A Pilot Study to evaluate 18F Florbetapir binding to cardiac amyloid in patients undergoing chemotherapy.

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Abbreviations:
PET-CT – Positron emission tomography and computerized tomography
MRI – Magnetic resonance imaging
H&P- history & physical
CBC- complete blood count
β-HCG- Beta human chorionic gonadotropin
AL amyloidosis- immunoglobulin light chain amyloidosis
ATTR- transthyretin amyloidosis
AA – serum amyloid protein A amyloidosis
LV – left ventricle
RV – right ventricle
CMR – cardiac magnetic resonance imaging
LGE – late gadolinium enhancement
HIPAA – health insurance portability and accountability act
EOS – end of synthesis
PI – principle investigator
CMP – comprehensive metabolic panel.

Schema

A Pilot study to evaluate ^{18}F Florbetapir binding to cardiac amyloid in patients undergoing chemotherapy.

PRIMARY OBJECTIVES:

1. Determine the relative difference in quantitative uptake of ^{18}F Florbetapir in patients with cardiac amyloidosis at the onset of chemotherapy and after 6 months of chemotherapy.

SECONDARY OBJECTIVES:

1. Evaluate correlation between 6-minute walk test and changes in quantitative uptake of ^{18}F Florbetapir in patients with cardiac amyloidosis at the onset of chemotherapy and after 6 months of chemotherapy
2. Evaluate correlation of hematologic treatment response and changes in quantitative uptake of ^{18}F Florbetapir in patients with cardiac amyloidosis at the onset of chemotherapy and after 6 months of chemotherapy
3. Evaluate correlation of cardiac response and changes in quantitative uptake of ^{18}F Florbetapir in patients with cardiac amyloidosis at the onset of chemotherapy and after 6 months of chemotherapy
4. Evaluate correlation of cardiac and hematologic response to results of patient outcome surveys at 6 and 12 months post onset of chemotherapy.

STUDY DESIGN:

This is a pilot study designed to assess for relative change in cardiac ^{18}F Florbetapir uptake in patients with AL cardiac amyloidosis after 6 months of appropriate chemotherapy.

PATIENT ELIGIBILITY CRITERIA:

1. Patients aged > 18 years
2. Patient must provide informed consent to participate in the study protocol.
3. All patients must have clinical diagnosis of cardiac AL amyloidosis (typical echocardiographic or cardiac MRI findings, NT-ProBNP levels above 332 pg/mL, cardiac or extra cardiac histological evidence of light chain amyloidosis).
4. Planned plasma cell-directed chemotherapy.
5. A negative pregnancy test will be required for all women of child bearing potential, breast feeding is not permitted.
6. Patients must be able to undergo PET-CT imaging
7. Patients must be able to complete 6-minute walk test

