Clinical Study Report Synopsis **GE Healthcare**
GE-067-010

Title: A PRINCIPAL, OPEN-LABEL, SINGLE CENTER STUDY TO VALIDATE THE DETECTION OF CEREBRAL CORTICAL AMYLOID WITH FLUTEMETAMOL F 18 INJECTION IN SUBJECTS PREVIOUSLY BIOPSIED

This is an exact copy of the synopsis from the final clinical study report for the study GE-067-010. The final clinical study report (document-identifier: GE-067-010 CREP) was authorized for use by the Head of Global Medical on 04-Jan-2012 (Version 2.0).

2 SYNOPSIS

Name of Sponsor/Company: GE Healthcare Ltd. and its Affiliates	**Individual Study Table Referring to Part of Dossier in Which the Individual Study or Study Table is Presented:**	**(For National Authority Use only)**
Name of Finished Product: Flutemetamol F 18 Injection		
Name of Active Ingredient: [^{18}F]flutemetamol	**Volume:**	
	Reference:	

Title of Study: A Principal, Open-Label, Single Center Study to Validate the Detection of Cerebral Cortical Amyloid with Flutemetamol F 18 Injection in Subjects Previously Biopsied.

Investigators and Study Center: Coordinating investigator Professor Juha Rinne, Turku PET Centre, Kiinamyllynkatu 4-8, 20520 Turku, Finland; 1 recruiting site, 1 imaging site in Finland

Center for Independent Evaluation of Images: Image Review Center, GE Healthcare.

Publication (reference): None

Study Period: 15 June 2010 to 17 November 2010 | **Phase of Development:** 3

Objectives:

The objectives are as stated in the statistical analysis plan (SAP) rather than the protocol.

Primary:

To determine the level of association between the quantitative estimates of brain uptake of [^{18}F]flutemetamol and the quantitative estimates of amyloid β levels in biopsy samples (standard of truth [SoT]) previously obtained during intracranial pressure measurement in patients who have normal pressure hydrocephalus (NPH). The quantitative estimates of brain uptake of [^{18}F]flutemetamol standard uptake value ratios (SUVR) were made from the analysis of positron emission tomography (PET) images at a location on the contralateral brain hemisphere that corresponds to (i.e., mirrors) the biopsy site using the cerebellum as the reference region. The quantitative immunohistochemical (IHC) estimates of amyloid β levels were estimated for each biopsy sample obtained using a monoclonal antibody (4G8) raised against amyloid β as the SoT.

Secondary:

1. To determine the level of association between SUVR and the quantitative estimates (area percentages) of amyloid levels for the following regions based on the cerebellum as the reference region:
   IHC measures (4G8):
   IHC estimates of amyloid β levels in the biopsy samples (4G8) compared to:
   - Ipsilateral SUVR estimates compared to IHC estimates of amyloid β levels in the biopsy samples
   - Composite SUVR estimates compared to IHC estimates of amyloid β levels in the biopsy samples
2. To determine the level of association between SUVR and the quantitative estimates (area percentages) of amyloid levels for the following regions based on the pons as the reference region:
   IHC measures (4G8):
   IHC estimates of amyloid β levels in the biopsy samples (4G8) compared to:
   - Contralateral SUVR estimates
   - Ipsilateral SUVR estimates
   - Composite SUVR estimates
3. To determine the levels of association between the blinded visual assessment of PET images (normal/abnormal amyloid levels) and the assessment of normal and abnormal amyloid levels from the quantitative histochemical (HC) and IHC estimates of amyloid levels in the biopsy samples. The blinded visual assessment of Flutemetamol F 18 Injection brain PET images was performed by 3 independent blinded readers trained in the evaluation of PET amyloid imaging.
4. To determine the inter-reader (between-reader) agreement of blinded visual image assessment, as measured by Fleiss' kappa (>70% = good; >80% = very good; >90% = excellent).
5. To determine the intra-reader (within-reader) reproducibility of blinded visual image assessment, as measured by their percentage self-consistency.

