**First in human evaluation and dosimetry calculations for peptide $^{124}$I-p5+14 - A novel radiotracer for the detection of systemic amyloidosis using PET/CT imaging**

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***Abstract*** *(Max 300)*

**Purpose**: Accurate diagnosis of amyloidosis remains a significant clinical challenge and unmet need for patients. The amyloid-reactive peptide p5+14 radiolabeled with iodine-124 has been developed for the detection of amyloid by PET/CT imaging. In a first-in-human evaluation, the dosimetry and tissue distribution of $^{124}$I-p5+14 peptide in patients with systemic amyloidosis. Herein we report the dosimetry and dynamic distribution in the first three enrolled patients with light chain-associated (AL) amyloidosis.

**Procedures**: The radiotracer was assessed in a single-site, open-label Phase 1 study (NCT03678259). The first three patients received a single intravenous infusion of $^{124}$I-p5+14 peptide (≤37 MBq). Serial PET/CT imaging was performed during the 48-h post infusion. Dosimetry was determined as a primary endpoint for each patient and gender-averaged mean values were calculated. Pharmacokinetic parameters were estimated from whole blood radioactivity measurements and organ-based time activity data. . Lastly, the biodistribution of radiotracer in major organs was assessed visually and compared to clinically appreciated organ involvement.

**Results**: Infusion of the $^{124}$I-p5+14 was well tolerated with rapid uptake in the heart, kidneys, liver, spleen, pancreas, and lung. The gender-averaged whole-body effective radiation dose was estimated to be 0.23 (± 0.02) mSv/MBq with elimination of the radioactivity via renal and gastrointestinal routes. The whole blood elimination $t_{1/2}$ of 21.9 ± 7.6 h. Organ based activity concentration measurements indicated that $AUC_{last}$ tissue:blood ratios generally correlated with the anticipated presence of amyloid. Peptide uptake was observed in 4/5 clinically suspected organs, as noted in the medical record, as well as six anatomic sites generally associated with amyloidosis in this population.

**Conclusion:** Peptide $^{124}$I-p5+14 rapidly distributes to anatomic sites consistent with the presence of amyloid in patients with systemic AL. The dosimetry estimates established in this cohort are acceptable for whole body PET/CT imaging. Pharmacokinetic parameters are heterogeneous and consistent with uptake of the tracer in an amyloid compartment. PET/CT imaging of $^{124}$I-p5+14 may facilitate non-invasive detection of amyloid in multiple organ systems.

## Introduction

Systemic amyloidosis is a multi-organ disease characterized by the extracellular deposition of protein fibrils, extracellular matrix components, and proteins sequestered from the circulation [1-2]. The progressive accumulation of amyloid results in architectural damage leading to organ dysfunction and ultimately death. More than 30 proteins have been identified in the fibril associated with amyloid; however, patients with immunoglobulin light chain- (AL), variant or wild type transthyretin- [ATTRv and ATTRwt], and leukocyte chemotactic factor 2- (ALECT2) associated amyloid comprise ~91% of the cases diagnosed in the US [3]. Almost all patients with systemic amyloidosis present with either cardiac or renal symptoms, but numerous other organs and tissues, including liver, spleen, and lung are often involved.

Anatomic imaging can reveal structural sequelae associated with amyloidosis, notably in the heart using echocardiography or cardiac magnetic resonance (CMR) imaging [4-5]; however, these techniques are not generally amyloid specific. Nuclear imaging can be used to specifically detect amyloid, and agents such as $^{99m}$Tc-pyrophosphate (PyP), in the US, and $^{123}$I-serum amyloid P component (SAP) or $^{99m}$Tc-3,3-diphosphono-1,2-propanodicarboxylic acid (DPD), in Europe, are in routine use. However, $^{123}$I-SAP cannot detect cardiac amyloid, and the bone-seeking agents $^{99m}$Tc-PyP and $^{99m}$Tc-DPD preferentially detect ATTR-associated cardiac amyloid but not AL [6]. Agents developed for detecting Aβ amyloid in patients with Alzheimer's disease also image systemic amyloid deposits in experimental studies, albeit with limitations [7-8].

Here we present data from a first-in-human study of a novel amyloidophilic peptide radiotracer, designated $^{124}$I-p5+14 (also known as AT-01), for positron emission/x-ray computed tomographic (PET/CT) detection of diverse forms of systemic amyloidosis. The peptide binds via electrostatic interactions to electronegative glycosaminoglycans (GAGs) and protein fibrils, two ubiquitous components of amyloid deposits [9]. In preclinical studies, radioiodinated p5+14 imaged systemic amyloid in a murine model of serum amyloid protein A (AA) associated amyloidosis and was shown to specifically co-localize with Congo red-positive deposits in all tissues [10]. Additionally, the peptide was

shown by use of immunohistochemistry to bind many diverse forms of human amyloid, regardless of the precursor protein from which the fibrils were formed [9].