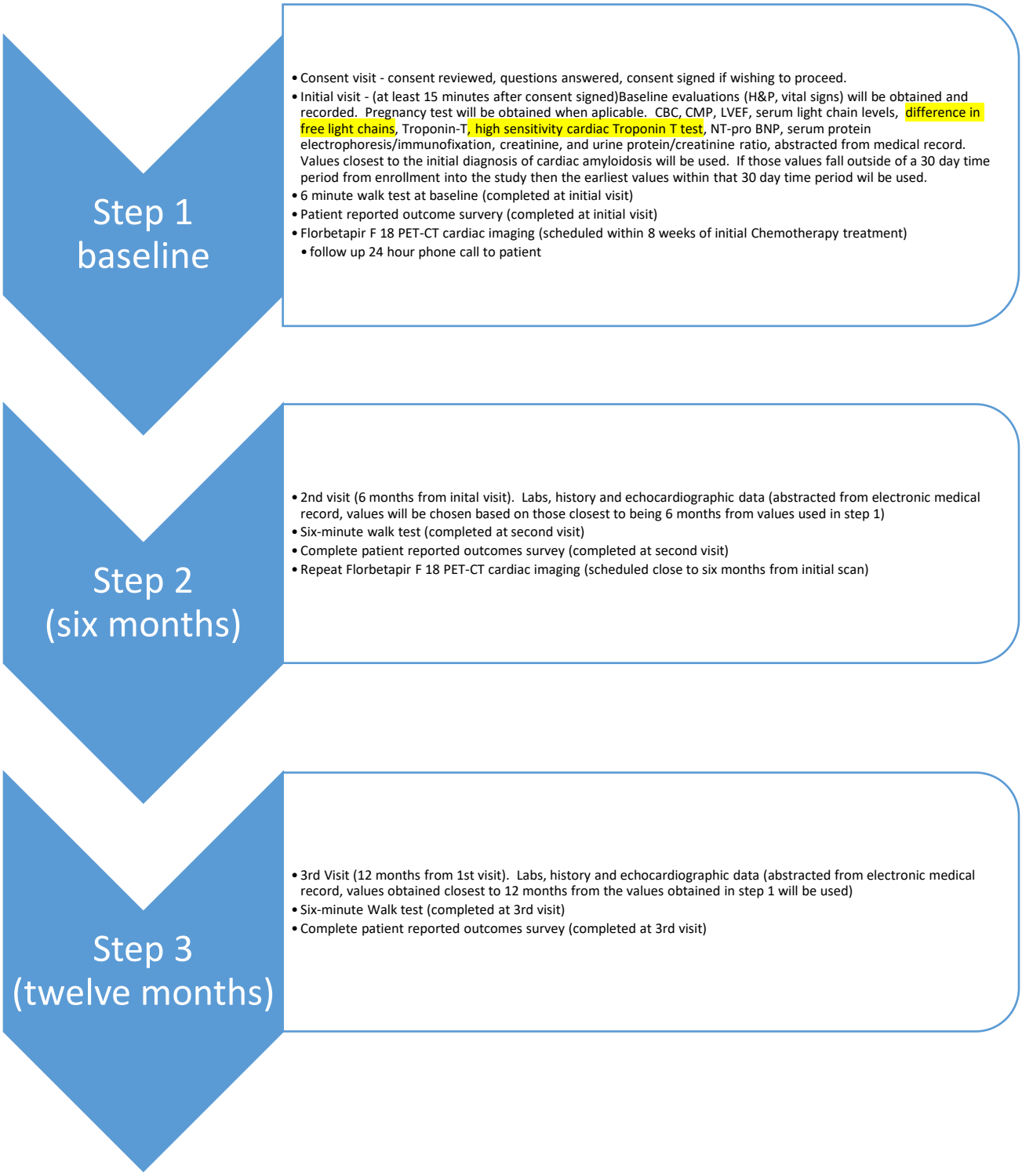

Step 1
baseline
• Consent visit - consent reviewed, questions answered, consent signed if wishing to proceed.
• Initial visit - (at least 15 minutes after consent signed)Baseline evaluations (H&P, vital signs) will be obtained and recorded. Pregnancy test will be obtained when aplicable. CBC, CMP, LVEF, serum light chain levels, difference in free light chains, Troponin-T, high sensitivity cardiac Troponin T test, NT-pro BNP, serum protein electrophoresis/immunofixation, creatinine, and urine protein/creatinine ratio, abstracted from medical record. Values closest to the initial diagnosis of cardiac amyloidosis will be used. If those values fall outside of a 30 day time period from enrollment into the study then the earliest values within that 30 day time period wil be used.
• 6 minute walk test at baseline (completed at initial visit)
• Patient reported outcome survery (completed at initial visit)
• Florbetapir F 18 PET-CT cardiac imaging (scheduled within 8 weeks of initial Chemotherapy treatment)
• follow up 24 hour phone call to patient
Step 2
(six months)
• 2nd visit (6 months from inital visit). Labs, history and echocardiographic data (abstracted from electronic medical record, values will be chosen based on those closest to being 6 months from values used in step 1)
• Six-minute walk test (completed at second visit)
• Complete patient reported outcomes survey (completed at second visit)
• Repeat Florbetapir F 18 PET-CT cardiac imaging (scheduled close to six months from initial scan)
Step 3
(twelve months)
• 3rd Visit (12 months from 1st visit). Labs, history and echocardiographic data (abstracted from electronic medical record, values obtained closest to 12 months from the values obtained in step 1 will be used)
• Six-minute Walk test (completed at 3rd visit)
• Complete patient reported outcomes survey (completed at 3rd visit)

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1. Introduction:

1.1 Amyloidosis:

The amyloidoses are a diverse group of protein misfolding diseases wherein proteins aggregate and form fibrillar deposits (amyloid) in tissues[1]. Examples of amyloid diseases include Alzheimer's disease, hereditary transthyretin-associated familial amyloid polyneuropathy, dialysis related amyloidosis, AA amyloidosis with chronic systemic inflammatory states and immunoglobulin-derived AL amyloidosis.

AL amyloidosis is a malignant plasma cell disease characterized by the formation of amyloid from immunoglobulin light chains produced by clonal plasma cells[1]. The amyloid deposits into vital organs such as the heart, liver, kidney, and nerves resulting in inexorable systemic decline in function and culminating in death. Of the 27 known proteins resulting in amyloidosis in humans, AL amyloidosis is the most common and the most rapidly fatal with a median survival of 6 months in advanced disease[2].

Clinically significant cardiac involvement is manifest in approximately one-third to one-half of all AL patients at the time of diagnosis[3]. The predominant presenting symptom is rapidly progressive heart failure with preserved ejection fraction. Unfortunately, this is also a major risk factor for mortality during the first year[4]. Kyle et. al showed that in 168 patients with systemic amyloidosis, those who presented with congestive heart failure had a median survival of 4 months[4]. In those patients presenting without heart failure, ultimately 40% would end up with cardiac involvement as a cause of their death.

1.2 Mechanism of cardiac dysfunction:

The exact mechanism of cardiac dysfunction secondary to light chain amyloid tissue disposition has not been entirely determined. The deposition of large amounts of fibrillary material can subvert the tissue architecture and consequently cause organ dysfunction[5]. It is also thought that there is a direct cytotoxic effect of amyloidogenic light chains which may play more of a role in organ dysfunction[6].

