2-1-2016

Evaluation of a PACAP Peptide Analogue Labeled with (68)Ga Using Two Different Chelating Agents.

Pardeep Kumar  
*Thomas Jefferson University*

Sushil Kumar Tripathi  
*Thomas Jefferson University*

Chang-Po Chen  
*Henan Normal University*

Neil Mehta  
*Thomas Jefferson University*

Bishnuhari Paudyal  
*Thomas Jefferson University*

Follow this and additional works at: [https://jdc.jefferson.edu/radiologyfp](https://jdc.jefferson.edu/radiologyfp)

Let us know how access to this document benefits you

See next page for additional authors

**Recommended Citation**

Kumar, Pardeep; Tripathi, Sushil Kumar; Chen, Chang-Po; Mehta, Neil; Paudyal, Bishnuhari; Wickstrom, Eric; and Thakur, Mathew L., "Evaluation of a PACAP Peptide Analogue Labeled with (68)Ga Using Two Different Chelating Agents." (2016). *Department of Radiology Faculty Papers*. Paper 44.

[https://jdc.jefferson.edu/radiologyfp/44](https://jdc.jefferson.edu/radiologyfp/44)
Evaluation of a PACAP peptide analogue labeled with $^{68}$Ga using two different chelating agents.

Pardeep Kumar¹, Sushil Tripathi¹, Chang-Po Chen², Neil Mehta¹, Bishnuhari paudyal¹, Eric Wickstrom³, Mathew L. Thakur*¹,4,5.

ABSTRACT

Objective: The authors have conjugated chelating agents (DOTA and NODAGA) with a peptide (PACAP analogue) which has a high affinity for VPAC1 receptors expressed on cancer cells. In order to determine a suitable chelating agent for labeling with $^{68}$Ga, we have compared the labeling kinetics and stability of these peptide conjugates.

Methods: For labeling, $^{68}$GaCl₃ was eluted in 0.1 M HCl from a $[^{68}$Ge-$^{68}$Ga] generator. The influences of peptide concentration, pH and temperature on the radiolabeling efficiency were studied. The stability was evaluated in saline, human serum, DTPA, Transferrin and metallic ions (FeCl₃, CaCl₂ and ZnCl₂). Cell binding assay was performed using human breast cancer cells (T47D). Tissue biodistribution was studied in normal athymic nude mice.

Results: Optimal radiolabeling (> 95.0%) of the DOTA-peptide conjugates required higher (50°C–90°C) temperature and 10 min incubation at pH 2–5. The NODAGA-peptide conjugate needed incubation only at 25°C for 10 min. Both radio-complexes were stable in saline, serum as well as against transchelation and transmetallation. Cell binding at 37°C for 15 min incubation with $^{68}$Ga-NODAGA-peptide was 34.0% as compared to 24.5% for $^{68}$Ga-DOTA-peptide. Tissue biodistribution at 1 hr post-injection of both $^{68}$Ga labeled peptide conjugates showed clearance through the kidneys.

Conclusions: NODAGA-peptide showed more convenient radiolabeling features than that of DOTA-peptide.

Key words: Breast cancer, Prostate Cancer, chelating agent, peptides, radiopharmaceuticals.

¹Department of Radiology, Thomas Jefferson University, Philadelphia, Pennsylvania. ²School of Chemistry and Chemical Engineering, Henan Normal University, Xinxiang, China. ³Department of Biochemistry and Molecular Biology, ⁴Kimmel Cancer Center, ⁵Department of radiation oncology, Thomas Jefferson University, Philadelphia, Pennsylvania.

For correspondence or reprints contact: Mathew L. Thakur, Thomas Jefferson University, 1020 Locust St., Suite: 359 JAH, Philadelphia, PA-19107. E-mail: madhukar.thakur@jefferson.edu
Introduction

Positron emission tomography (PET) has become a prominent imaging modality in the field of oncology.\textsuperscript{1} PET has several technical advantages over the single photon emission computed tomography (SPECT) like attenuation correction, and higher spatial resolution.\textsuperscript{2-3} Small molecules such as sugars, amino acids, nucleic acids, or receptor-binding ligands are labeled with positron-emitting radionuclides for PET imaging to study \textit{in vivo} visualization of physiological processes on a molecular level.\textsuperscript{4-7} There are several positron emitting radionuclides like fluorine-18 $^{18}\text{F}$ ($t_{1/2} \approx 110$ min), copper-64 $^{64}\text{Cu}$ ($t_{1/2} \approx 12.7$ hr), carbon-11 $^{11}\text{C}$ ($t_{1/2} \approx 20$ min), and oxygen-15 $^{15}\text{O}$ ($t_{1/2} \approx 2$ min). However, their production requires in-house cyclotron, not available at every academic institution and hospital.

