart to liver and lung uptake ratios were significantly higher for LMI1195 than $^{123}$I-MIBG. In rabbits, desipramine (1 mg/kg), a selective NET inhibitor, blocked LMI1195 heart uptake by 82%, more effectively than $^{123}$I-MIBG (53%), at 1-hour post dosing. Sympathetic denervation with 6-hydroxydopamine, a neurotoxin, resulted in a marked (79%) decrease in LMI1195 heart uptake. Cardiac PET imaging with LMI1195 in rats, rabbits and nonhuman primates (NHP) revealed clear myocardium with low radioactivity levels in the blood, lung and liver. Imaging in rabbits pretreated with desipramine showed reduced heart radioactivity levels in a dose dependent manner. Additionally, imaging in sympathetically denervated rabbits resulted in low cardiac image intensity with LMI1195 but normal perfusion images with flurpiridaz F 18, a PET myocardial perfusion imaging agent. In NHPs pretreated with desipramine (0.5 mg/kg), imaging with LMI1195 showed a 66% decrease in myocardial uptake. In a rat model of HF, the LMI1195 cardiac uptake decreased as HF progressed.

**Conclusions**—LMI1195 is a novel $^{18}$F imaging agent retained in the heart via the NET and allowing evaluation of the cardiac sympathetic neuronal function by PET imaging.

**Key Words:** positron emission tomography, cardiac neuronal imaging, norepinephrine transporter, heart failure, 18F
Heart failure (HF) is associated with abnormalities of the cardiac sympathetic system including increased norepinephrine (NE) release and impaired cardiac neuronal norepinephrine transporter (NET) function.\textsuperscript{1,2} Nuclear cardiac imaging has been widely used as a unique tool for evaluation of molecular changes in the heart, including NET, in a non-invasive and repeatable manner. False neurotransmitters labeled with radioisotopes like \textsuperscript{11}C-meta-hydroxyephedrine (HED) and \textsuperscript{123}I-meta-iodobenzylguanidine (MIBG) are substrates for the NET and allow cardiac imaging of the sympathetic neuronal function.\textsuperscript{3-5} Imaging with \textsuperscript{123}I-MIBG or \textsuperscript{11}C-HED has been shown for prediction of HF progression,\textsuperscript{6-9} assessment of the effectiveness of treatment and providing information for patient management.\textsuperscript{10-12} Patients with the lowest heart uptake tend to have the poorest prognosis. In the recent ADMIRE-HF trial, the heart to mediastinum uptake ratio derived from \textsuperscript{123}I-MIBG cardiac images independently predicted cardiac events including HF progression, arrhythmia and cardiac death in HF patients with a low ejection fraction.\textsuperscript{13}

However, the quality of \textsuperscript{123}I-MIBG cardiac image is generally poor due to low resolution and inaccurate attenuation correction associated with gamma camera imaging and a low level of high energy emissions of \textsuperscript{123}I.\textsuperscript{4,14} In addition, MIBG liver accumulation is high and can interfere with cardiac visibility, particularly in planar imaging which is frequently the procedure of choice. In contrast, imaging with agents labeled with positron-emitting isotopes provides high spatial and temporal resolution along with better attenuation correction and allows regional definition of tracer kinetics.\textsuperscript{3,15} While these advantages are seen with the NET substrates labeled with \textsuperscript{11}C, the short half life limits their widespread clinical application. LMI1195 was designed like \textsuperscript{123}I-MIBG as a
benzylguanidine based substrate for the NET but to incorporate $^{18}$F with a longer half life for PET imaging. The imaging profile of this agent was evaluated in the present study both in-vitro and in-vivo.

**Methods**

**Binding Assay in Cell Membrane**

NET binding affinity of LMI1195 and NE was determined in competition experiments using cell membranes overexpressing the human NET (hNET membrane, PerkinElmer, Boston, MA) and $^3$H-desmethylimipramine hydrochloride ($^3$H-desipramine, PerkinElmer). Aliquots of membrane suspensions (~6 μg protein) were incubated for 2 hours at 4°C with 2 nM $^3$H-desipramine and various concentrations (0.005 to 400 μM) of $^{19}$F-LMI1195 (non-radioactive form of LMI1195) or NE in a final volume of 200 μl incubation buffer (50 mM Tris-HCl, 120 mM NaCl and 5 mM KCl). After incubation, the solution was passed through filters. The filters were washed and counted for radioactivity (Microbeta TriLux 1450 LSC and Luminescence counter, PerkinElmer, Shelton, CT). Each assay was performed in triplicate and the $K_i$ value of each agent was calculated using GraphPad Prism (v4, GraphPad Software, CA).