The current report describes kinetic biodistribution data obtained from the first three patients with systemic AL amyloidosis who completed an open label Phase 1/2 evaluation (NCT03678259) of $^{124}$I-p5+14 using PET/CT imaging performed at a single site. The overall study objective was to assess the ability of the radiotracer to detect systemic amyloid. In total, 57 subjects completed the study. Following evaluation of futility in the target population, five healthy volunteers were added to the protocol by amendment. The study design required estimation of dosimetry in three initial patients with AL amyloidosis, a primary endpoint, before increasing the injected dose to 74 MBq in subsequent participants. Serial PET/CT data were acquired in the initial three patients, and the organ specific dosimetry and pharmacokinetic parameters were evaluated. Visual evaluation of the PET/CT images was compared to the known or anticipated distribution of amyloid in the patients, obtained from the medical record to assess sensitivity. This analysis will be performed on all participants. Herein we describe the dosimetry analysis and pharmacokinetic parameters obtained from evaluation of the first three patients who completed the study .

## Methods

### Patients

The first three patients, from a total of 57, enrolled in the study were diagnosed with systemic AL amyloidosis (Table 1). Informed consent was obtained from all individual participants included in the study. The patients were evaluated to determine the time-dependent tissue biodistribution and to assess dosimetry of $^{124}$I-p5+14 injection. The time from diagnosis was 4 y (P02 and P03) and 11 y (P01). Patient P01 had elevated creatinine, glucose, N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and reduced estimated glomerular filtration rate (eGFR) (Table 1). Patient P01 had had a heart transplant due to amyloidosis in 2008, and patients P01 – P03 had undergone autologous stem cell transplantation in 2009, 2015 and 2015, respectively. The PET/CT image data from the first healthy subject enrolled in the trial (a male aged 68, enrollee number 43) is included to demonstrate the normal physiological distribution of radioactivity associated with the infusion of $^{124}$I-p5+14.

### Peptide, radiolabeling and characterization of iodination

Peptide p5+14 was synthesized under cGMP conditions by AmbioPharm Inc. (North Augusta, SC) and supplied as a lyophilized powder (3 mg per vial) and stored at -20°C. Iodine-124 was supplied by 3D Imaging (Little Rock, AR 72205; DMF# 025853). Peptide p5+14 was radioiodinated using soluble Iodogen as an oxidant, in the UT Radiochemistry Facility, and used within 5 h of synthesis (Supplementary Methods and Supplementary Fig. 1).

Preclinical evaluation of the iodination state of p5+14 was performed with iodine-127 using ultraviolet photodissociation coupled mass spectrometry (Supplementary Methods) [11].

### Study design and oversight

The ongoing Phase 1/2 trial is a single-site, open-label, study to assess the dosimetry, safety and biodistribution of a single IV bolus injection of $^{124}$I-labeled peptide p5+14. The initial three patients, reported here, were required to have a confirmed diagnosis of AL amyloidosis, with at least two organs

clinically involved. Images from one healthy subject (patient P43), recruited later in the trial (in accordance with FDA guidance) have been included for normal physiological distribution.

Initially, three patients with biopsy proven AL amyloidosis, where the clinically suspected distribution of amyloid was known prior to imaging, were recruited for time-dependent biodistribution studies. On day one, patients were consented for the imaging study followed by a routine physical examination and venous phlebotomy. The patients began a seven-day course of 130 mg potassium iodide per day (iOSAT, ANBEX INC., Livingston, NJ) to protect the thyroid.

On day two, subjects received 650 mg acetaminophen and 25 mg diphenhydramine (or alternative). Thirty minutes thereafter, a 30-mL dose of $^{124}$I-p5+14 was administered IV over 10 min followed by a 30 mL saline flush. Vital signs were monitored before, during, and for 50 minutes after the infusion. A radiation dose escalation was performed where the first subject P01 received 11.5 MBq (0.84 mg of peptide), and patients P02 and P03 received 38.9 (1.06 mg of peptide) and 38.1 MBq (0.95 mg of peptide), respectively (Tables 1 and 2). Safety follow-up was performed by telephone interview at days 9 and 28 of the protocol. The healthy subject, recruited as patient P43 in the study, received 74 MBq (1.4 mg of peptide) of I-124.

The study protocol was approved by the US Food and Drug Administration and performed under the auspices of Investigational New Product (IND) No. 132282. Approval was obtained from the Institutional Review Board (protocol #4386) at the UT Graduate School of Medicine (Knoxville, TN). All patients provided informed written consent for prescreening of medical records and for participation in the imaging study.