1.3 Goals of systemic therapy

The current paradigm of AL amyloid treatment is focused upon eradicating the underlying malignant plasma cell clone with cytotoxic agents. This produces a hematologic response by clearing circulating immunoglobulin light chains which would have eventually been deposited into amyloid. Treatments range from high dose chemotherapy with autologous stem cell transplantation in eligible patients, or anti-myeloma chemotherapy regimens such as melphalan/dexamethasone[7]. Newer anti-myeloma agents such as thalidomide,[8,9] lenalidomide,[10,11] pomalidomide,[12] and bortezomib[13] have excellent anti-plasma cell efficacy in amyloidosis but are also associated with organ and tissue toxicity making their use in clinical practice challenging, particularly in advanced AL disease[14]. Treatment of AL amyloidosis thus remains a frustrating journey for patients and their treating physicians, where clinically tenuous and frail patients with compromised vital organ function are treated with cytotoxic chemotherapy that often rapidly clears light chains from serum (i.e. hematologic response) but does not rapidly improve organ function. Many of these patients succumb to organ failure despite hematologic response. Thus, consideration of therapies that complement current cytotoxic anti-amyloid treatment by hastening organ response is required.

Fibril-directed treatment is an area of active research; there have recently been several clinical trials to look at agents such as doxycycline, Epigallocatechingallate (EGCG), CPHPC/anti-SAP and NEOD001 and their ability to augment organ response. To date, there has not been much success with cardiac imaging modalities with regards to determining cardiac response to therapy in patients who have shown hematologic response after anti-myeloma therapy. The most recent recommendations suggest that the accepted standard for cardiac response has primarily been a decrease in NT-proBNP level[15]. This puts less

emphasis on previous decrease in wall thickness measurements by echocardiogram. However, NT-proBNP levels may be elevated in patients with renal disease. In addition, certain anti-myeloma therapy (such as lenolidomide and bortezomib) cause an initial worsening in cardiac biomarkers and even cell death[14,16,17]. Martinez-Naharro et. al. were able to show a decrease in cardiac amyloid burden after hematologic response through cardiac MRI by looking at extracellular volume which served as an estimate of amyloid burden[18]. However, the use of extracellular volume measurement obtained by cardiac MRI requires contrast administration, something which precludes its use in patients with significant renal disease. Given that > 60% of systemic AL patients have renal involvement at the time of diagnosis, extracellular volume calculation by cardiac MRI might not be an option in these patients. Therefore, we need an imaging modality that is not hampered by the presence of kidney disease. In this setting it would be useful to establish a method to directly measure a change in amyloid deposition within the heart. PET/CT imaging with newly developed radiopharmaceuticals has the potential to fill this gap.

1.4 PET/CT imaging in cardiac amyloidosis.

PET/CT imaging has advantages over conventional nuclear imaging with improved spatial resolution and potential for quantitative measurements. There is currently emerging research to define the roles of newer radiopharmaceuticals to identify cardiac amyloidosis. Recent attention has been paid to using radiopharmaceuticals which have been developed for imaging Beta-amyloid in the brain. [^{18}F]-florbetapir (Amyvid) was approved by the FDA in 2012 for imaging of the brain to estimate Beta-amyloid neuritic plaque density in adult patients with cognitive impairment who are being evaluated for Alzheimer's Disease and other causes of cognitive decline. The approval of this radiopharmaceutical has opened the door to research in other organ systems with amyloid involvement. In a recent pilot study, [^{18}F]-florbetapir has been used to show cardiac uptake in fourteen patients with biopsy-proven cardiac amyloidosis compared to five control subjects[19]. Osborne et al. recently published a PET/CT protocol with streamlined calculations for assessing cardiac Amyloidosis using [^{18}F]-florbetapir[20]. In their study, they could adequately differentiate amyloid positive patients from healthy controls in a small cohort of 11 patients.

1.5 Study Rationale

Current research involving [^{18}F]-florbetapir in relationship to cardiac amyloid has taken advantage of the ability of the radiopharmaceutical to bind specifically to amyloid fibrils. It has previously been established that the degree of radiotracer uptake correlates with the density of Beta-amyloid plaque[21]. The ability to quantify this uptake makes [^{18}F]-florbetapir imaging a potential tool to evaluate cardiac response in patients undergoing therapy for AL amyloidosis. It would provide a much-needed tool to monitor cardiac response to chemotherapy to potentially adjust chemotherapy if response was not adequate. It could also provide a tool by which we would be able to evaluate new therapies designed to decrease the burden of formed amyloid fibrils within the heart.

2. Objectives:

2.1 Primary Objective:

1. Determine the relative difference in quantitative uptake of ^{18}F Florbetapir in patients with cardiac amyloidosis at the onset of chemotherapy and after 6 months of treatment.

2.2 Secondary Objectives:

1. Evaluate correlation between 6-minute walk test and changes in quantitative uptake of ^{18}F Florbetapir in patients with cardiac amyloidosis at the onset of chemotherapy and after 6 months of chemotherapy
2. Evaluate correlation of hematologic treatment response and changes in quantitative uptake of ^{18}F Florbetapir in patients with cardiac amyloidosis at the onset of chemotherapy and after 6 months of chemotherapy
3. Evaluate correlation of cardiac response and changes in quantitative uptake of ^{18}F Florbetapir in patients with cardiac amyloidosis at the onset of chemotherapy and after 6 months of chemotherapy
4. Evaluate correlation of cardiac and hematologic response to results of patient outcome surveys at 6 and 12 months post onset of chemotherapy.