**Uptake Kinetics in Cells**

Human neuroblastoma cells (SK-N-SH, 1x10$^6$ cells/well in 2 mL of media) were incubated with LMI1195 or $^3$H-NE at ~0.1 μCi per well and increasing concentrations (0.1, 0.4, 1.6, 6.4, 12.8, 25.6 and 51.2 μM) of $^{19}$F-LMI1195 or NE in duplicate at 37°C.
for 30 minutes. Non-specific uptake was determined by including desipramine, a selective NET inhibitor, at 10 μM. Following incubation, cells were washed twice with PBS buffer, trypsinized and centrifuged at 1000g for 3 min to collect cell pellet. Radioactivity was measured with a γ-counter (Wallac Wizard 1480, PerkinElmer, Shelton, CT) or β-counter. The cell uptake kinetics (Vmax and Km) were determined using GraphPad Prism software.

Animal Preparation

Study protocols were approved by the Institutional Animal Care and Use Committee. Male Sprague Dawley rats (300-450 g, Harlan, Dublin, VA), male New Zealand rabbits (2.5-3.5 kg, Harlan, Oxford, MI) and both male and female NHPs (cynomolgus monkey, 4-6 kg, Charles River, TX) were used. Rats were anesthetized with sodium pentobarbital (40-60 mg/kg, i.p.) and the tail vein was cannulated for drug administration. Rabbits were anesthetized with ketamine (20 mg/kg, i.v.) and diazepam (2 mg/kg, i.v), and the marginal ear vein was canulated for drug injection. NHPs were initially anesthetized with ketamine (10 mg/kg, im.), acepromazine (0.3 mg/kg, im) and atropine (0.05 mg/kg im). The saphenous vein was catheterized for drug injection. For cardiac imaging, both the rabbit and NHP were further orally intubated and mechanically ventilated with isoflurane (0.4-1.5%).

Rabbit Model of Sympathetic Denervation

To determine the role of the sympathetic neuron in LMI1195 heart uptake, a rabbit model of chemical denervation with 6-hydroxydopamine, a neurotoxin, (6-OHDA, Sigma Aldrich, St. Louis, MO) was developed. Rabbits were administered either vehicle
(1mg/kg ascorbic acid in saline) or 25 mg/kg 6-OHDA i.v. on days 1, 2, 7 and 8. On day 15, these rabbits were used for tissue biodistribution and cardiac imaging studies.

**Rat Model of Heart Failure**

Inbred Dahl salt-sensitive (DSS/JrHsd, Harlan) rats fed either a low salt (0.1% salt) or high salt (8% salt) diet were used as control and HF groups. In a preliminary study, it was found that DSS rats fed a high salt diet developed early stage HF with myocardial hypertrophy in 4-5 weeks and late stage HF with myocardial hypertrophy and lung congestion in 9-10 weeks. Rats were anesthetized with ketamine (25-75 mg/kg, i.p.) and diazepam (2.2-10 mg/kg, i.p.) for imaging.

**Tissue Biodistribution**

Approximately 15 μCi LMI1195 or 25 μCi 123I-MIBG (for comparison) was injected intravenously in each rat. At 15, 60 or 120 minutes after the injection, rats were euthanized by barbiturate overdose and tissue samples of the blood, heart, lung, liver, spleen, kidney, muscle, femur and adrenal glands were collected. All samples were weighed and counted for radioactivity. The tissue biodistribution of the radiolabeled agent was determined as % injected dose per gram of tissue (%ID/g). A similar study was also performed in rabbits receiving about 50 μCi LMI1195 intravenously. In addition, to determine selectivity of LMI1195 for NET in comparison with 123I-MIBG (100 μCi), some rabbits were pretreated iv with vehicle (10% ethanol in saline) or desipramine (1 mg/kg) 10 minutes prior to administration of the radiotracer.
Cardiac Imaging