**Image acquisition**

Seven PET/CT images were acquired at approximately 25 min, 50 min, 2 h, 3 h, 6 h, 24 h, and 48 h post injection, for patients P01 – P03 using a Biograph 16 TruePoint (Siemens, Knoxville, TN) with a low dose CT (120 kVp, 50 effective mAs). Images were acquired from crown to thighs using 5 min bed positions. PET data were reconstructed using a 3DOSEM algorithm with attenuation weighting and prompt gamma correction yielding an image matrix of 168 x 168 and an image resolution of ~8 mm full

width half maximum. CT data were reconstructed using a medium smoothing kernel and a 4 mm reconstructions increment. The healthy subject was imaged once, at 5 h pi, with a Biograph mCT (Siemens) using a low dose CT acquisition.

**Biokinetic parameter evaluation**

Pharmacokinetic parameters were evaluated using data generated from venous blood samples that were drawn at intervals over 48 h pi (Supplementary Table S1). The radioactivity in 1 mL of whole blood was measured using a calibrated well-detector (Tennelec Nucleus, Oak Ridge, TN). The data expressed as counts per minute (cpm) were converted to Bq/cc by using a known standard of I-124. The heart, liver, spleen and kidneys, which represent the major amyloid-associated abdominothoracic organs in patients with AL were chosen specific evaluation of time-dependent biodistribution. Activity concentration (Bq/cc) measurements, for these four organs, were made using three independent axial (heart, liver, spleen) or coronal image slices (kidney) using 2D ROI placement performed by a single reviewer and the mean and SD values was used for analysis. The data were analyzed using a non-compartmental pharmacokinetic model (Phoenix® WinNonLin®, Version 8.3).

**Dosimetry and image analysis**

Image data from patients P01 - P03 were analyzed using the MIPAV software (National Institutes of Health) to obtain organ activity at each time point. Compartmental modeling was performed using the SAAM II software [12]. Time integrals of radioactivity were entered into the OLINDA/EXM software [13], using the adult male and female models, as appropriate, to generate a mean gender-averaged estimated radiation dose. Excretion was assumed to be principally via the urinary pathway, with some attributed to the gastrointestinal tract.

PET/CT images were evaluated using the XD General Oncology Review application in Mirada Medical DBx (Build 1.2.0.59) by a Nuclear Medicine physician blinded to the clinical assessment of amyloid distribution. Maximum intensity projections (MIPs) were prepared using RadiAnt DICOM viewer (ver. 2020.2.2, Medixant) or Inveon Research Workplace (IRW) software (Ed. 4.2 [4.2.0.15],

Siemens Preclinical Solutions). Organ retention of $^{124}$I-p5+14 was quantified from PET/CT images using region-of-interest (ROI) analysis employing a 2D freehand ROI and expressed as Bq/cc using IRW.

**Statistics**

The organ-specific radioactivity data are expressed as the mean and standard deviation (SD) of three independent ROIs. the three patients, unless otherwise noted. The gender average dosimetry was determined by calculating the mean and standard deviation for each organ and tissue of the three patients. The coeeficient of variation (COV) was expressed as (SD/mean)*100.

**Study oversight**

The study protocol was approved by the US Food and Drug Administration and performed under the auspices of Investigational New Product (IND) No. 132282. Approval was obtained from the Institutional Review Board (protocol #4386) at the UT Graduate School of Medicine (Knoxville, TN). All patients provided informed written consent for prescreening of medical records and for participation in the imaging study.

## Results

### Dosing and safety

There were no serious adverse events associated with infusion of the radiotracer in these subjects. Self-reported adverse events reported on day 9 or day 28 of the protocol, which also included a seven day series of oral 130 mg potassium iodide, were for P01, sore throat; P02, paresthesia, salivary duct inflammation (metallic taste), bruising at injection site and anxiety. Patient P03 reported no adverse events. All events were of mild severity and resolved without sequelae.

### Radioiodination of peptide p5+14

Oxidative labeling of p5+14 (at a peptide to $Na^{127}I$ molar ratio of 1000:1) yielded mostly mono iodotyrosine with trace amounts of di-iodotyrosine (Supplemental Figure 1A). However, using a molar ratio of 2000:1, as in our clinical study, only mono-iodotyrosine was detected in the product (Supplemental Figure 1B). The remaining ion abundance in the precursor region does not correspond to labeled p5+14 but is largely isobaric chemical noise.