3. Eligibility Criteria:

3.1 Patient Eligibility Criteria:

1. Patients aged > 18 years are eligible.
2. Patient must provide informed consent to participate in the study protocol.

3. All patients must have clinical diagnosis of cardiac AL amyloidosis (typical echocardiographic or cardiac MRI findings, NT-ProBNP levels above 332 pg/mL, cardiac or extra cardiac histological evidence of light chain amyloidosis).
4. Planned plasma cell-directed chemotherapy.
5. A negative pregnancy test will be required for all women of child bearing potential, breast feeding is not permitted.
6. Patients must be able to undergo PET-CT imaging
7. Patients must be able to complete 6-minute walk test

3.2 Exclusion Criteria:

1. Severe claustrophobia
2. Pregnancy
3. Allergy to F-18 florbetapir
4. Age < 18 years.

4. **Registration Procedure:**

- All source documents that support eligibility including a signed informed consent/HIPAA and signed eligibility checklist, should be available for review and eligibility verification.
- At the point of registration, the research nurse or data manager will register the patient in the electronic database, including demographic, consent and on-study information. The patient will be assigned a unique sequence number for the study.

5. **Diagnostic Plan:**

This is a pilot study designed to assess the relative change in Florbetapir F 18 cardiac uptake on PET-CT imaging at baseline and after 6 months from initiation of active anti-myeloma therapy.

Enrollment Discussion Plan (Script)

1. Patients identified with AL amyloidosis and cardiac involvement with plans for anti-myeloma therapy will be approached for consideration of enrollment in trial.

2. Patients who elect to speak with a trial representative will have their name and telephone number passed on to the research staff
3. Patients will be contacted by research staff to explain Florbetapir F 18 PET-CT imaging protocol, benefits and risks. Those wishing to participate will complete the informed consent.
4. Legally appointed representatives will not be used to enroll patients into the research study

Screening procedures:

Potential subjects will be referred to the study team by the treating Oncologist or study team doctors who are involved in clinical care of the subject for preliminary eligibility determination though review of medical record. Preliminary determination will include confirmation of Cardiac AL Amyloidosis and assess if it is appropriate for subject to receive 18F-florbetapir, and additional PET/CT scans.

Treating doctors will obtain permission to begin discussions about possible study participation with a member of the study team if preliminary eligibility determination is supportive of subject enrollment.

Research Study Groups:

Each study participant who is eligible and enrolls in the research study will have the same study procedures.

Summary of Study Procedures:

The study team will utilize available test results performed for clinical care during initial and follow up visits for this research study. Participation in this study will include 3-5 research related visits over 12 months of study participation; followed by 12 months medical records surveillance for patient outcomes.

Baseline Visit:

Prior to completion of any study procedures, subject will complete the consent signature process. Baseline procedures may be completed at two separate visits, depending on subject's chemotherapy initiation.

Initial Baseline visit:

- Vital signs including blood pressure, heart rate, respiratory rate, and pulse oximetry.
- Weight and Height.
- Medical History Review.
- Physical Examination.

- Confirmation of subjects plan to receive plasma cell-directed chemotherapy treatment.
- Pregnancy test (woman of child bearing potential only).
- Blood tests results will be collected from medical records, to eliminate the need to draw extra blood for the study. These include complete blood count, liver function tests, B-HCG, Troponin-T, high sensitivity cardiac Troponin T test, NT-pro BNP, serum protein electrophoresis/immunofixation, creatinine, urine protein/creatinine ratio serum free light chains, difference in free light chains.
- 6-minute hall walk
- Patient reported outcomes survey: This survey will provide the study with information about subject's ability to complete routine daily activities over the next 12 months.

Final Baseline Visit:

Includes the 18F Florbetapir PET/CT scan, to be completed within 8 weeks of starting chemotherapy treatment. It is likely that subject will need to return to Froedtert Hospital for completion of the PET/CT scan at a second baseline visit.

Florbetapir F 18 PET-CT imaging will take place at the radiology department of Froedtert Hospital. A peripheral intravenous catheter (IV) is placed for injection of the 18 F contrast. An initial, brief computed tomography (CT) scan will be completed to identify the basic heart structure. Followed by subject injection with approximately 10 mCi of Florbetapir F 18 (Amyvid), a standard dose; imaging is completed over 20 minutes images by the PET-CT scanner. Subject will be asked to lay on their back for this procedure. All imaging procedures will take less than 1 hour.

Follow-up Phone Call:

The study team will contact subject 24 hours after completing the PET/CT scan procedure to confirm subject has not had issues or problems.

Six Months following the start of Chemotherapy

- Vital signs including blood pressure, heart rate, respiratory rate, and pulse oximetry.
- Weight and Height.
- Physical Examination.
- Blood tests results will be collected from medical records, to eliminate the need to draw extra blood for the study.
- Echocardiogram completed for clinical care will be included in the study.
- 6-minute hall walk.
- Patient reported outcomes survey
- Repeat Florbetapir F 18 PET-CT imaging scans. This procedure can be scheduled at subject convenience but should be close to the 6-month mark from initial imaging procedure.

Follow-up Phone Call:

The study team will contact subject 24 hours after completing the PET/CT scan procedure to confirm subject has not had issues or problems.