After anesthesia, the animal was placed in a supine position on the bed of a microPET camera (Focus220, CTI Molecular Imaging, Knoxville, TN). About 1, 1.5 and 1.5 mCi LMI1195 was injected i.v. in rats, rabbits and NHPs respectively and the heart was imaged for 60 minutes. To determine NET selectivity, desipramine was administered at 0.1 or 1 mg/kg i.v. in rabbits and 0.5 mg/kg i.v. in NHPs 10 and 40 minutes respectively before LMI1195 injection. For comparison in NHPs, static planar cardiac imaging with $^{123}$I-MIBG (about 3.5 mCi) was also performed using a dual head Isocam II SPECT camera (Parkmed Medical Systems, Quebec, Canada). To ensure the myocardial perfusion was not altered in sympathetically denervated rabbits, the 6-OHDA pretreated animals were also imaged with flurpiridaz F 18 (formerly known as BMS747158, 1.5 mCi, i.v), a PET myocardial perfusion imaging agent.19,20

Image Reconstruction and Analysis

Following acquisition, PET images were reconstructed in a matrix of 256 x 256 pixels with 95 transverse slices using the OSEM2D algorithm and decay corrected (microPET Manager and ASIPro, CTI Molecular Imaging). The pixel size was 0.47 mm and the slice thickness was 0.80 mm. The images were reoriented regarding cardiac axis and serial tomographic cardiac image frames were then generated. Regions of interest (ROI) in the anterior left ventricular wall and liver were drawn from these cardiac slices and activities in these ROIs were quantified as video intensity per volume (VI/cc) and time activity curves (TAC) were generated. In planar $^{123}$I-MIBG images of the NHP, ROIs were drawn for the whole heart and liver. The average activity per pixel in each organ was generated.
and expressed as VI/pixel. The total activity in the whole organ was calculated by multiplying the total pixels of each organ in the image and expressed as VI/organ (assuming the activity levels in front of and behind the organ were low enough to be neglected). VI/gram tissue in each organ was estimated by dividing the VI/organ by the heart or liver weight reported in the literature.\(^2\)

**Radiochemistry**

Chemical structures of LMI1195 and \(^{123}\)I-MIBG are presented in Figure 1. LMI1195 was synthesized by our chemistry group using a brosylate precursor and single step \(^{18}\)F displacement reaction with HPLC separation. The radiochemical purity of LMI1195 used in imaging studies was consistently >98% and specificity was \(\geq3500\) Ci/mmol. The agent was formulated in a 10% ethanol/saline solution for injection. \(^{123}\)I-MIBG was purchased from Cardinal Health (Woburn, MA).

**Statistical Analysis**

Data are expressed as mean ± SD. LMI1195 heart uptake in HF rats was compared using one-factor ANOVA, and uptake ratios of the heart to adjacent organs between MIBG and LMI1195 at various timepoints in rats were analyzed using two-factor repeated measures ANOVA (one factor repetition, SigmaPlot v12). Normality and homogeneity of variance were assessed by Shapiro-Wilk and Levene median tests, and only data of the heart to liver uptake ratio failed the equal variance test and were transformed with the square root function to achieve homogeneity of variance. The post-hoc comparisons were performed with Bonferroni test. Data of LMI1195 heart uptake levels with and without NET
blockade in rabbits and NHPs, and in sympathetic denervated rabbits passed the normality test and were compared using equal or unequal variance t-test depending on homogeneity of variance. \( p<0.05 \) was considered statistically significant.

Results

In-vitro Affinity and Uptake Kinetics

As shown in Table 1, \(^{19}\)F-LMI1195 associated with NET with a Ki value similar to that of NE. In SK-N-SH cells, NET mediated \(^{19}\)F-LMI1195 uptake kinetics (\(K_m\) and \(V_{max}\)) were also comparable to that of NE.