For patient studies, peptide p5+14 was radiolabeled with $Na^{124}I$. Analysis of the intermediate (pre-formulation) indicated >99% radiopurity, >99% peptide identity, and 94.0 ± 0.7% peptide purity by reverse phase-HPLC. Each patient batch of $^{124}$I-p5+14 was assessed for reactivity with amyloid-like fibrils with the mean percent bound-peptide equal to 96.5 ± 0.8% (Table 2). All other release criteria; pyrogenicity, color, pH, peptide identity, peptide purity, and particulates were within accepted limits for each patient dose.

### Time-dependent biodistribution of $^{124}$I-p5+14

PET/CT images were acquired at approximately 25 min, 50 min, 2h, 3 h, 6 h, 24 h, and 48 h post injection. The distribution of $^{124}$I-p5+14 at 25 min post infusion was heterogeneous in the three patients (Fig 1.). In P01 the thyroid gland, spleen, kidneys and pancreas exhibited significant uptake of the radiotracer with weak but positive uptake in the heart and liver. At later time points the radioactivity in the liver progressively decreased but all other organs retained significant radioactivity up to 48 h pi. In Patient P02, at this initial time point, the parotid and salivary glands, liver, kidneys, heart and spleen were

positive at early time points (up to 50 min pi). Over the following 6 h the activity in all sites decreased, except that in the thyroid. left ventricular wall of the heart, kidneys, and lung. In addition, radioactivity in the lumen of the stomach became apparent at 50 min pi, peaked at 3 h pi and decreased thereafter. A similar time-dependent biodistribution was seen in patient P03; however, at 6 h pi only the heart, lung, stomach, parotid, salivary and thyroid glands were positive.

**Pharmacokinetics and radiation dosimetry**

The radiotracer cleared rapidly from the circulation, with estimated elimination $t_{1/2}$ values of 30.5 h, 16.6 h and 18.5 h for patients P01 – P03, respectively (Fig. 2A-C, Table 3). Dose normalized maximum radioactivity ($C_{max}$/Dose) trended lower than dose proportional in P01 compared to P02 and P03. Differences in parameter estimates between P02 and P03 were proportional to their individual bodyweights. Overall radioactivity exposure (AUCs) followed $C_{max}$ results. Whole blood radioactivity half-life was similar following a 37 MBq infusion in P02 and P03, it was approximately 2-fold longer in P01 who was administered 11.1 MBq and demonstrated rapid and intense uptake of the radiotracer in the spleen.

Changes in radioactivity concentration were assessed in the heart, liver, kidney, and spleen of each patient following ROI analysis of PET/CT images (Fig. 2D). Activity concentration (Bq/cc) measurements were performed using three independent axial (heart, liver, spleen) or coronal image slices (kidney) using 2D ROI placement performed by a single reviewer. The mean and SD were plotted (Fig. 2D). With the exception of the spleen, organ-specific $t_{1/2}$ values could not be determined. Activity in the heart (P01 - P03), liver (P01 and P02) and kidney (P01 and P02) increased or remained stable up to 24 h pi (Fig. 2D). The rapid accumulation of radiotracer in the spleen of P01 was followed by a biexponential loss of radioactivity from 2 h- 48 h pi.

Non compartmental analyses indicated that for the heart, the dose normalized (DN) $C_{max}$ ($C_{max}$/Dose) was similar among subjects as were $C_{max}$ tissue:blood (T:B) ratios; however, $AUC_{last}$/Dose and $AUC_{last}$ T:B ratios for P01 (22.1) and P02 (14.5) were notably higher than P03 (8.1) (Supplementary

table S2). Similar observations we made for the liver where the $AUC_{last}$ T:B ratio for P01 (17.7) was more than two-fold higher than P02 (7.4) and P03 (4.3) (Supplementary table S3). This trend was more significant in the kidney where he $AUC_{last}$ T:B ratio for P01 (52.1) was greater than P02 (12.9) and P03 (5.2) (Supplementary table S4). In the spleen the $AUC_{last}$ was greater for patient P01, where rapid accumulation of the $^{124}$I-p5+14 was observed, resulting in $C_{max}$ T:B and $AUC_{last}$ T:B ratios that were an order of magnitude greater in P01 as compared to the other patients (Supplementary table S5)

Time-activity data in P01-P03 were used to determine organ-specific and whole body dosimetry estimates for each patient (Table 4; these data for individual patients are shown in Supplementary table S6). The highest radiation dose was received by the urinary bladder wall with a gender-averaged estimated value of 0.94 ± 0.25 mSv/MBq. The gender-averaged, whole-body, effective radiation dose for $^{124}$I-p5+14 in these three patients was estimated to be 0.24 ± 0.02 mSv/MBq.