Study Design: This was a single-center, open-label PET study to evaluate the efficacy and safety of a single intravenous (i.v.) dose of Flutemetamol F 18 Injection for the detection of cortical amyloid. Frontal lobe

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biopsies had been obtained prior to study enrollment from 15 subjects during intracranial pressure measurement for suspected NPH. Subjects who underwent such biopsies were contacted to evaluate their interest in the study. If interested, these subjects signed an informed consent form, underwent a magnetic resonance imaging (MRI) scan, were administered Flutemetamol F 18 Injection, and underwent a PET scan. The level of amyloid in each biopsy sample was quantified using immunohistochemistry and histochemistry.
The subject allocation to Flutemetamol F 18 Injection was non-randomized: each subject was dosed. Image processing and SUVR determination were conducted blinded to the IHC and HC results.

Selection of Subjects:
Inclusion Criteria:
(1) Informed consent was signed and dated by the subject and/or the subjects' legally acceptable representative, if applicable, in accordance with local regulations.
(2) The subject was at least 50 years of age.
(3) The subjects' general health was adequate to comply with study procedures.
(4) The subject had a frontal lobe cortical biopsy adequate for the detection and quantitation of amyloid.
(5) For women who were either surgically sterile (have had a documented bilateral oophorectomy and/or documented hysterectomy) or were postmenopausal (cessation of menses for more than 2 years), enrollment in the study without a pregnancy test at screening was allowed. For women of childbearing potential, the results of a serum and urine human chorionic gonadotropin pregnancy test (with the results known on the day of and before the Flutemetamol F 18 Injection was given) were negative.

Exclusion Criteria:
(1) The subject had a contraindication for MRI or PET.
(2) The subject was pregnant or lactating.
(3) The subject had participated in any clinical study using an investigational agent within 30 days of dosing with the exception of the PET tracer, ^{11}C-labelled Pittsburgh Compound B ([^{11}C]PiB).
(4) The subject had a known or suspected hypersensitivity/allergy to [^{18}F]flutemetamol or to any of the excipients.

Number of Subjects (planned and analyzed):
Sixteen NPH subjects who underwent biopsy at Kuopio University Hospital were available for recruitment. Fifteen subjects agreed to participate, were enrolled and completed the study.

Treatment of Subjects :
Investigational Medicinal Product: All subjects received an i.v. dose of Flutemetamol F 18 Injection, administered within approximately 40 seconds. The activity of a single administration of Flutemetamol F18 Injection was approximately 185 Megabecquerels (MBq), corresponding to an effective dose of approximately 6 milliSieverts.
Imaging: PET imaging was conducted for 30 minutes, starting approximately 90 minutes after Flutemetamol F18 Injection.
Duration of Treatment: The study involved a maximum of 3 visits for each subject. Each subject attended a screening visit during which the subjects signed a consent form. Once all entrance criteria were met, an MRI scan was performed at a subsequent visit. The third visit consisted of the Flutemetamol F 18 Injection administration followed by PET imaging. Subjects were contacted approximately 24 hours later to collect data on potential adverse events (AEs). Reports of serious AEs for which a causal relationship to Flutemetamol F 18 Injection could not be ruled out were accepted for up to 30 days after injection.

Endpoints:
Efficacy: The primary endpoint was the SUVR obtained at a location on the contralateral brain hemisphere that corresponded to (i.e., mirrored) the biopsy site based on the cerebellum as the reference region.
The secondary endpoints were:

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Based on the cerebellum as the reference region:

- SUVR ipsilateral to the biopsy site;
- Composite SUVR.

Based on the pons as the reference region:

- SUVR determined at a location on the contralateral brain hemisphere that corresponded to (i.e., mirrored) the biopsy site;
- SUVR ipsilateral to the biopsy site;
- Composite SUVR

Classification of a subject's PET images as normal or abnormal based on the blinded evaluation of PET images by 3 independent blinded readers trained in the evaluation of PET amyloid imaging was another secondary endpoint.