Tissue Uptake and Heart to Lung and Liver Uptake Ratios

In rats as shown in Table 2, LMI1195 heart uptake was high, second to adrenal glands, among organs evaluated and the uptake levels were similar to that of \(^{123}\)I-MIBG. The activity in the heart washed out slowly over time, also comparable with \(^{123}\)I-MIBG. However, compared to \(^{123}\)I-MIBG, the LMI1195 retention was lower and washed out more rapidly in non-target organs, resulting in significantly higher target to non-target uptake ratios of the heart to blood, lung and liver over 2-hour period. Some LMI1195 bone accumulation was observed. Similar to rats, LMI1195 heart uptake in rabbits was high, the highest among organs examined, and the uptake in adjacent non-target organs, like blood, lung and liver, was low. Consequently, the uptake ratio for LMI1195 as a cardiac imaging agent is considered desirable.
Cardiac Imaging in Normal Animals

Representative cardiac images of LMI1195 in a rat, rabbit and NHP are shown in Figure 2 and 3. In cardiac short- and long-axis images, the myocardium was well defined with homogeneous distribution of radioactivity and remained clear for at least 1 hour. Consistent with tissue biodistribution (Table 2), radioactivity levels in the adjacent liver and lung were low and washed out over time. In NHPs as shown in the TACs (left panels in Figure 3), the heart activity was high initially and remained consistent over 50 minutes after injection, whereas the activity in the liver declined markedly. At 30 minutes post injection, the heart to liver activity (expressed as VI/cc) ratio was 3.5±1.9 (n=4). High accumulation of $^{123}$I-MIBG was also observed in the heart, liver and kidneys (right panels in Figure 3). However, the liver activity as shown in the TAC remained high for 2 hours. Additionally, organ activity (derived from the total video intensity in the organ) per gram of tissue was calculated using the organ weight from literature. Assuming heart and liver weights were 18 and 134 grams for a 5-kg cyno monkey, the activity/gram was 1667 and 1570 VI/g (arbitrary video intensity scaled unit) in the heart and liver respectively with a heart to liver ratio of 1.1 at 30 minutes post injection. This ratio was much lower than the ratio with LMI1195 (3.5±1.9) at same time point.

Selectivity for Cardiac NET

The selectivity of LMI1195 for cardiac NET was examined using the NET inhibitor, desipramine. In cardiac images of rabbits (upper panels in Figure 4), radioactivity levels in the heart were reduced following increasing doses of desipramine. At the highest dose (1mg/kg), the heart was barely visible. Consist with the imaging findings, direct tissue
radioactivity measurement by a γ-counter showed that desipramine (1 mg/kg) inhibited approximately 82% of LMI1195 heart uptake. This inhibition was higher than that of $^{123}$I-MIBG heart uptake (~53%, lower panel in Figure 4). Similarly in NHPs, desipramine visibly reduced LMI1195 radioactivity in the heart (Figure 5). Based on quantitative image analysis, desipramine (0.5 mg/kg) inhibited about 66% of LMI1195 heart uptake at 1-hour post dosing.

**Cardiac Imaging in Rabbit Model of Sympathetic Denervation**

When imaged with LMI1195 in the sympathetically denervated rabbit (Figure 6), the activity in the heart was markedly reduced and the heart was barely visible in comparison to that of the control rabbit. However, imaging with flurpiridaz F 18 showed no difference in heart activity between the control and sympathetically denervated rabbits. In addition, direct tissue sampling to count radioactivity indicated that sympathetic denervation decreased LMI1195 heart uptake by about 79%.

**Cardiac Imaging in Rat Model of HF**

As shown in Figure 7, imaging with LMI1195 in the control DSS rat allowed clear visualization of the left ventricular wall. The activity in the heart decreased progressively in DSS rats fed high salt diet from early to late stage HF. In addition, the radioactivity levels in the heart, which were quantified from the cardiac images acquired at 50-60 minutes, were reduced following the development of HF and about 69% lower in the DSS rats at late stage HF.
Discussion

In the present study, LMI1195 has been examined as a potential PET imaging agent targeting NET for assessment of cardiac sympathetic neuronal function. The in-vitro assays showed that LMI1195 had a similar cell uptake profile as NE, comparable NET binding affinity and NET mediated cell uptake kinetics. In-vivo studies demonstrated high cardiac uptake that allowed high quality PET imaging. Inhibition studies with desipramine in rabbits and primates indicated the cardiac uptake was NET mediated. Experiments in chemically denervated rabbits confirmed changes in sympathetic innervation could be detected. Imaging in HF rats showed LMI1195 heart uptake levels decreased following HF progression.

Cardiac neuronal NET is responsible primarily for NE clearance at the synaptic cleft. Impaired NET function in the heart has been identified in HF of animals and human with diverse etiologies including hypertension, valvular and ischemic heart disease and idiopathic hypertrophic cardiomyopathy. In contrast, increased NET function by overexpressing NET in the heart using adenoviral gene transfer improved cardiac function in animals with HF. Therefore, measuring cardiac NET function may be useful for monitoring the disease progression and cardiac events in HF patients.