Twelve Months following the start of Chemotherapy:

- Blood tests results will be collected from medical records, to eliminate the need to draw extra blood for the study.
- Echocardiogram completed for clinical care will be included in the study.
- 6-minute hall walk.
- Patient reported outcomes survey

This will conclude subject study participation.

Early Withdrawal from Study:

If subject decides to stop participating in this study (withdraw) or a Study Stopping Rule is met prior to the 12-month visit, the study team will request return to research clinic one more time to assess subject's health. This would be most important if subject were having issues that were thought to be related to Florbetapir F 18 PET-CT imaging.

5.2 Data Safety Monitoring Plan

This study will be reviewed by the Medical College of Wisconsin / Froedtert Hospital Institutional Review Board. (MCW/FMLH IRB). A summary of the MCW/FMLH IRB activities are as follows:

- Review the clinical trial for data integrity and safety
- Review all unexpected grade 3, and all grade 4, and 5 adverse events, as well as any others requiring expedited reporting as defined in this protocol. (Grades 4 & 5 events must be reported to the MCW/FMLH IRB within 5 calendar days of study staff's knowledge.) Review all MCW/FMLH IRB reports
- Submit a summary of any recommendations related to study conduct
- Terminate the study if deemed unsafe for patients

A copy of all MCW/FMLH IRB correspondence and membership roster will be maintained in the study research file and updated as membership changes. The committee will review reports from the study PI annually (or more frequently if needed) and provide recommendations on trial continuation, suspension or termination as necessary.

6. Drug Formulation and Procurement

6.1 Florbetapir F 18 Injection (Amyvid)

6.1.1 Drug description[22]

Amyvid is a radioactive diagnostic agent for Position Emission Tomography (PET) imaging. Amyvid contains florbetapir F 18, a molecular imaging agent that binds to B-amyloid aggregates, and is intended for use with PET imaging. Chemically, florbetapir F 18 is described as (E)-4-(2-(6-(2-(2-(2[^{18}F] fluoroethoxy)ethoxy)ethoxy)pyridine-3-yl)vinyl)-N-methylbenzamine. The molecular weight is 359 and the structural formula is shown in Figure 1:

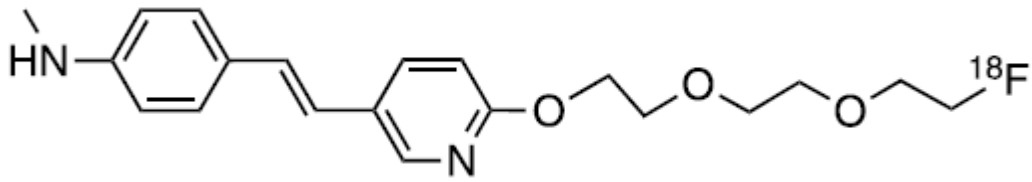


Figure 1: structure of Amyvid

Amyvid is a sterile, non-pyrogenic radioactive diagnostic agent for intravenous injection. The clear colorless solution is supplied ready to use and each milliliter contains 0.1 to 19 micrograms of florbetapir and 500-1900 MBq (1.35-51 mCi) florbetapir F18 at End of Synthesis (EOS), 4.5 mg sodium ascorbate USP and 0.1 mL dehydrated alcohol USP in 0.9% sodium chloride injection USP. The pH of the solution is between 5.5 and 7.5.

Amyvid is radiolabeled with [^{18}F] fluorine (F 18) that decays by positron (B+) emission to 0 18 and has a half-life of 109.77 minutes. The principal photons useful for diagnostic imaging are the coincident pair of 511 keV gamma photons, resulting from the interaction of the emitted positron with an electron. The principal radiation produced from decay of Fluorine 18 is shown in table 1[16].

Radiation	Energy Level (keV)	Abundance (%)
Positron	249.8	96.9
Gamma	511	193.5

Table 1: the principal radiation produced from decay of Fluorine[16].

6.1.2 Clinical pharmacology

Mechanism of Action

Florbetapir F 18 binds to B-amyloid plaques and the F 18 isotope produces a positron signal that is detected by a PET scanner. The binding of florbetapir F 18 to B-amyloid aggregates was demonstrated in postmortem human brain sections using auto radiographic methods, thioflavin S and traditional silver staining correlation studies as well as monoclonal antibody B-amyloid-specific correlation studies. Park et al. showed that florbetapir F 18 bound specifically to myocardial AL and ATTR deposits in humans using autopsy myocardial specimens of 20 subjects with documented cardiac amyloid and 10 controls[23].

6.1.3 Pharmacodynamics

Following intravenous injection, florbetapir F 18 diffuses through the system is taken up by the heart. Cardiac perfusion decreases the florbetapir F 18 content with differential retention of the drug in areas which contain B-amyloid aggregates compared with areas that lack the aggregates. Clinical studies have suggested that visual assessments are able to indicate differences in uptake between healthy controls and patients with cardiac amyloid involvement in as little as 10 minutes after injection[14].