**Tissue biodistribution of $^{124}$I-p5+14 and comparison with clinical manifestation**

Organ-specific binding of $^{124}$I-p5+14 to amyloid was assessed by visual analysis of PET/CT images acquired at 6 h pi (Fig. 3 and Table 5) – a time point where significant washout had occurred from organs deemed clinically amyloid-free (notably the kidneys of P03). At this time-point, peptide uptake in P01 was observed in kidneys, pancreas, spleen, and heart (Fig. 3A – 3C). In contrast, the clinical record indicated amyloid present only in the heart and lung of this subject (Table 5). Cardiac and intestinal amyloidosis were the main clinical features for P02; however, radiotracer uptake was detected in the left and right ventricular walls of the heart, as well as the lung, kidneys, and liver (Fig. 3A – 3C). Uptake was also noted in the liver, muscle, joints, pancreas and lymph nodes (Table 5). Cardiac and pulmonary amyloid was noted clinically for P03 and uptake in these anatomic sites was observed in the $^{124}$I-p5+14 PET/CT images (Fig. 3A – 3C). The latter also suggested possible hepatic uptake (Table 5). For all three patients, 4/5 clinically involved organs were imaged and amyloid in the heart was detected in all three. An additional six organs, not suspected of containing amyloid, were deemed positive by PET/CT imaging (Table 5).

In contrast to the patients with amyloidosis, activity was only observed in PET/CT images of the healthy at 5 h pi, in the parotid, salivary and thyroid glands, saliva, stomach lumen and urine in the ureters and bladder, consistent with the biodistribution of free radioiodide; however, specific binding of the radiotracer to amyloid in these sites, or accumulation of radiolabeled N-terminal peptide fragments (metabolites) cannot be excluded. No activity was observed in the heart, kidneys, spleen, pancreas, liver or lung (Fig. 3A).

**Discussion**

The systemic amyloidoses are a heterogeneous group of diseases with complex and variable clinical presentations making rapid and accurate diagnosis of the disease challenging. At present, two radiotracers, $^{123}$I-serum amyloid P component (SAP) and technetium-99m-labeled bone-seeking agents (e.g. $^{99m}$Tc-PyP), are used clinically for the detection of systemic and cardiac ATTR amyloidosis, respectively. However, $^{123}$I-SAP is used only in the UK and Netherlands and is incapable of detecting cardiac amyloidosis of any type using the short-lived iodine-123 radionuclide and imaging at 24 h pi [14]. Bone-seeking agents, used for detecting ATTR cardiac amyloidosis, likely bind to the microcalcifications in these deposits, rather than the proteinaceous amyloid components which may complicate quantitative interpretation of organ-specific amyloid load [15]. Moreover, the bone seeking agents, used specifically for the detection of cardiac ATTR are not exclusively specific for ATTR amyloidosis [16]. Other limitations of bone seeking agents are the inability to image certain ATTR variants, and the requirement to rule out AL prior to use. Experimental use of the radiofluorinated Aβ amyloid imaging agents, e.g. $^{18}$F-florbetapir, in patients with systemic amyloidosis has shown potential for imaging amyloid in the myocardium as well as other anatomic sites [17]. However, the liver, as the site of catabolism, cannot be imaged reliably, and clinically-evident renal amyloidosis is underestimated using $^{18}$F-florbetapir [7]. At present, there are no FDA-approved agents for detecting systemic amyloidosis.

In the current study, we present the first in human evaluation of $^{124}$I-p5+14 as a radiotracer for detecting amyloid in patients, specifically characterizing the kinetic distribution of the radiotracer over 48 h pi. In this initial cohort of three patients with AL amyloidosis. The blood elimination $t_{1/2}$ was similar in two patients, but was extended in patient P01, who also demonstrated rapid uptake in the spleen, an organ often associated with clinically unappreciated amyloid deposition in patients with AL-associated amyloidosis. This may indicate that tissue amyloid can serve as a significant third PK compartment that may be in equilibrium with the central compartment, thus providing a reservoir of peptide that reenters the blood and extends the apparent $t_{1/2}$; However, this phenomenon did not increase the exposure in patient P01 ($AUC_{last}$/Dose = 131 hr*Bq*mL$^{-1}$*MBq$^{-1}$) relative to the other patients. Such a mechanism

needs to be validated further in a cohort of AL amyloid patients with diverse abdominothoracic amyloid loads. Interestingly the $AUC_{last}$ T:B ratios for the kidney and spleen correlated with the visual accumulation of radiotracer in these organs, where P01>P02>P03. This was less evident for the heart and liver data. The efficiency of renal clearance of the peptide from the central compartment, as well as organ-specific amyloid load will impact interpretation of these data.