Several NET substrate based imaging agents, particularly the benzylguanidine based agent $^{123}$I-MIBG, have been extensively investigated in humans. Patients with the lowest MIBG heart uptake tend to have the poorest prognosis. However, MIBG has limitations associated with gamma camera imaging, radioisotope $^{123}$I and high liver retention as described in the introduction. In this study, LMI1195 heart uptake was
comparable with $^{123}$I-MIBG in rats and the highest among organs examined in rabbits (Table 2). The uptake ratios of heart to adjacent organs like blood, lung and liver for LMI1195 were high in rabbits, and higher than for $^{123}$I-MIBG in rats. Moreover, imaging in NHPs, LMI1195 uptake ratio of heart to liver showed superior to $^{123}$I-MIBG (3.5 vs. 1.1). In contrast to the high and sustained $^{123}$I-MIBG liver retention, LMI1195 washed out to a low level shortly after administration. These findings suggest that cardiac images of LMI1195 should have less interference by the background activity in organs, such as the liver.

Indeed, cardiac imaging with LMI1195 showed well-defined myocardium with low background activity in the lung and liver and quick radioactivity clearance from the blood in all species imaged (Figures 2 and 3). Due to the high image quality and quantification capability offered by PET, more parameters, such as retention index, absolute heart uptake and heart to blood ratios, could be derived regionally and globally from LMI1195 imaging for better quantification of sympathetic neuronal function. In NHPs, a slight increase in LMI1195 myocardial activity after the initial rapid washout was observed (left lower panel in Figure 3). The exact reason is unclear. It could be due to distribution of the activity in different compartments over time with various $K_1$, $K_2$ and $K_3$ values (rate constants for uptake into the extracellular space, clear from the extracellular space into the blood and uptake into neuronal cells from the extracellular space). The TAC of LMI1195 seems different from that of $^{11}$C-HED reported (heart uptake peaks and then extends into a plateau phase without rapid washout) and may indicate improved uptake kinetics for evaluation of NET function as suggested by Raffel
et al. Future examination of uptake kinetics in an isolated heart preparation may assist to address this issue.

Cardiac imaging with LMI1195 was also evaluated in a salt-sensitive rat model of HF. DSS rats fed a high salt diet have been characterized for the development of HF, from compensatory myocardial hypertrophy to late-stage dilated, failing HF, which is consistent with our findings. Imaging in these DSS rats showed reduced LMI1195 heart uptake following progression of HF development (Figure 7). The reduced LMI1195 uptake is consistent with assessments by others measuring $^3$H-NE tissue uptake directly in isolated heart preparations and MIBG heart uptake in DSS rats of late-stage HF. However, MIBG uptake was found normal at myocardial hypertrophy stage of DSS rats, even though the heart NE content decreased. The difference between reduced LMI1195 and normal MIBG heart uptakes at myocardial hypertrophy could be due to experimental conditions, such as elevated plasma NE levels found in our study and others but not in the MIBG study, and/or selectivity of the agent for NET (desipramine blocked 82% LMI1195 heart uptake vs. 53% $^{123}$I-MIBG in rabbits).

In recent studies to assess inducibility of ventricular arrhythmias and appropriate implantable cardioverter defibrillator (ICD) therapy in HF patients, both planar and SPECT imaging procedures with $^{123}$I-MIBG were performed. The results indicated that only regional, instead of global assessed by the planar, cardiac denervation scored by SPECT imaging predicted the clinical events. These findings emphasize the pivotal role of tomographic imaging for HF prognosis. However, SPECT imaging with $^{123}$I-MIBG is challenging particularly in patients with severe HF. Detection of cardiac sympathetic
denervation was evaluated in this study. Tomographic images of the denervated heart clearly showed significant reduction of LMI1195 uptake (79%), but normal myocardial perfusion assessed by flurpiridaz F 18 imaging (Figure 6). These findings suggest that reduced LMI1195 heart uptake is due to sympathetic denervation, not variations in perfusion. Even in images with low LMI1195 heart uptake seen in denervated rabbits and late-stage HF rats, the liver activity was still low enough for heart delineation.