6.1.4. Pharmacokinetics

Following the intravenous administration of 370 MBq (10mCi) of florbetapir F 18 to healthy volunteers, the drug was distributed throughout the body with less than 5% of the injected F 18 radioactivity present in the blood by 20 minutes following administration, and less than 2% present by 45 minutes after administration. The residual F 18 in circulation during the 30-90-minute imaging window as principally in the form of polar F 18 metabolites. Whole body scanning following the intravenous injection showed

accumulation of radioactivity in the liver within four minutes post-injection, followed by elimination of the radioactivity predominantly through the biliary/gastrointestinal tract with much lower radioactivity detected in the bladder. Essentially all radioactivity collected in the urine was present as polar metabolites of florbetapir F 18.

The FDA indicated application for florbetapir F 18 in imaging of the brain for amyloid plaque describes a protocol in which 370 MBq (10mCi) of florbetapir F-18 is administered as a single intravenous bolus in a total volume of 10 mL or less. 10 minute PET images are obtained 30-50 minutes after the intravenous injection. Our protocol uses the same bolus dose and only varies in the timing and focus of PET/CT imaging (timing of image acquisition). The use of a CT scan to calculate attenuation correction for reconstruction of Amyvid images will focus on cardiac imaging and not brain imaging.

6.1.5. Radiation Dosimetry

The estimated radiation absorbed doses for adults with intravenous injection of Amyvid are shown in table 2.

ORGAN/TISSUE	MEAN ABSORBED DOSE PER UNIT ADMINISTERED ACTIVITY(µGy/MBq)
Adrenal	14
Bone - Osteogenic Cells	28
Bone - Red Marrow	14
Brain	10
Breasts	6
Gallbladder Wall	143
GI[a] - Lower Large Intestine Wall	28
GI - Small Intestine	66
GI - Stomach Wall	12
GI - Upper Large Intestine Wall	74
Heart Wall	13
Kidneys	14
Liver	64
Lungs	9
Muscle	9
Ovaries	18
Pancreas	14
Skin	6
Spleen	9
Testes	7
Thymus	7
Thyroid	7
Urinary Bladder Wall	27
Uterus	16
Total Body	12
Effective Dose (µSv/MBq)[b]	19

[a] Gastrointestinal

[b] Assumed radiation weighting factor, w_r, (formerly defined as quality factor, Q) of 1 for conversion of absorbed dose (Gray or rads) to dose equivalent (Sieverts or rem) for F 18. To obtain radiation absorbed dose in rad/mCi from above table, multiply the dose in µGy/MBq by 0.0037, (e.g., 14 µGy/MBq x 0.0037 = 0.0518 rad/mCi)

Table 2: Estimated radiation absorbed dose, Amyvid (Florbetapir F 18 Injection)[16]

The effective dose resulting from a 370 MBq (10 mCi) dose of Amyvid is 7.0 mSv in an adult per exam. The use of a CT scan to calculate attenuation correction for reconstruction of Amyvid images (as done in PET/CT imaging) will add radiation exposure. The total radiation exposure from Amyvid administration and subsequent scan on a PET/CT scanner is estimated to be 9 mSv per exam.

6.1.5. Adverse reactions

In clinical studies, 555 patients were exposed to Amyvid. Amyvid caused no serious adverse reactions in the studies and the reported adverse reactions were predominantly

mild to moderate in severity. The adverse reactions reported in more than one subject within the studies are shown below in table 3.

Adverse Reactions	N (Percent of patients)
Headache	10 (1.8%)
Musculoskeletal pain	4 (0.7%)
Blood pressure increased[a]	4 (0.7%)
Nausea	4 (0.7%)
Fatigue	3 (0.5%)
Injection site reaction[b]	3 (0.5%)
Anxiety	2 (0.4%)
Back pain	2 (0.4%)
Claustrophobia	2 (0.4%)
Dizziness	2 (0.4%)
Feeling cold[c]	2 (0.4%)
Insomnia	2 (0.4%)
Neck pain	2 (0.4%)

[a] Includes the terms blood pressure increased and hypertension.
[b] Includes the terms injection site haemorrhage, injection site irritation, and injection site pain.
[c] Includes the terms feeling cold and chills.

Other adverse reactions occurred at lower frequencies and included infusion site rash, dysgeusia, pruritis, urticaria, and flushing.

Table 3: Adverse reactions reported in clinical trials (N=555 patients)[16]

6.2 How supplied/storage and handling

Amyvid is supplied in 10 mL, 30 mL, or 50 mL vials containing 10 mL, 10-30 mL, or 10-50 mL, respectively, of a clear, colorless solution at a strength of 500-1900 MBq/mL (13.5-51 mCi/mL) florbetapir F 18 at EOS. Each vial contains multiple doses and is enclosed in a shielded container to minimize external radiation exposure. Amyvid is Stored at 25 degrees Celsius, extrusions permitted to 15-30 degrees Celsius. The product does not contain a preservative.

6.3 Methods of procurement

The Froedtert Hospital Departments of Radiology will order Amyvid dose per procedure ordered. The will be paid for by study funds.

7. **Statistical Considerations:**

This is a prospective pilot study to assess the relative change in ^{18}F-Florbetapir in patients with cardiac amyloidosis at the onset of chemotherapy and after 6 months of treatment using a paired t-test. Given a sample size of 6 patients we estimate a minimum detectable effect

size of 4.7 with two-sided type I error of 10% and statistical power of 80%, assuming the standard deviation of the change in standard up-take value (SUV) is 4. The number of patients that is to be loss to follow-up is negligible. Therefore, this is not considered in our sample size estimation.