In recent years, Gallium-68 $^{68}\text{Ga}$ - a positron emitting radionuclide conveniently available in the form of Germanium-68 ($t_{1/2} \approx 270$ days)-Gallium-68 ($t_{1/2} \approx 68$ min) $^{68}\text{Ge} \rightarrow ^{68}\text{Ga}$ generator has drawn considerable attention for oncologic diagnostic applications. The short physical half-life of $^{68}\text{Ga}$ induces low radiation burden on patients and makes it an ideal radionuclide for diagnostic use.\textsuperscript{8-10} The labeling of biomolecules with $^{68}\text{Ga}$ is achieved by using bi-functional chelators (BFC) such as DTPA (diethylene triamine pentaacetic acid), DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid), or NODAGA (1,4,7-triazacyclononane-1-glutamic acid-4,7-diacetic acid). BFCs differ from each other in their physio-chemical properties which can influence radiolabeling kinetics. They can also influence overall charge of the complex, biodistribution and more importantly uptake in the tumor site.\textsuperscript{11-13}

In recent years, there has been increasing interest in the receptor targeted scintigraphy in which peptides have emerged as promising biomolecules.\textsuperscript{14} Their properties such as fast clearance, rapid tissue penetration, low antigenicity, and relatively easy synthesis have added to their applicability.\textsuperscript{15} In past few years, our laboratory has designed, synthesized and radiolabeled peptide conjugates which have high affinity for VPAC-1 receptors, over-expressed in many malignant tumors including those of the breast and prostate.\textsuperscript{16-20} The peptides are analogues of Pituitary adenylate cyclase-activating peptide (PACAP) - a 27 amino acid peptide and vasoactive intestinal peptide (VIP)- 28 amino acid. Both have similar biochemical properties and bind to VPAC1 receptors expressed in high density on the surface of certain cancers such as of the breast, prostate and urinary bladder (100%), colon (96%), pancreas (65%), lung (58%), stomach (54%), and liver (49%).\textsuperscript{21-24}

The present study was aimed to investigate the influence of these two BFC agents on the PACAP analogue, which differs in terms of their radiolabeling kinetics, biological activity and stability of the resultant product.
Methods and materials

Reagents

Fmoc amino acid, solvents and reagents for peptide chelator synthesis, was purchased from Fluka Chemicals (St. Louis, MO). The BFC DOTA was purchased from Macrocyclics (Dallas, TX) and NODAGA was purchased from Chematech (Dijon, France). Sodium acetate, diethylenetriamine pentaacetic acid (DTPA), calcium chloride (CaCl2), zinc chloride (ZnCl2), Ferric chloride (FeCl3), hydrochloric acid solutions were purchased from Fisher Scientific, Inc. (Waltham, MA). Transferrin was purchased from Sigma-Aldrich (St. Louis, MO). A 370MBq [68Ge-68Ga] generator was purchased from Eckert and Zeigler (Berlin, Germany). Sodium chloride solution (0.9%) was prepared in this laboratory using deionized water. Human serum was prepared from whole blood drawn from a volunteer. All chemicals were used without further purification.

Instruments

Peptide was purified using a preparative column on high-pressure liquid chromatography (HPLC) system (Shimadzu Corporation, Kyoto, Japan) equipped with gradient pumps and UV/VIS detector, a NaI(Tl) radioactivity monitor, and a rate meter. The reverse phase C18 microbound column (4.6 mm × 250 mm) served as the stationary phase, and two solvents: 0.1% Trifluoroacetic acid (TFA) in H2O and 0.1% TFA in acetonitrile as mobile phase. The gradient was such that 10% CH3CN in aqueous 0.1% CF3COOH to 100% CH3CN in 0.1% CF3COOH at a flow rate of 1 mL/min over 28 min at 22°C. For thin layer chromatography (TLC), pre-coated aluminum sheets with silica gel were purchased from Merck (Kenilworth, NJ) and radioactivity was measured on Perkin Elmer 2480 wizard² automatic gamma counter (Waltham, MA). The pH of the solutions was measured using either a pH meter (Sartorius, Gottingen, Germany) or pH strips (Fisher scientific Inc.)