Assessment of cardiac sympathetic denervation with an appropriate PET agent would provide higher spatial and temporal resolution along with better attenuation correction than 123I-MIBG, and allow regional definition of tracer kinetics. Several 11C labeled PET tracers for cardiac neuronal imaging have been evaluated in animals and humans, including 11C-HED, 11C-epinephrine, and 11C-phenylephrine.3-5 Studies have confirmed the value of PET imaging and shown imaging with these agents can predict progression of HF, ventricular arrhythmia and/or reinnervation after heart transplantation by using retention index, denervation/perfusion mismatch.12,32,33 However, the short isotopic half-life of 11C (20 min) requires on-site cyclotron and limits its broad clinical application. 18F isotope has the advantage of long half-life (110 min) and can be synthesized centrally for commercial use. 18F-agents, such as 18F-6-fluorodopamine, 18F-para-fluorobenzylguanidine and 18F-fluoriodobenzylguanidine, have been investigated, but not emerged in clinical development, possibly due to rapid metabolism by monoamine oxidase (18F-6-fluorodopamine), difficulty in radio-synthesis and/or low specific activity (900-1500 Ci/mmol for 18F-para-fluorobenzylguanidine and 18F-fluoriodobenzylguanidine).34-36 LMI1195 is an 18F labeled benzylguanidine and not a
substrate for monoamine oxidase. Radiolabeling is a one-step $^{18}$F displacement reaction with high specific activity (4500-5500 Ci/mmol to ensure $\geq 3500$ Ci/mmol at injection).

Cardiac imaging with LMI1195 is proposed to be used in patients with HF. These patients may take various drugs for treatment, such as $\beta$-receptor blockers, angiotensin converting enzyme inhibitors, calcium channel blockers and/or diuretics. Effects of these drugs on LMI1195 heart uptake have not been investigated in this study. Future assessment of the drug interaction is needed to determine if temporary cessation of a drug intake is required before LMI1195 imaging.

**Clinical Implications**

Assessment of cardiac NET function using molecular imaging has demonstrated the potential to stratify HF patients for treatment. In addition to drug therapies, device-based interventions, including cardiac resynchronization therapy (CRT) and ICD, have been used increasingly in recent years due to the clinically proven benefit to prevent cardiac mortality in HF patients. However, patient selection for these interventions is mainly based on clinical signs and physical measurements, such as ejection fraction and ECG. Consequently, only a few ICD implanted patients benefit from the implantation.$^{37}$ The remaining majority may suffer from complications such as bleeding, infection and inappropriate ICD activation. Indeed, suppressed cardiac NET function in HF patients assessed by $^{123}$I-MIBG imaging has been demonstrated to predict ventricular arrhythmia induced appropriate ICD therapy$^{31}$ and suggested for patient selection for CRT.$^{38}$ In this study, LMI1195 has demonstrated an improved imaging profile compared with MIBG for evaluation of cardiac NET using PET technology. Collectively, LMI1195 cardiac
imaging should be able to provide more detailed information to better stratify patients for treatments, particularly in selecting patients for some costly and invasive procedures, such as ICD implantation.

Conclusions

LMI1195 is a new $^{18}$F labeled NET substrate with good heart uptake, superior heart to adjacent organ uptake ratios and high NET selectivity that enables PET imaging of the cardiac neuronal function. This agent may allow the sensitivity, resolution and quantification of PET to measure changes in the cardiac sympathetic innervation/denervation associated with HF for treatment stratification.
Disclosures

All the work in this manuscript was performed by Lantheus Medical Imaging employees.
References


Table 1. Affinity and Uptake Kinetics of LMI1195 and Norepinephrine

<table>
<thead>
<tr>
<th></th>
<th>$K_i$ (μM)</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (pmol/10^6 cells/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine</td>
<td>3.36±2.77</td>
<td>2.01±0.85</td>
<td>6.23±1.52</td>
</tr>
<tr>
<td>$^{19}$F-LMI1195</td>
<td>5.16±2.83</td>
<td>1.44±0.76</td>
<td>6.05±3.09</td>
</tr>
</tbody>
</table>

$K_i$ was assessed in cell membrane overexpressed with human norepinephrine transporter. $K_m$ and $V_{max}$ were determined in SK-N-SH cells. (n=7/agent for $K_i$, and n=5/NE and 6/LMI1195 for $K_m$ and $V_{max}$)
Table 2. Tissue Biodistribution (%ID/g) and Uptake Ratio in Rat and Rabbit