Visual analysis of PET/CT images indicated that $^{124}$I-p5+14 partitioned rapidly (within 25 min pi) into organs in a patient-dependent fashion, suggesting specific retention in amyloid deposits, notably in the heart, kidneys, pancreas and spleen and the thyroid gland (in P01). Patients P02 and P03 exhibited very little splenic and thyroid gland uptake at the early time point, but instead the liver was readily visible. In P03 the radioactivity in the liver and kidneys decreased rapidly as the peptide was catabolized such that at 2 h pi, the urinary bladder was visible as was the gastric lumen. The latter indicative of renal dehalogenation of the catabolized peptide and redistribution of the free radioiodide to the stomach, and parotid, salivary and thyroid glands. These sites are known to scavenge free radioiodide and correlate with the physiological distribution of radioactivity seen in exemplary images of a healthy subject imaged with $^{124}$I-p5+14 (Fig. 3A) [18]. Rapid dehalogenation of unbound radioiodinated p5+14 related peptides has been previously reported in healthy, disease-free mice [19].

As a result of the relatively rapid dehalogenation of catabolized $^{124}$I-p5+14, evidenced principally by the appearance of radioactivity in the stomach lumen as early as 50 min pi, we posit that renal retention of radioactivity seen in images at greater than 3 h pi is indicative of radiotracer bound to extracellular amyloid. It has been demonstrated in preclinical studies that the amyloid bound half-life of radioiodinated p5+14 in organs with amyloid is much longer than that in healthy amyloid-free mice (Molecules paper). In support of this hypothesis, patient P03 exhibited no evidence of renal radioactivity after 2 h pi (Fig. 2), whereas both patients P01 and P02 exhibited visually positive focal renal retention of radiotracer.

Accumulation of $^{124}$I-p5+14 in abdominothoracic organs that are commonly involved with AL amyloidosis was rapid, notably in the spleen, kidneys, pancreas and potentially the thyroid of P01.

Retention of radioactivity persisted visually in PET/CT images in these anatomic sites for at least 48 h pi. The salivary, parotid and thyroid glands were visualized in all subjects, albeit to varying degrees, consistent with the physiologic distribution of liberated $^{124}$I. However, amyloid-associated uptake in these anatomic sites, notably the thyroid in P01, cannot be excluded as $^{18}$F-florbetapir, which is less susceptible to the activity of dehalogenases, also accumulates in these glands in patients with AL amyloidosis [17].

The major limitation of this study with respect to estimating early kinetic distribution of the radiotracer is that each dose was administered as a slow infusion over 10 min, rather than a bolus administration. Additionally, the first imaging time point was 25 mins pi; therefore, the rapid distribution phase of the kinetics is not available for analysis. As anticipated, the heterogeneous organ distribution and varied amyloid load in patients with AL contributed to the heterogeneity of the results, in such a way as to make each analysis relatively patient-specific. Thus, the gender-averaged dosimetry estimates and organ-specific time dependent-biodistribution data serve as a general guidance for this population.

These studies indicate that PET/CT imaging of $^{124}$I-p5+14 is safe with acceptable radiation absorbed dose exposure. The radioiodine is cleared rapidly from the patient unless bound to amyloid deposits. Moreover, $^{124}$I p5+14 was retained in clinically anticipated amyloid deposits in multiple organ systems with additional uptake of radiotracer in organs not clinically appreciated but consistent with the distribution of amyloid in this patient population , including the heart, kidney and spleen. Given these promising data, we anticipate that $^{124}$I-p5+14 imaging could provide a facile, non-invasive method for detecting whole body amyloid load in patients with systemic amyloidosis and thereby accelerate accurate diagnosis of this disorder.

**Figure Legends:**

**Figure 1.**

Time-dependent biodistribution of radioactivity following IV infusion of $^{124}$I-p5+14 in patients with AL amyloidosis. Maximum intensity projections (MIPs) for P01 (image window level (WL) = 1670 and window width (WW) = 370); P02 (where WL = 4150 and WW = 10290), and; P03 (where WL = 2930 and WW = 7700). Images were prepared using RadiAnt DICOM viewer (ver. 2020.2.2).

**Figure 2.**

Blood and organ time-dependent biodistribution of radioactivity in patients P01 – P03. Whole blood clearance of radioactivity, assessed as counts per minute per unit volume (cpm/cc) for P01 (A), P02 (B), and P03 (C). (D) Time activity curves of radioactivity concentration (Bq/cc divided by injected dose [Bq/cc] for major abdominothoracic organs for P01 (white), P02 (gray) and P03 (back). Organ data represent the mean ± SD of three independent ROIs for each time point.