Synthesis of peptide conjugate

Briefly, the PACAP analogue with C-terminal NODAGA/DOTA chelators was synthesized on a Wang resin using an ABI 341A peptide synthesizer (Applied Biosystems, Foster City, CA). Fmoc-Lys (ivDde) was first introduced at the C terminus of the peptide, followed by 4-aminobutyric acid (y-Aba). The 27-amino-acid-long PACAP sequence was then assembled by standard Fmoc coupling with the final histidyl residue, being a t-Boc–protected His(Trt) derivative. The capping t-Boc function was necessary to ensure that the N-terminal amino group remained protected during
subsequent deprotection and coupling cycles performed at the C-amino group of the C-terminal lysine. The ivDde group at the C-terminal lysine was then selectively removed with 2% hydrazine, followed by coupling with DOTA or NODAGA. The peptide was cleaved from the resin using TFA/water/phenol/thioanisole/ethanedithiol (82.5:5:5:5:2.5) and precipitated with diethyl ether. The crude peptide was purified to homogeneity by reversed phase HPLC using a preparative column. The peptide was characterized by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry.

**Radiolabeling**

For radiolabeling, 300 µL (14.5 – 18.5 MBq) of $^{68}$GaCl$_3$ was added in a clean glass test tube to which were added varying amounts of acetate buffer to study the influence of pH (1.0 – 6.0) and varying amount of a 4 mg/mL peptide-chelators water solution (1-20 µg). The mixture was heated at predetermined temperatures (25, 50, 70 and 90 °C) for pre chosen time periods (10, 15, 20 and 30 min) to study the kinetics of the radiolabeling reactions with respect to temperature and time. The radiolabeling was evaluated by Radio-HPLC.

**Preparation of free $^{68}$GaCl$_3$, $^{68}$Ga-hydrolyzed, $^{68}$Ga-DTPA and $^{68}$Ga-Tf as reference standard**

To identify HPLC peaks of the $^{68}$Ga species, HPLC peaks, that may have formed during the chelation experiments, free $^{68}$GaCl$_3$ (as eluted from generator with 0.1M HCl), $^{68}$Ga-hydrolyzed products, $^{68}$Ga-DTPA and $^{68}$Ga-Transferrin (Tf) were prepared as described$^{25-26,13}$ and their retention times were recorded on radio-HPLC.

**Stability (in vitro), transchelation and transmetallation studies**

For the stability, transchelation and transmetallation studies, $^{68}$Ga-peptide-chelators were prepared by mixing 300 µL (14.5 – 18.5 MBq) of $^{68}$GaCl$_3$ in 0.1 M HCl, 120 µL of 1.0 M sodium acetate and 5 µL (20 µg) of peptide-chelators. The preparation was incubated at 90°C for 30 min. Aliquots of $^{68}$Ga-peptide-chelators solutions (0.3 mL, approx. 15 MBq) were alternatively mixed with (1) 0.3 mL of a 0.9% NaCl, (2) 0.3 mL of a 100 nM Fe$^{3+}$/Zn$^{2+}$/Ca$^{2+}$ (for transmetallation study), (3) 0.3 mL of a 200 µM DTPA solution, (4) 0.3 mL of a 50 µM transferrin solution (for transchelation study) and (5) 0.3 mL of human serum solution. The mixtures were incubated at 37 °C and analyzed after 0, 10, 30, 60 and 120 min of incubation, respectively. A 10 µl (in 100µL of normal saline) aliquot preparations of (1), (2) and (3) were injected in the radio-HPLC while preparations (4, 5) were analyzed by ITLC-SG using 0.1M citric acid as a solvent.
**Cell binding (in vitro) assay**