<table>
<thead>
<tr>
<th>Tissue</th>
<th>123I-MIBG (Rat n=6-12/timepoint)</th>
<th>LMI1195 (Rat n=6-12/timepoint)</th>
<th>LMI1195 (Rabbit n=4-10/timepoint)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
<td>60 min</td>
<td>120 min</td>
</tr>
<tr>
<td>Blood</td>
<td>0.18±0.02</td>
<td>0.19±0.06</td>
<td>0.25±0.15</td>
</tr>
<tr>
<td>Heart</td>
<td>2.14±0.30</td>
<td>2.19±0.27</td>
<td>1.77±0.39</td>
</tr>
<tr>
<td>Lung</td>
<td>3.55±0.93</td>
<td>2.13±0.45</td>
<td>1.13±0.42</td>
</tr>
<tr>
<td>Liver</td>
<td>0.90±0.20</td>
<td>0.61±0.17</td>
<td>0.40±0.25</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.84±0.11</td>
<td>0.91±0.19</td>
<td>0.79±0.07</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.79±0.21</td>
<td>0.54±0.24</td>
<td>0.33±0.16</td>
</tr>
<tr>
<td>Adrenal</td>
<td>1.45±0.23</td>
<td>1.21±0.63</td>
<td>1.32±0.15</td>
</tr>
<tr>
<td>Femur</td>
<td>0.18±0.03</td>
<td>0.30±0.27</td>
<td>0.13±0.04</td>
</tr>
</tbody>
</table>

Uptake ratio

<table>
<thead>
<tr>
<th></th>
<th>Heart : blood</th>
<th>Heart : lung</th>
<th>Heart : liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>11.97±1.60</td>
<td>2.42±0.41</td>
<td>5.43±1.93</td>
</tr>
<tr>
<td></td>
<td>12.34±2.81</td>
<td>3.80±0.93</td>
<td>6.21±1.68</td>
</tr>
<tr>
<td></td>
<td>8.45±4.62</td>
<td>5.43±1.93</td>
<td>14.93±4.22</td>
</tr>
<tr>
<td></td>
<td>15.56±3.61</td>
<td>6.21±1.68</td>
<td>19.69±2.00</td>
</tr>
<tr>
<td></td>
<td>20.72±5.74*</td>
<td>14.93±4.22*</td>
<td>31.21±4.91</td>
</tr>
<tr>
<td></td>
<td>17.66±3.37*</td>
<td>19.69±2.00*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.45±6.48</td>
<td>3.61±0.97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.66±8.60</td>
<td>6.04±1.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>31.21±4.91</td>
<td>7.50±2.68</td>
<td></td>
</tr>
</tbody>
</table>

Tissue uptake of radiotracer was measured at 15, 60 and 120 minutes after injection and expressed as % injected dose per gram tissue (ID/g). * indicates p<0.05 vs. 123I-MIBG at same timepoint.
Figure Legends

Figure 1. Chemical structures of LMI1195 (N-[3-Bromo-4-(3-[18F]fluoro-propoxy)-benzyl]-guanidine) and MIBG (meta-iodobenzylguanidine).

Figure 2. Cardiac PET images of LMI1195 in rats and rabbits at two timepoints post injection.

Figure 3. Left panels: cardiac PET images of LMI1195 in a nonhuman primate (NHP) and the time-activity curve (TAC) generated from the images. Right panels: whole body planar images of $^{123}$I-MIBG in a NHP and the image derived TAC. Tissue activity levels are expressed as video intensity per tissue volume (VI/cc) and video intensity per pixel respectively. In contrast to $^{123}$I-MIBG, LMI1195 liver radioactivity washed out rapidly over time.

Figure 4. Upper panel: cardiac PET images of LMI1195 in rabbits pretreated with vehicle or desipramine at two doses to block NET. These images were acquired at 50-60 minutes post injection. Lower panel: summarized $^{123}$I-MIBG (n=6/group) and LMI1195 (n=4-5/group) heart uptake (% injected dose/g tissue) by tissue sampling at 60 minutes post injection in rabbits with and without NET blockade using desipramine (1 mg/kg). * indicates $p<0.05$ vs. control group.
**Figure 5.** Upper panel: cardiac PET images of LMI1195 in nonhuman primates (NHP) pretreated with vehicle (control) or desipramine to block NET. These images were acquired at 50-60 minutes post injection. Lower panel: summarized heart activity of control and NET blocked NHPs. Heart activity levels were expressed as video intensity unit (VIU) per injected dose (µCi) corrected by body weight [VIU/(ID/kg)*10^6]. * indicates p<0.05 vs. control group.