**Figure 3.**

Biodistribution of $^{124}$I-p5+14 in patients (P01-P03) with AL amyloidosis and an exemplar healthy subject (H1). (A) Maximum intensity projections for P01 – P03 (acquired at 6 h pi) showing heterogeneous uptake of radiotracer in amyloid-laden organs, including the heart, spleen, pancreas, lung, and kidneys. Image data from an amyloid-free, healthy subject (H1; acquired at 5 h pi) is presented to show the physiological distribution or radioactivity. Coronal and axial PET/CT data showing cardiac uptake of radiotracer in the heart (B) and kidneys or spleen (C) of patients P01 – P03. Coronal and axial PET/CT Image intensity scales are as shown in A. For B and C, lower and upper image windows of 400 Bq/cc and 1e4 Bq/cc were used except B (P01) which was windowed at 400 Bq/cc – 4000 Bq/cc to show cardiac uptake..

**Author Contributions**: JSW, EBM, ACS, BW, SJK and RRJ designed the study. AE, DP, SH, TRL, MS RHL and JSW performed data analyses. ACS, BW, TRL EBM, ADW and SJK performed the studies and acquired the data. JSW, AE, SJK, RRJ and EBM wrote and edited the manuscript.

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**Conflicts of Interest Statement:** EBM, TR, AS and SJK are shareholders, and JSW is interim CSO, cofounder and shareholder of Attralus Inc which licensed patents related to $^{124}$I-p5+14 (AT-01). AE provides contracted drug development consulting support for Attralus Inc through Certara. JSW and SJK are inventors and have patent rights in peptide p5+14 for imaging amyloid.

**Ethical approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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**Tables**

**Table 1**. Demographics and Clinical Data

| Patient | Amyloid type | Sex | Age (y) | Diagnosis date | Creatinine (mg/dL) | eGFR (mL/min/1.73) | NTproBNP (pg/mL) | Glucose (mg/dL) |
|---|---|---|---|---|---|---|---|---|
| P01 | ALλ | F | 67 | 2008 | 1.2 | 47 | 476 | 241 |
| P02 | ALκ | F | 67 | 2015 | 1.0 | 58 | 114 | 79 |
| P03 | ALκ | M | 71 | 2015 | 0.9 | 86 | 115 | 77 |

**Table 2**. Summary of $^{124}$I-p5+14 Dose Characteristics

| Patient | Injected dose (MBq) | Injected peptide (mg) | Radiopurity intermediate (%) | Bioactivity (%) |
|---|---|---|---|---|
| P01 | 11.5 | 0.84 | >99 | 95.8 |
| P02 | 38.9 | 1.06 | >99 | 96.5 |
| P03 | 38.1 | 0.95 | >99 | 97.3 |

**Table 3**. Summary of noncompartmental blood PK analysis

| Subject | $C_{max}$ (Bq*mL$^{-1}$) | $C_{max}$ / Dose (Bq*mL$^{-1}$*MBq$^{-1}$) | t ½ (hr) | $AUC_{last}$ (hr*Bq*mL$^{-1}$) | $AUC_{last}$ / Dose (hr*Bq*mL$^{-1}$*MBq$^{-1}$) | $AUC_{inf}$ (hr*Bq/mL) |
|---|---|---|---|---|---|---|
| P01 | 126 | 11.0 | 30.5 | 1510 | 131 | 2300 |
| P02 | 968 | 24.9 | 16.6 | 9540 | 245 | 11000 |
| P03 | 654 | 17.2 | 18.5 | 6580 | 173 | 7940 |
| **Mean** | 583 | 17.7 | 21.9 | 5880 | 183 | 7100 |
| **SD** | 426 | 7.0 | 7.6 | 4060 | 57.7 | 4430 |