Standard of Truth:

Each subject's biopsy sample was fixed and then cut into microscopic sections, which were mounted on microscope slides. The slides containing tissue sections were then exposed to the appropriate IHC or HC stain and associated reagents for the analysis of interest. The number of slides analyzed and the analytical method varied by stain used, as follows:

4G8 Immunohistochemistry: The SoT for the amyloid level in a subject's brain tissue was the percentage of total area of brain-tissue microscopic section staining positive for amyloid β after exposure to IHC reagents that targeted amyloid β through use of a monoclonal antibody raised against amyloid β (4G8). Up to 3 slides per subject were analyzed for percent area of tissue section staining amyloid-positive, using an automated slide reading process. The average result was taken to be the overall assessment for the biopsy specimen and thus the subject.

Thioflavin S: Up to 3 slides per subject were read under a microscope by a neuropathologist using fluorescent light, who examined each slide as having none, sparse, moderate or frequent amyloid plaque loads (modified Consortium to Establish a Registry for Alzheimer's Disease [CERAD] scoring system).

Bielschowsky silver stain: Up to 3 slides per subject were read under a microscope by a neuropathologist, who examined up to 5 fields of view (FOV) per slide and assessed each FOV as having none (scored as 0), sparse (scored as 1), moderate (scored as 2) or frequent (scored as 3) neuritic plaque loads (modified CERAD scoring system). The FOV scores were averaged for each slide. The mean FOV scores generated from each slide (up to 3) were averaged to give an overall mean FOV score for the biopsy specimen. We assume that the overall mean FOV score is representative of the biopsy sample and therefore, of the brain region from which the biopsy was taken. If this overall mean score was >1.5 the subject was classified as abnormal; otherwise, he/she was classified as normal.

Overall Pathology Assessment: The above IHC and HC assessments were used to determine a single overall pathology assessment of each subject's biopsy sample as normal or abnormal based on the level of amyloid. Any abnormal assessment rendered the overall pathology assessment as abnormal; only if all IHC and HC assessments were normal was the subject's biopsy specimen classified as normal.

Safety: Vital signs, clinical laboratory assessments, an electrocardiogram (ECG), a physical/neurological examination, and AEs were monitored and evaluated.

Statistical Analyses:

Primary Efficacy Analysis: In the primary analysis, the SUVR obtained at a location on the contralateral brain hemisphere that corresponds to (i.e., mirrors) the biopsy site was compared to the level of amyloid β determined from IHC in the biopsy sample, across all subjects. The Pearson correlation coefficient was calculated. The null hypothesis of no correlation between SUVR and IHC-4G8 estimates of amyloid β in the biopsy sample was tested. A statistical significance level of 0.05 was used for the correlation. This relationship was also analyzed

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using a regression model with SUVR as the independent variable, the amyloid β level from the biopsy sample as the dependent variable, and a factor for time from biopsy to PET scan.

Secondary Efficacy Analyses:

The level of association between SUVR and the amyloid level in the biopsy sample was analyzed using a regression model similar to that used in the primary analysis. The following table provides the comparisons analyzed:

Standard of Truth	**SUVR from PET imaging**			
	Reference region for SUVR	**Contralateral to the biopsy site**	**Ipsilateral to the biopsy site**	**Composite region**
IHC 4G8	**Cerebellum**	(primary) % of tissue section area staining positive for amyloid β	% of tissue section area staining positive for amyloid β	% of tissue section area staining positive for amyloid β
	Pons	% of tissue section area staining positive for amyloid β	% of tissue section area staining positive for amyloid β	% of tissue section area staining positive for amyloid β

The blinded visual assessment of Flutemetamol F 18 Injection brain PET images was performed by 3 independent blinded readers who had been trained in the evaluation of PET amyloid imaging. Each reader assessed each subject's PET image set as normal or abnormal for amyloid. Each subject's PET image set assessment (as normal or abnormal for amyloid) was compared with the corresponding histopathological assessment of the subject's biopsy sample (as normal or abnormal). Results were provided in a two-by-two table with the sensitivity and specificity and 95% confidence intervals.