Estrogen receptor-positive T47D human breast tumor cells from American Type Culture Collection were maintained in RPMI 1640 medium with 2 mM L-glutamine contained 10 mM HEPES, 1.0 mM sodium pyruvate, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose and supplemented with 0.2 Units/ml 90 %bovine insulin, and 10% fetal bovine serum, at 37°C under 5% CO₂/95% air. The cells when confluent were detached using 0.25% trypsin-EDTA, washed and resuspended with RPMI-1640 medium at a concentration of ~ 5× 10⁶ cells/mL. Each test tube contained 1 mL of cell suspension into which 10 µL of ⁶⁸Ga-peptide-chelator (approx. 0.74 MBq) was added and incubated for 2 hr at 37°C in a water bath. The incubation was terminated by adding 0.5 ml of cold normal saline. The mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant from each wash was collected into marked test tubes. To the remaining pellet, 1.0 ml of normal saline was added and centrifuged again at 3000 rpm for 10 minutes. The supernatant was separated and collected in the marked test tube. The cells and supernatant were counted for radioactivity in a gamma counter and percentage of radioactivity bound on the cells was calculated.

**Biodistribution studies**

The biodistribution of ⁶⁸Ga labeled peptide was studied in normal (n = 10, athymic nude, female mice, 20-25 g). The resultant optimal preparation procedure was used which contained 300 µL of ⁶⁸GaCl₃ in 0.1 N HCl, 120 µL of 1.0 M sodium acetate solution and 20 µg of peptide conjugate, incubated for 10 min at RT (for NODAGA-peptide) and 90°C (for DOTA-peptide). Following determination of radiochemical purity (> 95%) by radio-HPLC analysis, ⁶⁸Ga-peptide (3.7 – 5.5 MBq) was administered intravenously through a lateral tail vein in 200 µL of the preparation (pH ≈ 5.5). Radioactivity in the syringe before and after administration was measured in an energy-calibrated dose calibrator (CRC-15; Capintec, Ramssey, NJ), and the exact quantity received by each animal was determined. A standard ⁶⁸GaCl₃ solution of a known quantity was prepared at the time of injection. The animals (n = 5, each study) were sacrificed at 1 h post injection by CO₂ inhalation. Various organs were removed, washed free of blood, blotted dry, and weighed. The associated radioactivity was counted in the γ-counter and results were calculated as percentage injected dose per gram (% ID/g) of tissue.
Results

Purity of peptide-chelators conjugates

NODAGA- and DOTA-peptide purity as determined by HPLC was 99%. The MALDI-TOF demonstrated that, for NODAGA-peptide observed molecular mass was 3716 (calculated molecular mass-3718) whereas for DOTA-peptide observed molecular weight was 3787 (calculated molecular mass-3785). The MALDI peaks for the peptide conjugates are given in Figure 1A and B.

Radiolabeling kinetics

Optimal radiolabeling (> 95.0%) of the DOTA-conjugated peptide required pH 2-5 and higher (50-90°C) temperature, while the NODAGA conjugated peptide needed incubation only at room temperature at same pH range. At higher temperature (90°C), the radiolabeling was not affected by pH for both peptide conjugates (Fig. 2A, B) and can be labeled at wide range of pH (1.0 – 6.0). NODAGA-peptide can bind 68Ga at a minimal quantity of 1 µg but DOTA-peptide required minimum of 20 µg to yield optimal (≥ 95%) radiolabeling (Fig. 2C). The minimum incubation time needed for optimal (> 95%) radiolabeling was 10 minutes at 90°C (Fig. 2D). Radio-HPLC showed retention time for both radio-complexes was 9.9 ± 0.3 minutes as compared to 3.1 ± 0.2 minutes for free 68Ga as shown in Fig. 3-b. The retention time was also determined for the reference standard like hydrolyzed 68GaCl₃, 68Ga-DTPA and 68Ga-transferrin and found to be 3.5 ± 0.2 minutes.

Stability studies

Both 68Ga labeled peptide conjugates were stable in 0.9% normal saline (Fig. 4A) and showed minimal (1-2%) degradation against (FeCl₃, CaCl₂ and ZnCl₂) transmetallation (Fig. 4B-D) up to 2 hours. The transchelation study with DTPA (Fig. 5A) and transferrin (Fig. 5B) solution showed that both radio-complexes were remained intact over 2 hours. Radio-TLC showed ratio factor (Rf) value of 0.0-0.1 for 68Ga labeled peptide and 0.9-1.0 for free 68GaCl₃.