**Figure 6.** Upper panel: cardiac PET images of LMI1195 and flurpiridaz F 18, a myocardial perfusion imaging agent, in rabbits with and without sympathetic denervation. These images were acquired at 20-30 minutes post injection. Lower panel: summarized LMI1195 heart uptake (% injected dose/g tissue) by tissue sampling at 60 minutes post injection in rabbits with and without sympathetic denervation (n=3/group). The decreased LMI1195 heart uptake in the sympathetically denervated rabbit is due to reduced neuronal function, not the changes in myocardial perfusion. * indicates p<0.05 vs. control group.

**Figure 7.** Upper panels: cardiac images of LMI1195 in Dahl salt sensitive (DSS) rats fed a low salt (control) or high salt diet for 5 (early stage HF) and 10 weeks (late stage HF). These images were acquired at 50-60 minutes post injection. Lower panel: summarized heart activity of control and high salt fed rats. Heart activity levels were derived from these cardiac images and expressed as video intensity unit (VIU) per injected dose (µCi) corrected by body weight [VIU/(ID/g)*10^6]. * indicates p<0.05 vs. control group. LS-5 and -10: low salt diet for 5 and 10 weeks; HS-5 and -10: high salt diet for 5 and 10 weeks.
PET imaging with LMI1195

Planar imaging with $^{123}$I-MIBG

<table>
<thead>
<tr>
<th>Time after injection</th>
<th>Tissue activity (V/ccc)</th>
<th>Tissue activity (V/pixel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>120</td>
<td>300</td>
</tr>
<tr>
<td>Liver</td>
<td>100</td>
<td>200</td>
</tr>
</tbody>
</table>

Heart
Liver
Vehicle

0.1 mg/kg

1 mg/kg

Desipramine

Heart uptake (%ID/g)

Control

NET blockade

Mean value

123I-MIBG

LMI1195
Short axis

Long axis

Control NET blockade

LMI1195 heart activity [VU/(lp/kg)x10^6]

Mean value

Control (n=4)  NET blockade (n=3)
LMI1195 Flurpiridaz
Control (same rabbit)

Sympathetic denervation (same rabbit)

<table>
<thead>
<tr>
<th>LMI1195</th>
<th>Flurpiridaz</th>
<th>LMI1195</th>
<th>Flurpiridaz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>Sympathetic denervation</td>
<td></td>
</tr>
</tbody>
</table>

Mean value

LMI1195 heart uptake (%ID/g)

Control

Sympathetic denervation

By guest on October 19, 2017 http://circimaging.ahajournals.org/ Downloaded from

Cardiovascular Imaging

Journal of the American Heart Association
Control (LS-5) | HF (HS-5) | Control (LS-10) | HF (HS-10)

Early stage HF

Late stage HF

LM1195 heart activity [VU/(ID/g)*10^6]

Mean value

control (n=4) | Early stage HF (HS-5, n=2) | Late stage HF (HS-10, n=3)
Evaluation of LMI1195, a Novel $^{18}$F Labeled Cardiac Neuronal PET Imaging Agent, in Cells and Animal Models

Ming Yu, Jody Bozek, Melanie Lamoy, Mary Guaraldi, Paula Silva, Mikhail Kagan, Padmaja Yalamanchili, David Onthank, Mahesh Mistry, Joel Lazewatsky, Matthias Broekemà, Heike Radeke, Ajay Purohit, Michael Azure, Richard Cesati, David Casebier and Simon P. Robinson

_Circ Cardiovasc Imaging_. published online May 9, 2011;
_Circulation: Cardiovascular Imaging_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-9651. Online ISSN: 1942-0080

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circimaging.ahajournals.org/content/early/2011/05/09/CIRCIMAGING.110.962126

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation: Cardiovascular Imaging_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation: Cardiovascular Imaging_ is online at: http://circimaging.ahajournals.org//subscriptions/