The inter-reader (between-reader) agreement and the intra-reader (within-reader) reproducibility were determined. A kappa coefficient with 95% confidence interval was determined for each reader comparison, a coefficient was determined for all readers and each reader's result was compared to the majority result. Intra-reader reproducibility was measured using a kappa coefficient. A comparison of the pathology results obtained with the different stains (Bielschowsky silver stain, Thioflavin S, and overall pathology) is provided.

Summary of Results

Efficacy: The contralateral SUVR was significantly correlated with the biopsy specimen amyloid β level (expressed as the percentage of brain tissue section area staining positive for amyloid with 4G8). The Pearson correlation coefficient was 0.858 ($p<0.0001$). The full model was significant ($R^2 = 0.74$; $p = 0.0003$) and the model factor for SUVR was significant with a slope of 12.6 and a p-value of 0.0003. The factor for time in months was not significant and indicates that the subject's amyloid load did not change substantially between biopsy and subsequent PET imaging. In the secondary efficacy analyses, ipsilateral and composite SUVR values based on the cerebellum and pons as the reference regions correlated significantly with the percentage of brain tissue section staining positive for amyloid β based on 4G8 as did the contralateral SUVR results based on pons as the reference region.

In the blinded visual assessments of PET images, using the overall pathology assessment as the reference standard for amyloid level, Readers 1 and 2 and the majority read showed 100% (4/4) sensitivity, whereas Reader 3 showed 50% (2/4) sensitivity; each reader and the majority read showed 100% (11/11) specificity. When Bielschowsky silver-stained tissue sections were used to establish the neuritic plaque levels, Readers 1

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and 2 and the majority read had 100% (3/3) sensitivity, and reader 3 had a sensitivity of 33% (1/3); all readers had a specificity of 91% (10/11). When Thioflavin S stained tissue sections were used to establish the amyloid levels, blinded visual assessments of PET images by Readers 1 and 2 and the majority read showed 67% (4/6) sensitivity, and Reader 3 showed a sensitivity of 33% (2/6); specificity was 100% (9/9) for all readers. These results suggest that raised [^{18}F]flutemetamol uptake ratios in PET images are highly specific for abnormal amyloid deposits in the brain.

Inter-reader agreement was good as evidenced by the kappa score ($\kappa = 0.74$). Reader 3 rated 2 images as normal while Readers 1 and 2 rated them abnormal. Intra-reader reproducibility was 100%.

Safety: Four (27%) subjects experienced a total of 4 AEs. All AEs reported were mild in intensity. Two of the AEs reported were considered to be possibly related to Flutemetamol F 18 Injection. There were no serious AEs or AEs leading to withdrawal or deaths during the study. All AEs reported resolved during the study period.

There were no clinically significant findings in serum chemistry, hematology or coagulation laboratory values from screening through imaging or clinically significant findings in changes of vital signs, ECG or physical examination.

Conclusions:

Efficacy:

- The cortical uptake of [^{18}F]flutemetamol, as measured by SUVR, correlates significantly with the percentage of tissue section area immunostaining positive for amyloid β with 4G8.
- In blinded visual interpretations of [^{18}F]flutemetamol images, individual readers showed sensitivity of 33% to 100%, and the majority reads showed sensitivity ranging from 67% to 100%. Specificity ranged from 91% to 100% for individual readers as well as the majority reads.
- Between-reader agreement was good (κ=0.74), with 2 of 3 readers being in full agreement in every subject's [^{18}F]flutemetamol PET image. Intra-reader reproducibility was 100%.
- Despite the modest size of the cohort, the GE-067-010 study shows moderate to high sensitivity and high specificity of [^{18}F]flutemetamol PET for detection of amyloid in the brain of living NPH subjects.
- These data confirm the concordance of [^{18}F]flutemetamol PET imaging with histopathology, supporting its sensitivity to detect amyloid in the brain of living subjects with NPH.

Safety:

- Single doses of [^{18}F]flutemetamol were generally well tolerated in NPH subjects.
- No clinically significant abnormalities were noted in clinical laboratory results, or results from the physical/ neurological examinations. There were no clinically significant changes in ECG results.