Safety/Stopping Rules:

1. Development of grade 3-4 adverse events deemed to be related to the study in 2 or more patients, will mandate halting further patient accrual.

Accrual Estimates:

25 patients consented to obtain number needed to fulfill enrolment in study given patient drop out and or patient death.

Accrual Period : Approximately 12-24 months

Follow-Up Period : One year to evaluate incidence of amyloid (hematologic and organ) response and relapse pattern.

8. Adverse Event Reporting Requirements

Definitions

The following are definitions of adverse events as defined by 21CFR312.32.

Types of Adverse Events

Adverse Event means any untoward medical occurrence associated with the use of a drug in humans, whether or not consider drug related.

Life-threatening adverse event or life-threatening suspected adverse reaction: An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse event or serious suspected adverse reaction: An adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator or sponsor, it results in any of the following outcomes:

- Death
- A life-threatening prolongation of existing hospitalization,
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent of the outcomes listed above.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse event or unexpected suspected adverse reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, it is not consistent with the risk information currently described.

Unexpected also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Adverse Event Grading

Grade	Description
0	**No AE** (or within normal limits).
1	**Mild**; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	**Moderate**; minimal, local or noninvasive intervention (e.g., packing cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL).
3	**Severe** or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
4	**Life-threatening** consequences; urgent intervention indicated.
5	**Death** related to AE

Adverse Event Attribution

Relationship	Attribution	Description
Unrelated to investigational agent/intervention	Unrelated	The AE *is clearly **NOT** related* to the intervention
	Unlikely	The AE *is **doubtfully related*** to the intervention
Related to investigational agent/intervention	Possible	The AE ***may be related*** to the intervention
	Probable	The AE ***is likely related*** to the intervention
	Definite	The AE ***is clearly related*** to the intervention

Reporting of Adverse Events

Unexpected adverse events will be reported throughout the study.

Unexpected Adverse Events

Unexpected Grades 3-5 (severe, life threatening, disabling, or fatal) require expedited reporting and will be submitted to the MCW/FH IRB within 5 calendar days of discovery for review.

Expected Adverse Events

Grades 4 and 5 (fatal) expected adverse events require expedited reporting and will be reported to the MCW/FH IRB within 5 calendar days for review. Expected adverse events that are being captured on the study toxicity form will be reported at the time of the form's scheduled due date.

Adverse Events Occurring after the End of the Study

Follow-up of AEs

Any unexpected AEs thought to be related to Florbetapir F-18 or study procedures ongoing at the time of study discontinuation will be followed until resolution or stable for at least 2 months.

FDA Reporting Procedures

Commercial Agents: Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. In some cases, an agent obtained commercially may be used for indications not included in the package label. The following procedures should be followed to determine if an adverse is reportable to the FDA:

Refer to the pharmaceutical section of the protocol to determine if an agent is investigational or commercial.

- **WHAT TO REPORT:** An unexpected, life-threatening (Grade 4) or unexpected, fatal (Grade 5) adverse event with an attribution of possible, probable or definite.
- **WHEN TO REPORT:** These events should be reported within (7) working days.

- **WHERE TO REPORT:** These adverse events with commercial agents must be reported to the FDA using the Med Watch form. A copy of the Med Watch form can be obtained from the FDA's Med Watch web site at www.fda.gov/medwatch. You can mail the reports to the address below or fax it 1-800-332-0178.

  Med Watch
  5600 Fishers Lane
  Rockville, MD 20852-9787

9. Patient Consent and Peer Review Statement

9.1 Subject Information and Informed Consent

Informed consent must be obtained from the subject prior to study participation. Legally appointed representatives will not be used to obtain consent from patients. The informed consent document must be signed and dated before initiation of any study procedures.

Subjects must be consented in accordance with all local regulatory and legal requirements. This process must include a verbal explanation of the nature, scope, and possible consequences of the study provided in plain language. The information should be presented by the investigator unless a designee is permitted by local regulations. The potential study subject should be encouraged to ask questions about the study.

The informed consent document must be prepared in accordance with GCP guidelines and with local regulatory and legal requirements. A copy of the signed consent form will be given to the subject and the original document must be safely archived by the investigator so that the forms can be retrieved at any time for monitoring, auditing, and inspection purposes.

The informed consent will be updated as appropriate (e.g., due to protocol amendment or if significant new safety information that may be relevant to consent of the subjects becomes available). If the informed consent is revised, it is the investigator's responsibility to ensure that an amended consent form is reviewed and signed by all subjects subsequently entered into the study and those currently in the study.

10. Patient reported outcomes survey

Patient reported outcomes survey was developed using PROMIS short form v2.0-Physical Function 8b, PROMIS short form v1.0 Dyspnea Severity 10a, and PROMIS short form v1.0 Fatigue 8a. Measures used in the patient survey were obtained from the health measures website at www.healthmeasures.net/promis, which can be accessed for further information.