**Table 4**. Summary of $^{124}$I-p5+14 dosimetry estimates

| | **Average** | | **SD** | | **COV** |
|---|---|---|---|---|---|
| | Gender Averaged | | Gender Averaged | | |
| | Estimated Dose | | Estimated Dose | | |
| | mSv/MBq | rem/mCi | mSv/MBq | rem/mCi | |
| Adrenals | 2.32E-01 | 8.60E-01 | 2.41E-02 | 8.92E-02 | 10.4 |
| Brain | 1.63E-01 | 6.02E-01 | 6.11E-02 | 2.26E-01 | 37.6 |
| Breasts | 1.65E-01 | 6.12E-01 | 6.00E-02 | 2.22E-01 | 36.3 |
| Esophagus | 1.81E-01 | 6.70E-01 | 5.72E-02 | 2.12E-01 | 31.6 |
| Eyes | 1.62E-01 | 5.99E-01 | 6.07E-02 | 2.24E-01 | 37.4 |
| Gallbladder Wall | 2.11E-01 | 7.81E-01 | 6.02E-02 | 2.23E-01 | 28.5 |
| Left colon | 2.97E-01 | 1.10E+00 | 1.23E-01 | 4.56E-01 | 41.5 |
| Small Intestine | 2.23E-01 | 8.24E-01 | 7.18E-02 | 2.66E-01 | 32.2 |
| Stomach Wall | 2.06E-01 | 7.61E-01 | 6.90E-02 | 2.55E-01 | 33.6 |
| Right colon | 2.60E-01 | 9.63E-01 | 1.03E-01 | 3.82E-01 | 39.7 |
| Rectum | 3.50E-01 | 1.30E+00 | 1.03E-01 | 3.83E-01 | 29.5 |
| Heart Wall | 2.03E-01 | 7.51E-01 | 7.19E-02 | 2.66E-01 | 35.4 |
| Kidneys | 3.85E-01 | 1.42E+00 | 3.03E-01 | 1.12E+00 | 78.7 |
| Liver | 1.65E-01 | 6.11E-01 | 6.24E-02 | 2.31E-01 | 37.8 |
| Lungs | 1.81E-01 | 6.71E-01 | 6.35E-02 | 2.35E-01 | 35.0 |
| Ovaries | 2.75E-01 | 1.02E+00 | 7.02E-02 | 2.60E-01 | 25.5 |
| Pancreas | 2.20E-01 | 8.14E-01 | 7.01E-02 | 2.60E-01 | 31.9 |
| Prostate | 2.47E-01 | 9.13E-01 | 4.85E-02 | 1.80E-01 | 19.7 |
| Salivary Glands | 4.24E-01 | 1.57E+00 | 4.42E-01 | 1.63E+00 | 104.2 |
| Red Marrow | 1.78E-01 | 6.57E-01 | 5.69E-02 | 2.11E-01 | 32.0 |
| Osteogenic Cells | 1.86E-01 | 6.87E-01 | 6.47E-02 | 2.40E-01 | 34.9 |
| Spleen | 2.10E-01 | 7.78E-01 | 5.23E-02 | 1.93E-01 | 24.9 |
| Testes | 1.72E-01 | 6.36E-01 | 5.39E-02 | 2.00E-01 | 31.4 |
| Thymus | 1.91E-01 | 7.05E-01 | 6.65E-02 | 2.46E-01 | 34.9 |
| Thyroid | 6.02E-01 | 2.23E+00 | 5.10E-01 | 1.89E+00 | 84.7 |
| Urinary Bladder Wall | 9.35E-01 | 3.46E+00 | 2.48E-01 | 9.16E-01 | 26.5 |
| Uterus | 3.25E-01 | 1.20E+00 | 4.79E-02 | 1.77E-01 | 14.7 |
| Total Body | 1.87E-01 | 6.92E-01 | 5.90E-02 | 2.18E-01 | 31.5 |
| **Effective Dose** | 2.35E-01 | 8.68E-01 | 2.24E-02 | 8.28E-02 | 9.5 |

**Table 5**. Summary of clinical manifestation of amyloid and $^{124}$I-p5+14 organ uptake based on visual read

| | P01 | | P02 | | P03 | |
|---|---|---|---|---|---|---|
| **Tissue** | **Clinical*** | **Imaging** | **Clinical** | **Imaging** | **Clinical** | **Imaging** |
| Tongue | 1 | 0 | 0 | 1 | 0 | 1 |
| Skin | 0 | 0 | 0 | 0 | 0 | 0 |
| Muscle | 0 | 0 | 0 | 1 | 0 | 0 |
| Joints | 0 | 0 | 0 | 1 | 0 | 0 |
| Heart | 2[1] | 2 | 2[1] | 2 | 2[2] | 2 |
| Lung | 2[1] | 0 | 0 | 2 | 2[3] | 2 |
| Liver | 0 | 0 | 0 | 2 | 0 | 1 |
| Spleen | 0 | 2 | 0 | 1 | 0 | 0 |
| Kidneys | 0 | 2 | 0 | 2 | 0 | 0 |
| Lymph nodes | 0 | 0 | 0 | 1 | 0 | 0 |
| Adrenal gland | 0 | 1 | 0 | 0 | 0 | 0 |
| Pancreas | 0 | 2 | 0 | 1 | 0 | 0 |

*0, negative; 1, suspected or patient reported; 2, positive

[1]Based on organ biopsy findings of Congo red-positive amyloid deposits. [2]Cardiac MR showed left ventricular thickening and moderate patchy delayed enhancement of the basal and mid septum, all strongly suggestive of cardiac amyloidosis. Echocardiography revealed increased left ventricular filling pressures, abnormal longitudinal peak systolic strain, felt consistent with cardiac amyloidosis. [3]Chest CT revealed bilateral peripheral interlobular septal thickening and peripheral basilar reticular ground-glass which may be indicative of early pulmonary amyloidosis.