Cell binding (in vitro) assay

Cell binding assay showed higher uptake for 68Ga-NODAGA-peptide, 34.0% ± 0.8% as compared with 24.5% ± 0.9% for 68Ga-DOTA-peptide at 15 minutes. However, at 2
hours of incubation at 37°C, the uptake was nearly same, 48.9% ± 3.9% for \(^{68}\)Ga-NODAGA-peptide and 45.2% ± 2.1% for \(^{68}\)Ga-DOTA-peptide (Table – I).

Biodistribution studies

The biodistribution pattern of the both \(^{68}\)Ga labeled peptide conjugate is shown in Figure 6. In both cases, a higher uptake is seen in kidneys followed by liver, blood and lungs. It was evident that the \(^{68}\)Ga-NODAGA-Peptide showed rapid blood clearance as compared to \(^{68}\)Ga-DOTA-peptide. Liver uptake was 8.8% for \(^{68}\)Ga-NODAGA-Peptide was lower (p = 0.01) as compared to 16.5% for \(^{68}\)Ga-DOTA-peptide. Most of the activity was excreted through kidney.

Discussion

With its several advantages such as the availability in a generator form, cyclotron independency, multi elution ability in a single day, simplified radiolabeling chemistry with several biomolecules using bifunctional chelating agents, the use of \(^{68}\)GaCl\(_3\) is rapidly growing for diversified clinical applications.\(^{27}\) The presence of even µg levels of metallic impurities (with ageing of generator) in \(^{68}\)GaCl\(_3\) or reagent used may hamper the radiolabeling complexation. The removal of radionuclide impurities however has helped expansion of its use for clinical application.\(^{28}\)

The eluate of \(^{68}\)GaCl\(_3\) from the generator may contain metal ion impurities such as Fe\(_{3+}\), Cu\(_{2+}\), Al\(_{3+}\), Zn\(_{2+}\) and Sn\(_{4+}\) which could hamper the radiolabeling of biomolecules. It is important therefore to identify the suitable chelating agent for the radiolabeling which is least affected by these impurities as well the stability of the labeled compound.\(^{29-30}\) DOTA is the most commonly used chelating agent and \(^{68}\)Ga-DOTATOC is accepted in the European Pharmacopeia.\(^{31}\) \(^{68}\)Ga labeled DOTA biomolecules conjugates (like peptides, antibody, and small molecules) have been successfully developed and used for many clinical applications. The disadvantage of DOTA conjugate is its slow reaction kinetics for complex formation with \(^{68}\)Ga.\(^{32}\) These limitations prompted us to investigate the use of NODAGA for comparison with DOTA.

Chelator-peptide conjugates were synthesized by solid phase peptide synthesis method. Both peptide conjugates were synthesized with high purity (\(~ 99.0\%\)). NODAGA-peptide conjugate showed faster kinetics as compare to DOTA- peptide conjugate and less influenced by the pH variation. NODAGA peptide conjugate yielded higher radiolabeling efficiency at minimal quantity of peptide (1 µg), at room temperature and within short incubation (5 min) time. For the preparations with short lived radionuclides like \(^{68}\)Ga, these characteristics of NODAGA are advantageous.
kinetics may be attributable to its ionic size compatibility with cavity size of the nine
membered triazomacrocycle of NODAGA and therefore, yielding a high radiolabeling in
5 min at room temperature. A low radiolabeling was observed in acidic (pH-1.0)
conditions for both peptide conjugates when incubated at temperature (25-70°C). It may
attribute to strongly acidic environment which might have protonated the donor atoms of
the ligand and destabilized the 68Ga complex. At high temperature (~ 90°C), the
radiolabeling was found to be independent of pH variation (Fig. 2 a-b). However at pH 7.0
and above, labeling decreases (data not included). This may be due to a fact that at acidic pH,
68Ga remains predominant in cation Ga3+ form (> 90%), though soluble complex ions Ga(OH)2+,
Ga(OH)3+ may be present in the solution. As the pH of the solution shifts towards basic (> 7.0),
the hydrolyzed form of Gallium, Ga(OH)4 increases and decreases the radiolabeling yield. Once
the peptide conjugate is radiolabeled, then adjusting the pH to physiological pH 7.0 does not
adversely influence the chelated Ga-68.