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Appendix A – PET CT imaging protocol

Appendix

A.1. Clinical Protocol

A.1.1. Imaging

- Place patient on PET/CT imaging pallet
- Acquire topogram of patient for selection of thoracic scan range
- Perform CT using institutional parameters
- Begin listmode acquisition and wait 5 s
- Inject patient with approximately 10 mCi of ^{18}F-Florbetapir
- Perform 20 min PET listmode acquisition
- Histogram data into two 5 min datasets: 0–5, 15–20 min
- Reconstruct histogrammed data using identical parameters.

Additionally, if the clinical viewing software supports visualization of dynamic data, the dataset may be histogrammed using the following parameters:

Frame 1: 0–5 min
Frame 2: 6–14 min
Frame 3: 15–20 min

The corresponding ROI analysis is then only performed on the first and third frames. For non-listmode enabled scanners, the scan may need to be divided into two distinct 5 min acquisitions separated by the appropriate time delay.

- first acquisition: 5 min acquisition beginning at injection
- second acquisition: 5 min acquisition beginning at 15 min p.i.

A.1.2. Analysis

- Draw an ROI of at least 1 cc volume in the septal wall on the first time point image
- Copy the ROI to the second time point image.
- Calculate the ratio between the regions of interest.

In both cases, users should check for appropriate registration between the two time points before performing the ROI ratio analysis.

A.2. Study Imaging Parameters

This section outlines the imaging parameters for each modality used in this study.

A.2.1. CT

- kVp: 120
- mAs: continuously modulated (CareDose, Siemens Medical Solutions USA, Inc.)
- Scan range: thoracic region from upper lung to just below dome of liver
- Contrast: non-contrast enhanced
- Slice thickness: 5 mm slice thickness
- Reconstructed image matrix: 512 × 512
- Pixel size: approximately 1.5 mm.

A.2.2. PET

- Acquisition type: listmode
- No. of beds: 1
- Scan time: 20 min
- Injection: approximately 10 mCi ^{18}F-Florbetapir
- Reconstructed image matrix: 200 × 200
- Pixel size: approximately 2 mm
- Reconstruction algorithm: OSEM + Resolution Recovery (TRUEX, HD-PET, Siemens Medical Solutions USA, Inc.).

Appendix B – Patient reported outcomes survey

		Not at all	Very little	Somewhat	Quite a lot	Cannot do
PFC12	Does your health now limit you in doing two hours of physical labor?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFB1	Does your health now limit you in doing moderate work around the house like vacuuming, sweeping floors or carrying in groceries?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFA5	Does your health now limit you in lifting or carrying groceries?.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFA4	Does your health now limit you in doing heavy work around the house like scrubbing floors, or lifting or moving heavy furniture?.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

During the past 7 days…

		Not at all	A little bit	Somewhat	Quite a bit	Very much
HI7	I feel fatigued	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
AN3	I have trouble <u>starting</u> things because I am tired.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
	In the past 7 days…					
FATEXP41	How run-down did you feel on average? ...	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATEXP40	How fatigued were you on average?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATEXP35	How much were you bothered by your fatigue on average?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATIMP49	To what degree did your fatigue interfere with your physical functioning?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Please respond to each question or statement by marking one box per row.

		Without any difficulty	With a little difficulty	With some difficulty	With much difficulty	Unable to do
PFA11	Are you able to do chores such as vacuuming or yard work?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFA21	Are you able to go up and down stairs at a normal pace?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFA23	Are you able to go for a walk of at least 15 minutes?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFA53	Are you able to run errands and shop?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

	In the past 7 days…	Never	Rarely	Sometimes	Often	Always
FATIMP3	How often did you have to push yourself to get things done because of your fatigue?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATIMP16	How often did you have trouble finishing things because of your fatigue?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Please respond to each question or statement by marking one box per row.

	<u>Over the past 7 days</u>, how short of breath did you get with each of these activities?…	No shortness of breath	Mildly short of breath	Moderately short of breath	Severely short of breath	I did not do this in the past 7 days
DYSSV001	Dressing yourself without help	☐ 0	☐ 1	☐ 2	☐ 3	☐ X
DYSSV002	Walking 50 steps/paces on flat ground at a normal speed without stopping	☐ 0	☐ 1	☐ 2	☐ 3	☐ X
DYSSV003	Walking up 20 stairs (2 flights) without stopping	☐ 0	☐ 1	☐ 2	☐ 3	☐ X
DYSSV004	Preparing meals	☐ 0	☐ 1	☐ 2	☐ 3	☐ X
DYSSV005	Washing dishes	☐ 0	☐ 1	☐ 2	☐ 3	☐ X

DYSSV006	Sweeping or mopping	☐ 0	☐ 1	☐ 2	☐ 3	☐ X
DYSSV007	Making a bed.....	☐ 0	☐ 1	☐ 2	☐ 3	☐ X
DYSSV008	Lifting something weighing 10-20 lbs (about 4.5-9kg, like a large bag of groceries).....	☐ 0	☐ 1	☐ 2	☐ 3	☐ X
DYSSV009	Carrying something weighing 10-20 lbs (about 4.5-9kg, like a large bag of groceries) from one room to another.....	☐ 0	☐ 1	☐ 2	☐ 3	☐ X
DYSSV010	Walking (faster than your usual speed) for ½ mile (almost 1 km) without stopping.....	☐ 0	☐ 1	☐ 2	☐ 3	☐ X