The use of sodium acetate buffer maintained the pH and allowed strong formation BFC-
68Ga complex. The other advantage of using sodium acetate buffer is its biocompatibility
for human use. DOTA peptide conjugate showed labeling at high temperature. Therefore, DOTA may not be useful for those peptides which can denature at higher
temperature and become unsuitable for molecular imaging. On the other hand, radiolabeling of NODAGA-peptide at room temperature ruled out major thermal
denaturation. Radio-HPLC showed that the formation of the 68Ga-NODAGA and DOTA
peptide conjugate has retention time of around 9.9 min whereas free 68Ga eluted at 3.6
min (Fig. 3-b) and complex formation between 68Ga and NODAGA-peptide conjugate is
shown in Fig. 3-a . HPLC UV detector also detected the UV peak for NODAGA and
DOTA peptide conjugate at around 9.9 min. Therefore, it confirmed the complexation of the
68Ga with peptide conjugate which leads to the retention of the 68Ga labeled peptide
approximately at the same time.

Both radio-complexes were found to be stable in normal saline (Fig. 4a) and human
serum over 120 minute. The stability in human serum (at 37°C) indicated its suitability
for in vivo applications and showed their inertness towards the metal ion/species that may
be present in the serum. The transmetallation study was important not because these ions
are present as impurities in the eluate but these ions are also present in serum in
appreciable quantity. 68Ga-NODAGA and DOTA peptide conjugates showed minimal
(1-2%) degradation in radiolabeling after 120 minute incubation with respective ion
solution at 37°C (Fig. 4-b-d). This could also provide an indication about the stability of
these radio-complexes in in vivo environment. Transferrin is the major protein in the
plasma which is known to form a strong complex with 67Ga-citrate. The minimal
transchelation (~1-2%) of 68Ga-peptide conjugates with transferrin showed the stability of
radio-complexation (Fig. 5-a-b).
The cell binding assay was performed on T47D breast cancer lines which express VPAC-1 receptors on their surface.\textsuperscript{19} \textsuperscript{68}Ga-NODAGA-peptide showed higher uptake in 15 minute as compared to \textsuperscript{68}Ga-DOTA-peptide but the uptake was almost same at 2hr (Table-1) which may be due to saturation of the receptor site. The biodistribution data (Fig. 6) showed that both radio-complexes were cleared through kidneys. Hence, renal system was the major route of clearance from the body. \textsuperscript{68}Ga-NODAGA-peptide was cleared rapidly from the blood and had significantly less liver uptake (p < 0.05) as compare to \textsuperscript{68}Ga-DOTA-peptide at 1 hr. The fast clearance from the blood may be advantageous in reducing the background radioactivity for PET imaging but it could also limit the accumulation of radioactivity at target (tumor) area. Hence, a detailed \textit{in vivo} investigation will be required further for any conclusion.

In the present study, we have showed that \textsuperscript{68}Ga-NODAGA-peptide exhibited most favorable features from radiochemistry point of view. Chelator NODAGA has flexibility over the influence of temperature and acidic environment.\textsuperscript{13} Labeling at room temperature provides an advantage and could prevent any deleterious effect on peptide conjugate due to heating. Both peptide conjugates were found to be stable in serum, against transchelation and transmetallation experimentations. A detailed \textit{in vivo} study is in progress to investigate the influence of chelators on the biodistribution and tumor targeting.

\textbf{Conclusions}

A comparison between NODAGA and DOTA revealed that NODAGA-peptide conjugate could be efficiently radiolabeled with \textsuperscript{68}Ga at room temperature with high yield. \textsuperscript{68}Ga-NODAGA-peptide conjugate remained stable in serum, in presence of major metal ions impurities, against transchelation with DTPA or transferrin. The biodistribution data showed its clearance from kidneys, which minimize the radiation burden on the organs. The clearance of \textsuperscript{68}Ga-NODAGA-peptide from blood was faster as compare to \textsuperscript{68}Ga-DOTA-peptide and would be advantageous for PET imaging.

\textbf{Acknowledgments}

The research, in part, was supported by NIH/NCI RO1 CA 157372 (M.L.T.), NIH/NCI 1S10OD012406 (M.L.T.), and NIH/NCI S10RR23709 (M.L.T.).

\textbf{Disclosure Statement}

M.L.T. is consultant to NuView and Zevacor, Inc. No other potential conflicts of interest relevant to this article are reported.
References


